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Protective effect of hydrogen rich saline solution on experimental ovarian ischemia reperfusion model in rats

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ABSTRACT

Background: The present study aimed to investigate the effects of hydrogen rich saline solution (HRSS) in a rat model of ovarian ischemia–reperfusion injury.
Methods: Thirty-six female Wistar-albino rats were grouped randomly, into six groups of six rats. The groups were classified as: sham (S), hydrogen (H), torsion (T), torsion/detorsion (TD), hydrogen-torsion (HT), and hydrogen-torsion/detorsion (HTD). Bilateral adnexal torsion was performed for 3 h in all torsion groups. HRSS was given 5 ml/kg in hydrogen groups intraperitoneally. Malondialdehyde (MDA) and glutathione-*S*-transferase (GST) levels were measured in both the plasma and tissue samples. Tissue sections were evaluated histopathologically, and the apoptotic index was detected by TUNEL assay. The results were analyzed by Kruskal–Wallis and Pearson chi-square tests using computer software, SPSS Version 20.0 for Windows.
Results: The MDA levels were higher and GST levels were lower in the torsion and detorsion groups when compared to other groups, but the differences were insignificant ($P > 0.05$). The MDA levels were lower and GST levels were higher in the HT and HTD groups compared with the T and TD groups ($P > 0.05$). Follicular injury, edema, vascular congestion, loss of cohesion and apoptotic index were higher in the torsion groups but decreased in the groups that received HRSS.
Conclusions: According to histopathological and biochemical examinations, HRSS is effective in attenuating ischemia–reperfusion induced ovary injury.

Adnexal torsion, although seen rarely, is a significant cause of emergency surgery in gynecology with a 2.7% prevalence [1]. Adnexal torsion (AT) can occur at any age and also in premenarchal girls, even though it is more commonly encountered in reproductive years [1]. In pediatric age groups, it is important to perform organ-preserving surgery since the masses causing torsion are mostly benign, and the ovaries are normal in 25% of the torsions [1–4]. The torsion/detorsion (T/D) of the ovary results in ischemia/reperfusion (I/R) damage [5]. During I/R, oxidative stress occurs owing to the increase in intracellular calcium (Ca^{+2}), decrease in adenosine triphosphate (ATP) and mitochondrial oxidative phosphorylation, and the generation of free oxygen radicals (FOR) as a result of the activation of phosphatase and protease that leads to the degeneration of the cytoskeleton and membrane phospholipids [6–8]. Low amounts of FOR occur during ischemia. During reperfusion, a larger amount of FOR occurs after re-oxygenation of the tissue and increases tissue damage by producing lipid peroxidation [9]. Hence, various chemicals are used as antioxidants to prevent I/R damage. Recently, tissue damage inhibitory and antioxidant hydrogen-rich saline solutions (HRSSs) have been used in several I/R models [10–13].

This experimental study aimed to investigate the probable protective effects of HRSS on I/R damage developed in the ovarian tissue of experimental AT in rats.

1. Materials and methods

Approval from Gazi University Ethical Committee was obtained before the study (ethical number:2015/012, ethical approval date: 5 March 2015/27,702). The study was carried out in the Animal Laboratory and Experimental Research Center of Gazi University in April 2015. Thirty-six healthy, adult, female, nonpregnant Wistar-Albino rats weighing between 200 and 250 g were used in the study. The animals were fed and kept in constant room temperature and humidity under 12-h light and dark standard laboratory conditions in a cage. There was no restriction of food or water supply prior to the experiment. These 36 animals are allocated into 6 groups, each group containing 6 animals.

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1.1. Experimental study model

All procedures were performed under general anesthesia and sterile conditions. Each rat was intramuscularly injected with 45 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg xylazine hydrochloride (Alfazyne 2%, Ege Vet, Turkey) for general anesthesia. The skin of the abdomen was shaved and cleaned with 10% povidone iodine. The lower abdomen was opened with a 2-cm midline incision, and the bilateral ovary and adnexa were exposed. The adnexa were rotated 720° clockwise and fixed to the abdominal wall for 3 h with 5/0 polydioxanone suture as AT. In the detorsion groups, the abdomen was reopened, and after the removal of the fixation sutures, the ovaries were brought to their former positions with detorsion [25,29]. To protect the rats from hypothermia, the operating table was heated with a lamp from above and a heater from below. During the waiting period, the incision line on the abdominal region was closed with 3/0 silk suture. HRSS was prepared according to the method reported by Cai et al. [16]. Hydrogen was dissolved in a saline solution for 6 h under high pressure (0.4 MPa). The saturated HRSS was stored under atmospheric pressure at 4 °C in an aluminum bag with no dead volume.

In the sham group (group S), laparotomy was performed and the incision was closed with a 3.0 nylon suture. In the hydrogen (H) group, 5 ml/kg of HRSS was given intraperitoneally (ip) 30 min before laparotomy, and after the uterus and adnexa were seen with laparotomy, the abdomen was closed. In the torsion (T) group, the AT was performed (3-h period of ischemia). In group TD, both AT and adnexal detorsion (AD) (3 h of reperfusion) were performed. In group HT, HRSS was given (5 ml/kg ip). After 30 min, AT was performed. In Ggoup HTD, HRSS was given (5 ml/kg ip). After 30 min, AT and AD were performed.

Relaparotomy was done after a 3-h period of all procedures in all groups, bilateral oophorectomy was performed, and the animals were sacrificed after their blood was taken and cardiac functions were tested at the end of the experiment. While the right ovary was kept in 10% formaldehyde in all groups for histopathological evaluation, the left ovary was frozen in −80 °C for MDA and GST analyses. The blood samples were centrifuged with a speed of 1710 g for 10 min, and their serums were placed in Eppendorf tubes and kept in −80 °C for MDA and GST analysis.

1.2. Histopathological evaluation

The ovaries of the rats were left in fixation within a 10% buffered formalin solution for 24 h. Following routine follow-ups, paraffin blocks and 4-μm-thick sections were prepared and dyed with hematoxylin eosin (H&E). Sections dyed with H&E were examined under light microscopically for I/R damage and evaluated semiquantitatively. While every ovary was classified as “0: no damage”, “1: slightly damaged (mild)”, “2: medium-damaged (advanced)”, or “3: severely damaged (serious)” in terms of edema in the ovary tissue, follicular cell damage, and vascular congestion, they were evaluated as “existent” and “nonexistent” in terms of hemorrhage, neutrophil infiltration, and cohesion loss [17]. One pathologist examined all of the ovarian sections in a blinded fashion.

1.3. Apoptosis index

To detect apoptosis, TUNEL assay was performed using an in situ apoptosis detection kit (ApopTaq Peroxidase In Situ Apoptosis Detection Kit; S7101-KIT, Millipore) by following the manufacturer's instructions. The paraffin blocks were cut into 5 μm. Apoptotic cells were counted according to percentage of TUNEL-positive apoptotic cells in 1000 granulosa cells from each ovarian section (3 sections/ovary; 4 ovaries/group). The sections were independently evaluated by two experienced observers concurrently blinded to the experimental course. The slides were observed under a light microscope at high magnification (400×).

1.4. Indication of GST and MDA in ovarian tissues and blood

The ovary tissue was 1/10 homogenized with cold 0.9% NaCl. The homogenate was centrifuged with a speed of 1710g for 10 min and its supernatant was separated. Tissue protein preparation was carried out according to the Lowry method by using Folin reactive, which is the final product of lipid peroxidation in ovary homogenates and serum samples. The MDA levels were studied according to the method described by Van Ye et al. [18,19]. Preparation of glutathione-*S*-transferase activity in ovary homogenates and serum samples was studied according to the method described by Habig et al. [20]. Tissue MDA and GST results were reported in units of nanomiligram per protein.

1.5. Statistical analysis

Statistical analysis of the data was performed on SPSS Version 20 for Windows on a computer. The biochemical results obtained from blood and tissue samples and the apoptosis index were stated in terms of the median ± range. The Kruskal–Wallis test was used to test the differences between the groups. The Pearson chi-square test was used to detect the differences of histopathological evaluation between the groups; $p < 0.05$ was considered as statistically significant.

2. Results

The histopathological evaluation of the ovary tissues is given in Table 1. Severe edema and follicular cell damage were significant in rat ovary tissues in groups T and TD and statistically significant in groups S and H ($p < 0.05$). Although groups HT and HTD were not as good as groups H and S in terms of edema, statistically significant improvement was observed in group T and TD ($p < 0.05$). Edema regressed to the degree of mild and medium from severe in groups HT and HTD, which were given HRSS treatment. In fact, edema disappeared in three rats in group HT and in one rat in group HTD. Follicular cell damage regressed to the degree of mild and medium from severe especially in group HTD, which was given HRSS treatment (Table 1), (Figs. 1 and 2). Damage was not statistically significant when the groups were investigated for vascular congestion, and similarly, while damage was detected in groups T and TD (in all rats), severe damage seen in group HTD regressed and disappeared ($P > 0.05$) (Fig. 3). Except for groups S and H, neutrophil infiltration was not seen in groups T, TD, HT, and HTD. Hemorrhage was seen in 4 rats in groups TD ($p < 0.05$) (Fig. 4), and in one rat in group T. While hemorrhage regressed in groups HT and HTD, which were given HRSS treatment, it disappeared in group HT and was seen in one rat in group HTD ($p < 0.05$). Cohesion loss was seen in 6 rats in group TD and in 5 rats in group T ($p < 0.05$). Cohesion loss totally disappeared in groups HT and HTD, which were given HRSS treatment ($p < 0.05$) (Fig. 5).

Apoptosis was not present in groups S and H, but present in other groups ($p < 0.05$). The highest apoptosis index was found in groups T and TD (20 ± 10 and 15 ± 290, respectively) (Table 1) (Fig. 6). In groups HT and HTD, which were given hydrogen therapy, significant fall was detected in apoptosis index (5 ± 20 and 10 ± 4.15, respectively) ($p < 0.05$).

Median oxidant MDA values in the ovary tissues were detected to be the lowest in group S with 6.01 ± 11.32 nmol/mg protein and highest in group TD with 25.40 ± 28.50 nmol/mg protein ($p = 0.001$). But the results were found to be statistically insignificant ($p = 0.78$) (Table 1). Compared to groups T and TD, the tissue MDA values in groups HT and HTD, which were given HRSS, were found to be low, especially in the HTD group. Moreover, tissue MDA value in group TD was higher than that in group T, which is evidential that the damage occurred after reperfusion (Table 2).

Median serum MDA values were found to be the lowest in group H with 0.73 ± 5.04 nmol/ml and highest in groups T and TD (4.77 ± 2.21 and 4.51 ± 1.89 nmol/ml) (Table 2). However, a statistical