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Original Article

Effect of combined anti-inflammatory and antioxidant therapy on ischemia-reperfusion injury in rat ovary: an experimental study

Murat Kaya[1], Aybars Ozkan[1], Recep Eroz[2], Tutku Soyer[3], Murat Kabaklioglu[1], Murat Oktay[4], Hilmi Demirin[5], Sengul Cangur[6], Hakan Uzun[7], Adem Kucuk[8]

Departments of [1]Pediatric Surgery, [2]Medical Genetics, Medical Faculty, [4]Pathology, [6]Biostatistics, [7]Pediatrics, Medical Faculty, Duzce University, Duzce, Turkey; [3]Department of Pediatric Surgery, Medical Faculty, Hacettepe University, Ankara, Turkey; [5]Department of Biochemistry, Medical Faculty, Turgut Ozal University, Ankara, Turkey; [8]Department of Pediatric Surgery, Muncurlu Ataturk State Hospital, Duzce, Turkey

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Abstract: An experiemental study was performed to evaluate the effect of combined anti-inflammatory and antioxidant theraphy on ischemia-reperfusion injury in rat ovary. Also the results of combined theraphy is aimed to compared with monotherapy. Fifty-four female Wistar rats were randomly divided into 9 equal groups (n=6). In Sham group right ovaries of the rats were sampled without generating ischemia and reperfusion injury via median laparatomy. Ischemia/Reperfusion (I/R) was performed by clamping the vascular supply of right ovary for 3 hours (I/R 1 group) and 6 hours (I/R 2 group), respectively. After one hour reperfusion, rats recieved 20 mg/kg Methylprednisolone (Pred 1; 3 hours ischemia and Pred 2; 6 hours ischemia) and 50 mg/kg Vitamin C (Vit C 1; 3 hours ischemia and Vit C 2; 6 hours ischemia). The combined therapy groups (Pred+Vit C 1 and Pred+Vit C 2) were administered same doses of both Methylprednisolone and Vitamin C. Rats were sacrifed after 24 hours of reperfusion and ovarian tissues were sampled for oxidative markers [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] biochemically. Histopathological findings of inflammation (follicular cell degeneration, vascular congestion, hemorrhage and infiltration by inflammatory cells) were also evaluated with an injury score grading normal findings to severe injury (Grade 0 to 3). The results were compared among groups. Mean levels of antioxidant enzymes and histopathologic grades showed significant difference among groups ($P<0.05$). MDA and CAT levels were lower in Pred+Vit C 1 than Pred 1, Vit C 1 and I/R 1 ($P<0.05$). SOD and CAT levels were lower in Pred+Vit C 2 than Pred 2 and I/R 2. Total injury scores were lower in Pred+Vit C 1 and Pred+Vit C 2 than I/R 1 and I/R 2 ($P<0.05$). The combined treatment of anti-oxidant and anti-inflammatory theraphy reduces the biochemical and histopathologic findings of I/R injury in rat ovary. These results are significantly comparable with the effect of monotheraphy.

Keywords: Ischemia-reperfusion, methyl prednisolone, vitamin C and ovary

Introduction

Adnexal torsion is an infrequent but serious surgical emergency. Early diagnosis and emergency surgical treatment (distortion) are important to preserve fertility and to prevent peritonitis or loss of the ovary. Tissue damage is more severe during reperfusion than ischemia because of oxygen derived radicals [1]. Arterial or venous blood flow decrease due to inadequate organ and tissue perfusion as a result of ischemia, defined as the deprivation of oxygen in the tissue or organ, deposition of discharge of toxic metabolites and cellular energy stores leads to cell death [2]. Ischemic cells and tissues must be cleaned toxic metabolites and restore blood flow. Paradoxically reperfusion causes to more serious damages than ischemic injury in tissues [3]. Reperfusion related cellular damages are caused by the rapid entry reactive oxygen species (ROS), which are rapidly generated in the tissue as a result of reperfusion with several mechanisms. Membrane lipids, proteins and nucleic acids are the cellular molecules, which easily affected from reperfusion-related damage [4].

Enzymatic antioxidant defences include glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Glutathione (γ-glutamyl-cysteinyl-glycine; GSH), ascorbic acid (vitamin C), α-tocopherol (vitamin E), carotenoids, flavonoids, polyphenols and other exogenous antioxidants are non-enzymatic antioxidants [5].

Vitamin C is the most potent water soluble antioxidant molecule. Since vitamin C is not synthesized in humans, it must be taken with diet. Due to vitamin C reduces oxygen free radicals in reperfusion injury; it has a positive contribution in wound healing. The redox form of vitamin C, ascorbate is a physiological antioxidant. It was hypothesized that the early administration of a high pharmacological dose of vitamin C can reduce oxidative damage to endothelial and other cells, and so enhances tissue perfusion and oxygenation, and reduces subsequent organ dysfunction after ischemia/reperfusion (I/R) [6, 7]. It also improves immune function, and synthesis of collagen (wound healing), catecholamines, cortisol and carnitine [5].

The high-dose vitamin C can prevent deterioration of microcirculatory flow by inhibiting inducible nitric oxide synthase, and nicotinamide adenine dinucleotide phosphate-oxidase. Also it prevents uncoupling of oxidative phosphorylation, augmenting tetrahydrobiopterin and decreasing the formation of peroxynitrite and superoxide by removing superoxide. It can also improve vascular responsiveness to vasoconstrictors, conserves endothelial barrier via maintaining cyclic guanylate phosphatise, occludin phosphorylation and prevents apoptosis [8]. Additionally, vitamin C can induce anti-inflammatory effects by owing to its ability to inhibit secretory phospholipase A2 (sPLA2) [9].

It is not exact that how the corticosteroids block ischemia-reperfusion injury and reduce inflammatory cytokines and correct the cellular reducing the blood flow. Methylprednisolone is a potent anti-inflammatory drug. Glucocorticoid steroids act as an antioxidant and membrane stabilizer via reducing the migration of macrophages and neutrophils to inflammatory sites. They also reduce lipid peroxidation, hydrolytic enzyme release, and oxygen radical production [10]. Even if the effects of methyl prednisolone on I/R injury have been widely studied [10-13], to the best of our knowledge its' combined using with vitamin C in I/R injury have not been reported, yet.

While methyl prednisolone is used because of the anti-inflammatory activity, ascorbic acid is used due to its antioxidant activity. Until this time, a lot of studies were performed including the many anti-inflammatory, antioxidant, vasodilator and other molecules such as sildenafil [14], resveratrol [15], methylene blue [16], vitamin C [17], selenium [3], methylprednisolon [12] and erythropoietin [18] that reduce ischemia-reperfusion injury and used for direction of this damage.

In the current study, we performed ovarian torsion model in rats and aimed to show whether anti-inflammatory and antioxidant combination therapy reduces ischemia-reperfusion injury in rat ovary more than monotherapy.

Materıals and methods

Animals

Animals were provided by Bolu Abant Izzet Baysal University Medical Experimental Application and Research Center. Fifty-four female Wistar rats, weighing 150 to 250 g, were randomly divided into 9 equal groups (n=6). The experimental protocols were in compliance with the Abant İzzet Baysal University Ethics Committee on Research in Animals as well as the internationally accepted principles for laboratory animal use and care.

Experimental design

After anestheziation with intramusculary ketamine hydrochloride (Ketalar®, 20 mg/kg, Eczacıbasi, Istanbul Turkey), median laparatomy was performed. For this purpose, the clamps were placed on vascular pedicle of right ovary for 3 and 6 hours to generate ischemia in groups and animals were sacrificed after 24 hours reperfusion period [19].

In Sham group right ovaries of the rats were sampled without generating an ischemia and reperfusion injury via median laparatomy. Ischemia/Reperfusion (I/R) was performed by clamping the vascular supply of right ovary for 3 hours (I/R 1 group) and 6 hours (I/R 2 group) respectively. After one hour reperfusion, rats recieved 20 mg/kg Methylprednisolone (Pred 1; 3 hours ischemia and Pred 2; 6 hours isch-

emia) and 50 mg/kg Vitamin C (Vit C 1; 3 hours ischemia and Vit C 2; 6 hours ischemia). The combined therapy groups (Pred+Vit C 1 and Pred+Vit C 2) were adminstered same doses of both Methylprednisolone and Vitamin C. Rats were sacrifed after 24 hours and ovarian tissues were sampled for oxidative markers [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] biochemically and for histopathologic findings of inflammation (follicular cell degeneration, vascular congestion, hemorrhage and infiltration by inflammatory cells).

Biochemical analysis of ovarian tissues

The level of antioxidants was analyzed from ovarian tissues. Ovaries of the rats were divided equally into two parts, one of which was used for biochemical analyses, the other for histopathological examination. Each organ section for biochemistry was readily put in a plastic tube containing phosphate buffer saline (pH: 7.4), and stored in fridge at -80°C (since oxidation continues in room temp) until analysis time. When the analyses were started, the ovaries were taken out, thawed, and each of them was immediately homogenized for three minutes (Ultra-Turrax T25, Janke&Kunkel GmbH&Co. KG, IKA® -Labortechnik, Staufen, Germany) in ice-cold phosphate buffer in order to provide a %10 homogenate. The homogenates were centrifuged at 6000 × g for 10 minute and supernatants were obtained.

Owing to the analysis process better reflects to the mass of the tissue, the levels of MDA and enzymes activities were detected per protein content of the each tissue. The levels of MDA, GPx, SOD, and CAT were detected in the supernatants. Protein contents of homogenates were detected by the Lowry method [20].

The tissue levels of MDA analysis

MDA levels were determined as a marker for lipid peroxidation using a high pressure liquid chromatography (HPLC) method improved by Mateos and modified by Akturk [21, 22]. The method is based on chromatographic separation of the pyrazole and hydrazone derivatives after derivatization of MDA with 2,4-dinitrophenylhydrazine (DNPH). Then 0.5 g ovarian tissue was added into 2.5 ml Trizma base buffers (0.25 mol/L), and 250 µL of 500 ppm butylated hydroxytoluene (BHT) was added on the tissue sample to prevent further oxidation, after which it was well mixed. Homogenization preceded a centrifugation for 30 minutes at 10,000 × g at 4°C, and followed by collecting of 250 µL supernatant in an Eppendorf tube. 55 µl NaOH (6 mol/L) was added to the Eppendorf tube, and incubated in a 60°C, and followed by collecting of 250 µL supernatant in an Eppendorf tube. 55 µl NaOH (6 mol/L) was added to the Eppendorf tube, and incubated in a 60°C, and followed by collecting of 250 µL supernatant in an Eppendorf tube. 55 µl NaOH (6 mol/L) was added to the Eppendorf tube, and incubated in a 60°C water bath for 30 minute water bath for 30 minute water bath for 30 minute (alkaline hydrolysis of protein-bound MDA). After hydrolysis, 125 µl of % 35 (v/v) per-chloric acids is added for precipitation of proteins and the mixture was centrifuged at 2800 × g for 10 minute. Then, 25 µL of DNPH solution was added to 50 µL of supernatant in an Eppendorf for derivatization, after which the mixture was vortexed and left in dark for 30 minute at room temperature for incubation. Then, 50 µL of sample was injected automatically to the HPLC system using a reversed-phase column with an acetonitrile (ACN)-H2O-acetate (38:62:0.2, V:V:V) mobile phase flow rate of 0.65 mL/minute. Wavelengths of 310 nm are used for spectrophotometric determination of the MDA derivative. The HPLC instrument used a Thermo Finnegan Spectra System with diode array detector. The concentration of MDA was given as nmol/ml [21].

The determination of GPx activity

The GPx activity was determined via Paglia and Valentine method [23]. According to this method, the oxidation of glutathione is catalyzed by GPx via cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. Wavelengths of 340 nm were used to measure the decrease in absorbance of NADPH.

The determination of CAT activity

Aebi method was used to measure the CAT activity [24]. The assay is based on the determination of the rate constant (k, s^{-1}) of decomposition of hydrogen peroxide by the enzyme catalase. The formulation of the rate constant calculation is $k=(2.3/\Delta t)(a/b)\log(A1/A2)$, where

Table 1. The mean values of biochemical parameters and total ovarian tissue damage scores in 3 hours ischemic groups (P<0.05 vs Sham group)

Groups		Parameters				
		MDA (nmol/mg prot)	GPx (U/mg prot)	CAT (kU/mg prot)	SOD (U/mg prot)	Total tissue damage score
Sham	Mean ± SD	0.74±0.06	0.22±0.03	0.12±0.03	15.12±1.08	1 (1)
Pred 1	Mean ± SD	1.24±0.09	0.30±0.06	0.36±0.04	22.17±1.73	5.5 (1.5)
Vit C 1	Mean ± SD	1.35±0.09	0.26±0.06	0.13±0.02	18.75±2.07	7.5 (1.5)
Pred+Vit C 1	Mean ± SD	1.36±0.05	0.27±0.05	0.15±0.03	19.99±1.72	5 (3.5)
I/R 1	Median (IQR)	1.19±0.03	0.30±0.08	0.34±0.04	21.34±0.9	8.5 (1.5)
	P	<0.001	0.103	<0.001	0.001	<0.001

P: *p* value of post-hoc comparisons; SD: Standard Deviation; IQR: Interquartile Range. GPx: Glutathione peroxidise; CAT: Catalase; SOD: Superoxide dismutase; MDA: Malondialdehyde; I/R: Ischemia/reperfusion; Pred: Methylprednisolone; Vit C: Vitamin C.

A1 and A2 are the absorbance values of hydrogen peroxide at t1 (0.second) and t2 (15.second), b is the protein level of the homogenate and it is the dilution factor.

The determination of SOD activity

The measurement of SOD was based on the principle that xanthine reacts with xanthine oxidase (XO) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the degree of inhibition of this reaction [25].

The absorbencies in SOD, GPx and CAT analyzes were taken within the same spectrophotometer mentioned above in protein analysis section. An autoanalyser (Aeroset, Abbott, Abbott Park, IL, USA), was used to determine the activities of SOD, GPx, and protein contents of the tissues; and a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) was used to estimate the activities of the enzyme CAT.

Histopathological evaluation

The ovarian tissue samples were obtained from the right ovary was embedded in the paraffin blocks and cut into 4-5 μm thickness. Before staining protocol with haematoxylin-eosin, the tissue sections were deparaffinised in xylene and then rehydrated in graded alcohol solutions. Then stained procedure was performed and the slides were examined using a light microscope (Eclipse 80i; Nikon, Tokyo, Japan) and photographed using a digital camera (Digital Sight DS-fi1; Nikon) by an expertise pathologist. The criteria for ovarian ischemia were follicular cell degeneration, vascular congestion, hemorrhage and infiltration by inflammatory cells. The sections were evaluated for the degrees of alveolar hemorrhage, vascular congestion, inflammation, and edema. Each slide was examined, and the severity of observed changes was scored using a scale of none (0), mild (1), moderate (2), and severe (3) damage [18].

Statistical analysis

The results were calculated with descriptive statistics for each variable (mean, standard deviation, median and interquartile range). The normality assumption for the continuous variables was analyzed by Shapiro Wilk test quality. Kruskal-Wallis test was used for group comparisons. A significant difference post-hoc test for detection of groups of Dunn's test was used. The degree and direction of the relationships between variables were determined by the Spearman correlation analysis. Statistical analysis was performed with PASW 18 v program. *P* values less than 0.05 to be considered as significant.

Results

Results of biochemical investigations

The mean levels of antioxidants were listed in **Tables 1** and **2**. MDA levels were significantly different between the groups (P<0.001). Sham group had lower MDA levels when compared to Vit C 1 (P=0.001), Pred 2 (P<0.001) and I/R 2 groups (P<0.001).

Table 2. The mean values of biochemical parameters and total ovarian tissue damage scores in 6 hours ischemic groups (P<0.05 vs Sham group)

Groups		Parameters				
		MDA (nmol/mg prot)	GPx (U/mg prot)	CAT (kU/mg prot)	SOD (U/mg prot)	Total tissue damage score
Sham	Mean ± SD	0,74±0.06	0.22±0.03	0.12±0.03	15.12±1.08	1 (1)
Pred 2	Mean ± SD	1.75±0.05	0.33±0.02	0.41±0.02	24.91±2.00	7 (1.25)
Vit C 2	Mean ± SD	1,43±0.09	0.27±0.06	0.19±0.05	19.93±1.63	7.5 (1.5)
Pred+Vit C 2	Mean ± SD	1,39±0.06	0.29±0.06	0.21±0.05	21.88±1.44	4.5 (4.25)
I/R 2	Median (IQR)	1,76±0.13	0.32±0.02	0.39±0.01	24.87±1.47	11 (2.25)
	P	<0.001	0.014	<0.001	0.001	<0.001

P: *p* value of post-hoc comparisons; SD: Standard Deviation; IQR: Interquartile Range. GPx: Glutathione peroxidise; CAT: Catalase; SOD: Superoxide dismutase; MDA: Malondialdehyde; I/R: Ischemia/reperfusion; Pred: Methylprednisolone; Vit C: Vitamin C.

When the antioxidant enzyme activities were compared, significant differences were found among the groups (P<0.05). When we evaluated GPx levels, there was no significant different among 3 hours ischemic groups (Vit C 1, Pred 1 and Vit C+Pred 1) (P>0.05), whereas significant difference was detected in Vit C 2, Pred 2 and Pred+Vit C 2 groups (P<0.05). GPx levels were significantly lower in Sham group than Pred 2 group (P=0.012).

The differences were statistically significant among the groups for CAT activities (P<0.001). CAT activities were significantly lower in Sham group than Pred 1 (P=0.003), Pred 2 (P<0.001), I/R 1 (P=0.013) and I/R 2 groups (P=0.013). Additionally, CAT activities were significantly lower in Vit C 1 than in Pred 1 group (P=0.015). Also, this activities were significantly lower in the Vit C 2 group than in Pred 2 group (P=0.048).

SOD activities were significantly difference between the groups (P<0.001). SOD activities were significantly lower in Sham group than Pred 2 and I/R 2 groups (P=0.001). This activities were also lower in Vit C 1 group than Pred 1 (P=0.001) and I/R 1 groups (P=0.005).

MDA and CAT levels were lower in Pred+Vit C 1 than I/R 1 group (P=0.237). MDA, SOD and CAT levels were lower in Pred+Vit C 2 than I/R 2 group (P=0.105; P=0.344 and P=0.882, respectively).

Comparison of MDA, GPx and CAT and SOD levels for all groups were given in **Figure 1**.

Histopathologic results

Edema, congestion, hemorrhage, inflammation and total injury scores were significantly different between the groups (P<0.001). Edema scores were significantly difference among the groups (P=0.001). The group/groups which cause this difference were evaluated by post-hoc test. Edema scores were significantly lower in Sham group than in I/R 1 (P<0.001), Pred 1 (P=0.020) and I/R 2 groups (P<0.001). Also, it was significantly lower in Pred+Vit C 2 group than I/R 2 group (P=0.039).

Congestion scores were significantly difference among the groups (Vit C 1, Pred 1 and Pred+Vit C 1) (P=0.010) and (Vit C 2, Pred 2 and Pred+Vit C 2) (P=0.001). This scores were significantly lower in Sham group than I/R 1 (P=0.011), Vit C 1 (P=0.032) and I/R 2 groups (P<0.001). Hemorrhage scores were significantly difference among the groups (P=0.001). This scores were significantly lower in Sham group than Vit C 1 (P=0.002), Vit C 2 (P=0.015), I/R 1 (P=0.002) and I/R 2 groups (P<0.001). Inflammation scores were significantly difference among the groups (P<0.001). This scores were significantly lower in Sham group than I/R 1 (P<0.001), Vit C 1 (P=0.029), Pred 1 (P=0.029), I/R 2 (P<0.001), Vit C 2 (P=0.004) and Pred 2 groups (P=0.018). Total injury scores were significantly difference among the groups (P<0.001). Total injury scores were significantly lower in Sham group than I/R 1 (P<0.001), Vit C 1 (P=0.010), I/R 2 (P<0.001) and Vit C 2 groups (P=0.027).

Edema, hemorrhage, inflammation and total injury scores were lower in Pred+Vit C 1 than I/R 1 (P=0.145; P=0.782; P=0.077 and P=0.074, respectively). Inflammation, edema, congestion, hemorrhage and total injury scores were lower in Pred+Vit C 2 than I/R 2 (P=0.540;

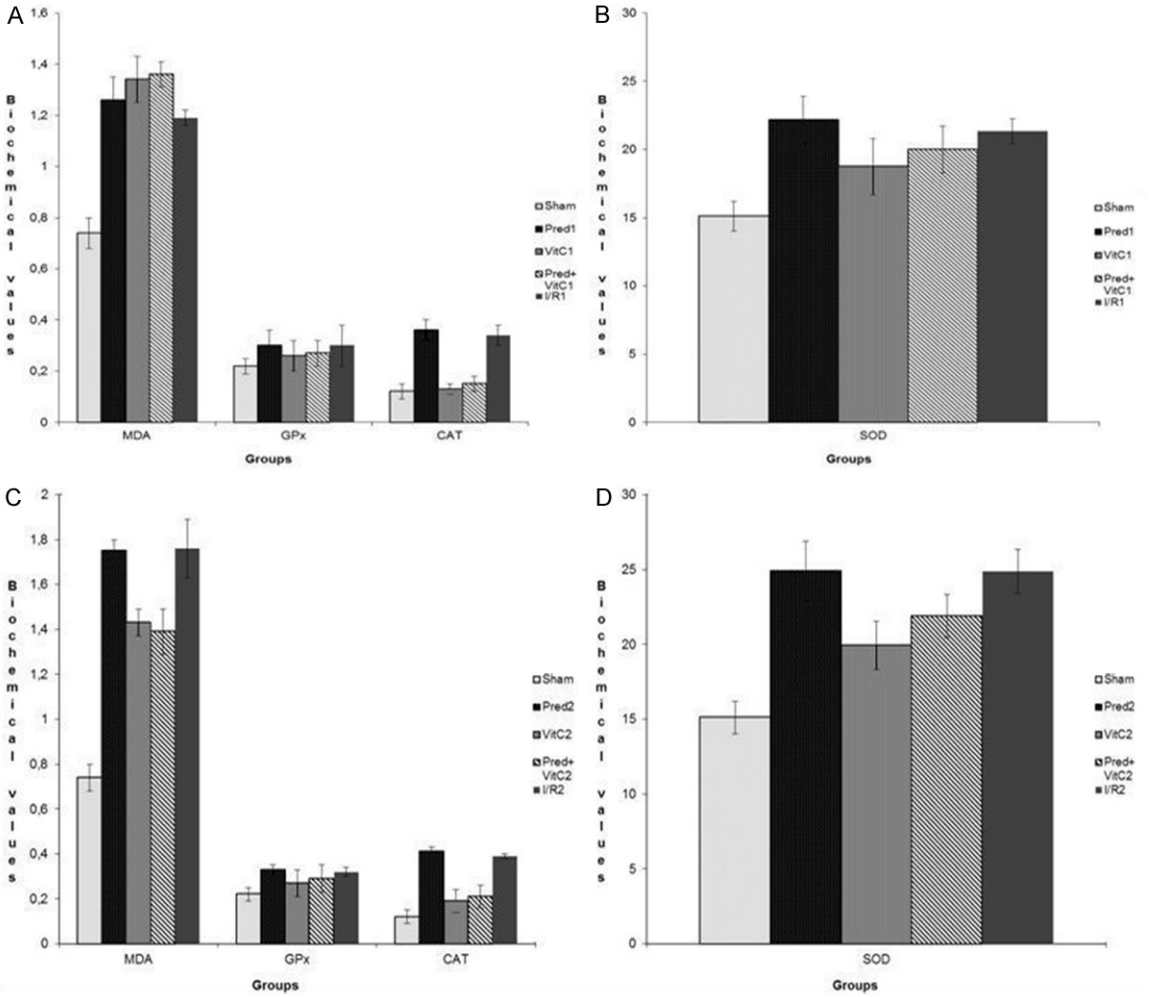

Figure 1. Comparison of MDA, GPx and CAT (A) and SOD (B) levels 3 hours and MDA, GPx and CAT (C) and SOD (D) levels 6 hours after ischemia (groups).

P=0.540; P=0.062; P=0.132 and P=0.057, respectively).

A significant positive correlation was found between MDA levels and both hemorrhage and total injury score in Vit C 1 group (r=0.837, P=0.038; r=0.880, P=0.021, respectively). In the same group, a significant positive correlation was found between CAT levels and inflammation (r=0.828, P=0.042). Additionally, there was a significant positive correlation between SOD levels and both inflammation and total injury score in Vit C 1 group (r=0.828, P=0.042; r=0.880, P=0.021, respectively). A significant positive correlation was found between MDA levels and both edema and congestion in Pred+Vit C 1 group (r=0.878, P=0.021). Conversely, a negative correlation was found between GPx levels and congestion in I/R 1 group (r=0.-845, P=0.034).

A significant positive correlation was found between MDA levels and hemorrhage in Pred 2 group (r=0.878, P=0.021). Conversely, a significant negative correlation was found between CAT levels and both hemorrhage and edema in Pred+Vit C 2 group (r=-0.828, P=0.042). Additionally, a significant negative correlation was found between SOD levels and both hemorrhage and edema in Pred+Vit C 2 group (r=-0.828, P=0.042).

Demonstrative example of the histopathological pictures was given in **Figure 2** for Sham and **Figure 3** for 6 hours ischemic combined therapy group.

Discussion

Ischemia is described as cell death caused by inadequate tissue perfusion because of the

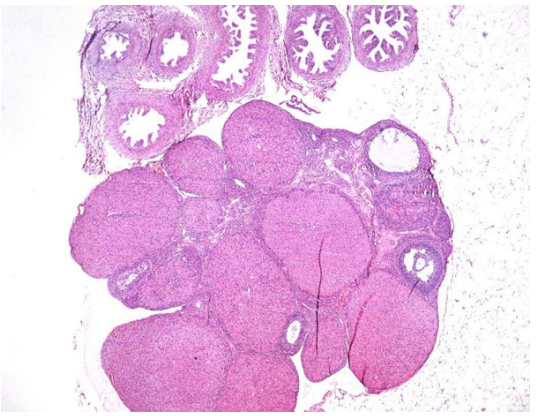

Figure 2. Usual follicular and corpus luteum does not contain the edema, hemorrhage and congestion from the Sham group (H&E × 40).

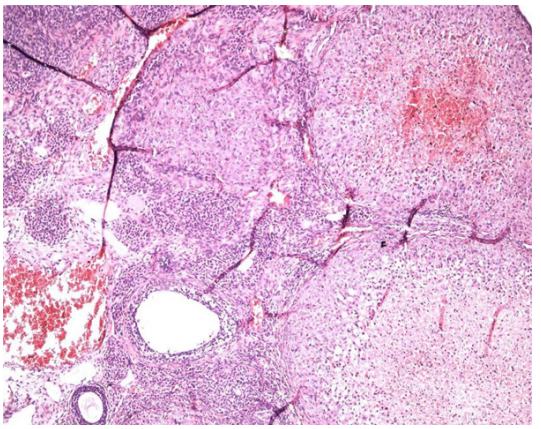

Figure 3. Mild congestion and hemorrhage, moderate edema from 6 hours ischemic combined therapy group (H&E × 100).

reduction in blood flow, depletion of cellular energy storages and accumulation of toxic metabolites. Ovarian torsion/distortion is the basic clinical pictures of I/R damage [4]. Recently, a lot of studies were performed about I/R injury on damage of renal, myocardial, cerebral, intestinal, testicular, ovarian and hepatic tissues. In the current study, we researched whether the anti-inflammatory (methyl prednisolone) and antioxidant (vitamin C) combination (methylprednisolone+vitamin C) therapy reduces ischemia-reperfusion injury more than when administered alone on ovarian torsion/distortion.

It was shown that methyl prednisolone and vitamin C decrease the ischemia-reperfusion injury in the rat ovary [12, 17]. In the studies, methyl prednisolone was used to prevent the I/R injury on hepatic [11], ovarian [12], lungs [13], cerebral [26] and myocardial damage [27]. Our results confirmed that methyl prednisolone prevented the I/R injury on ovarian torsion, too.

The useful effects of vitamin C were shown on hepatic [28], ovarian [17] damages after ischemia. Additionally, the useful effects of combination therapy of vitamin C with vitamin E on renal [29] and myocardial [30] were shown after the ischemic damage. According to our findings, alone and combination therapy of vitamin C (with methyl prednisolone) reduced the I/R damage, too.

It was reported that the use of vitamin C and methyl prednisolone after the I/R damage decrease the histopathological injury [12, 17]. We also found the similar results.

It was found that the use of anti-inflammatory and antioxidant drugs reduced MDA and antioxidant enzymes (CAT, GPx and SOD) levels after I/R [3, 12]. But in another study, it was shown that SOD levels were higher in the healthy group than ischemic group [4]. Conversely this result, we found the decreased antioxidant enzymes (CAT, GPx and SOD) and MDA levels in treated group than no treated group after I/R. We also use the combination therapy of Pred+Vit C (20 mg/kg+50 mg/kg) after the I/R injury. To the best of our knowledge this is the first study which carried out combination therapy of Pred+Vit C. According to our results; MDA, CAT and SOD levels were lower in the groups who given combination therapy (Pred+Vit C 1 and Pred+Vit C 2) than ischemic groups without drugs (I/R 1 and I/R 2). Also total histopathological score was lower in the groups who given combination therapy (Pred+Vit C 1 and Pred+Vit C 2) than ischemic groups without drugs (I/R 1 and I/R 2), too.

In the study which performed by Osmanoglu MA et al., higher doses methyl prednisolone (50 mg/kg) was used and statistically significant results to be obtained [12]. In the current study, according to ethical lows, it was not possible to use higher doses of methyl prednisolone. Therefore, we used the lower doses (20 mg/kg) of methyl prednisolone. According to our results; MDA, CAT and SOD levels were lower in the groups who given combination therapy (Pred+Vit C 1 and Pred+Vit C 2) than ischemic

groups without drugs (I/R 1 and I/R 2). Also, total injury scores were lower in the groups who given combination therapy (Pred+Vit C 1 and Pred+Vit C 2) than I/R 1 and I/R 2. Additionally, our results indicated that the use of the combination therapy was more effective in the long time ischemic groups than short time.

Because the long term ischemia causes the worse ischemic injury, early diagnosis and surgery are still important to prevent complications and loss of organs. May methyl prednisolone and vitamin C combination therapy which will be given before or/and after surgery such as bowel anastomosis in necrotizing enterocolitis, hypospadias and amputation surgery performed as a tourniquet application, ischemic cardiac and cerebral attacks, fetuses who have hypoxic-ischemic brain damage and especially delayed ovarian and testicular torsion reduce the damage and improve the prognosis.

We suggest that higher doses of the drugs in larger series of experiments may reveal a statistical difference. Also, preoperative use of vitamin C instead of methyl prednisolone may be more useful than combined treatment to prevent ischemic/reperfusion injury. The use of combined therapy (anti-inflammatory and antioxidant drugs) before surgery may increase the success rate of surgical treatment to prevent ischemic injury. Further studies are needed to have a firm conclusion about the use of combined therapy.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Murat Kaya, Department of Pediatric Surgery, Medical Faculty, Duzce University, Duzce 81820, Tuekey. Tel: +90 530 3244661; Fax: +90 380 5421387; E-mail: drkayam@gmail.com

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