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Dexpanthenol prevents ovarian ischemic damage via antioxidant mechanisms in rats

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Abstract

Ovarian torsion is a gynecological emergency characterized by ovarian ischemic damage. This study aimed to investigate the possible protective effects of dexpanthenol (DEX)-an antioxidant molecule-against ovarian ischemic damage in rats. The rats were simple randomly grouped into (1) Sham group (n=8); (2) Ischemia (I) group (n=8) (2 hours of I in ovaries); (3) DEX+I group (n=8) (500 mg/kg DEX administration 30 minutes before 2 hours of I.) The removed ovaries were examined histopathologically and biochemically. In the DEX+I group, necrosis-pycnosis severity was significantly reduced compared to the I group; but congestion-hemorrhage severity was similar. In the I group, malondialdehyde (MDA) and total oxidant status (TOS) levels were slightly increased, and glutathione (GSH) levels were significantly decreased compared to the Sham group. In the DEX+I group, MDA and TOS levels were slightly reduced and GSH levels were slightly increased compared to the I group. DEX prevents ischemic damage in rat ovaries via an antioxidant effect. Further research is needed to support our findings.

Keywords: Antioxidant, dexpanthenol, ischemia, oxidative stress, ovary

Introduction

Ovarian torsion is a gynecological emergency characterized by ovarian ischemia (I) [1]. Ischaemia causes congestion, hemorrhage, and necrosis in ovarian tissues, ultimately ovarian dysfunction [2,3]. These outcomes have accelerated drug research.

Dexpanthenol (DEX) is a precursor of pantothenic acid which increases the cellular glutathione (GSH) and coenzyme A levels [4]. Antioxidant, anti-inflammatory, and antiapoptotic effects of DEX were demonstrated in several models including methotrexate-induced liver oxidative toxicity [5], I/reperfusion (R)-provoked oxidative induced hepatic injury [6], streptozotocin-induced diabetic rats [7], cisplatin-induced ototoxicity [8], sciatic nerve injury [9], acetic acid-induced inflammatory bowel disease [10], carbon tetrachloride-induced myocardial toxicity [11], sepsis [12], lipopolysaccharide-induced acute lung injury [13], necrotizing enterocolitis [14], radiation-induced lung injury [15] and renal injury caused by I/R [16].

Based on the literature review, we found no study assessing the impact of DEX on the pure ischemic injury of the ovary. Soylu Karapinar et al. [3] evaluated the effects of DEX against ovarian I/R injury; however, they did not evaluate the effect of DEX on the ischemic ovary. This study aimed to investigate the effects of DEX in the rat model of bilateral ovarian I.

Material and Methods

Experiments

This study was approved by the Animal Ethics Committee of İnönü University (protocol number: 2016/A-55). The present study was conducted under the Animal Research: Reporting of In

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Vivo Experiments guidelines [17]. *Wistar albino* female rats (4-6 months, 200-250 g) were obtained from The Laboratory Animal Research Center of Inonu University. The rats were housed in cages under controlled room temperature (21±2°C) and relative humidity (60±5%) under 12-hour light/dark cycles. *Ad libitum* feeding with rat chow and water was provided. Simple random grouping was made as follows:

1. Sham group (n=8): The group that underwent sham surgery.
2. I group (n=8): The group that underwent 2 hours of bilateral ovarian I.
3. DEX+I group (n=8): The group in which 500 mg/kg DEX was administered intraperitoneally (i.p.) 30 min before 2 hours of bilateral ovarian I.

The dosage of DEX (Bepanthene ampule®, 500 mg, Bayer Corp., Istanbul, Türkiye) was determined according to an earlier work [18]. Bilateral ovarian ligation was conducted as previously described with some modifications [19,20]. Animals were anesthetized with ketamine (75 mg/kg, i.p.)+xylazine (8 mg/kg, i.p.). Hair removal and sterilization were performed at the laparotomy site. A longitudinal cut (2 cm) was made in the midline of the lower abdomen to reach the uterine horns. The ovarian pedicles were ligated about 1 cm above and below the ovary. The rats were i.p. injected with saline (1 ml) during surgery. The area of surgery is covered with a sterile pad. After a 2-hour ischemia period, all groups underwent bilateral oophorectomy (Figure 1). The skin was closed and sterilized. The Sham group underwent the same steps, apart from the ligation of ovarian pedicles. The right and left ovaries were reserved for histopathological and biochemical analyses, respectively. The right ovaries were fixed in 10% formaldehyde. The left ovaries were stored at -80°C until analysis. Animals were euthanized by over dose anesthesia.

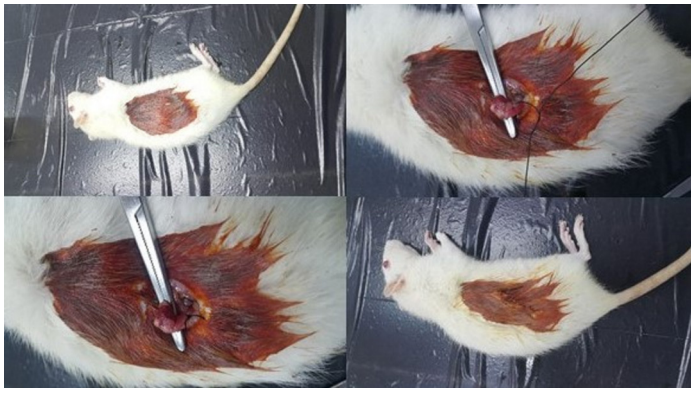

Figure 1. Laparotomy, ovarian ischemia, and oophorectomy methods

Biochemical analyses

Homogenization was performed by using a homogenizer inside the 20 mmol cold phosphate buffer with a pH of 7.4 and involving a protease inhibitor cocktail at 16,000 rpm for 3 min at +4°C. Centrifugation of homogenates implemented at 10,000×g for 20 min at +4°C. For measuring MDA, GSH, TAS, and TOS levels, the obtained supernatants were utilized.

To measure the malondialdehyde (MDA) levels, a microplate reader was used. MDA level was detected via thiobarbituric acid at 535 and 520 nm in a spectrophotometer as reported earlier [21]. The values were represented as nmol/g wet tissue.

The glutathione (GSH) levels of homogenates were determined as previously reported [22]. The values were presented as nmol/g wet tissue.

Total antioxidant status (TAS) and total oxidant status (TOS) levels were detected via spectrophotometer by using kits (Rel Assay Diagnostics, Gaziantep, Türkiye). The values were given as mmol Trolox Eqv/L for TAS and μmol H_2O_2 Eqv/L for TOS. The oxidative stress index (OSI, arbitrary unit) was calculated via the TOS/TAS ratio [23].

Histopathology

Ovarian sections were prepared and stained with hematoxylin-eosin (H-E) staining following previously described procedures [24] and then evaluated with light microscopy. The sections were assessed with a light microscope. Ovaries were evaluated in terms of congestion-hemorrhage and degenerative changes in the follicles and corpus luteum. Histopathological alterations were scored as 0 (no change), 1 (mild damage), 2 (moderate damage), and 3 (severe damage).

Immunohistochemistry

The sections were prepared and stained with caspase-3 (Thermo Scientific) immunostaining following previously described procedures [24] and then evaluated with light microscopy.

Statistical analysis

The necessary sample size was determined by an available software [25]. The minimum sample size was revealed as at least 7 in each group (21 in all), taking into account type I error (alfa)=0.05, power (1-beta)=0.8, and effect size=0.81 for vascular congestion. In this study, 8 animals were used in each group, considering the risk of mortality. Also, the power analysis of this study was carried out by an available software [25]. The calculated power (1-beta) of this study was 0.92, considering type I error (alfa) of 0.05, sample size of 8 in each group, and effect size of 0.81.

For analysis, a "Kruskal-Wallis" software [26] was used. Multiple comparisons were performed with the Conover test. Data were outlined as median (interquartile range [IQR]). Statistical significance was defined as $p<0.05$.

Results

Histopathology

The Sham group had normal histology (Figure 2A). Contrarily, intense congestion and hemorrhage were noted in the cortex and medulla in the I group (Figure 2B). Degenerative changes (pycnotic nuclei and necrotic cell groups) were observed in the follicles and corpus luteum (Figure 3A, 2B, and 2C). In addition, infiltrative cells were found around the ovary and in the cortex in some sections.

While degenerative changes were remarkably reduced in the DEX+I group contrary to the I group (Table 1) (Figure 3D and 3E), these two groups were similar regarding congestion and

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hemorrhage (Table 1) (Figure 2C).
Weak immunoreactivity of caspase-3 was observed in follicular and luteal cells in the Sham group (Figure 4A). Immunoreactivity was similar in all groups (Figure 4A, 4B, and 4C).

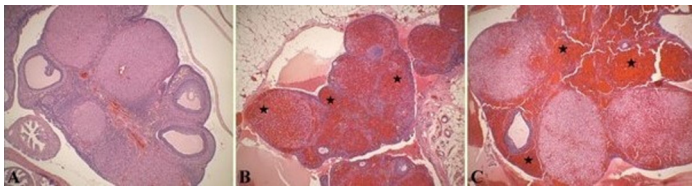

Figure 2. Ovary in Sham **2A.**, I **2B.**, and DEX+I **2C.** groups. Stars indicate congestion-hemorrhage. H-E x4

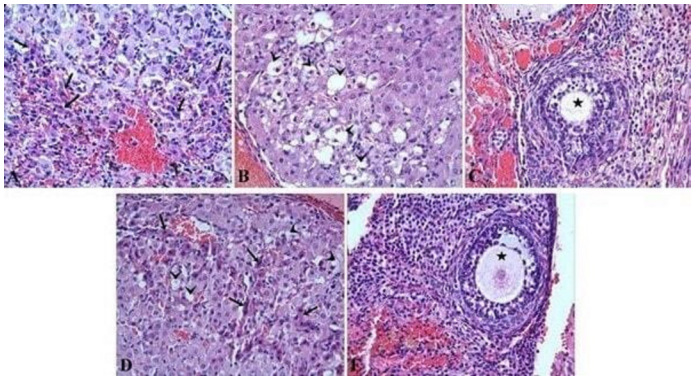

Figure 3. In the I group; **3A.** follicle with pycnotic nuclei (**3A.**, arrows) and necrotic (**3B.**, arrowheads) cells with increased eosinophilia and degenerative changes in oocyte and follicular cells (**3C.**, star). In the DEX+I group; degenerative changes decreased in luteal cells (**3D.**) and follicle (**3E.**) compared to the I group. H-E x40

Table 1. Comparison of the groups regarding the histopathological score

Groups	Congestion-hemorrhage	Necrosis-pycnosis
Sham	0.0 (1.0)	0.0 (0.0)
I	2.5 (2.0)[a]	2.0 (2.0)[a]
DEX+I	2.0 (2.0)	1.0 (2.0)[b]

DEX: dexpanthenol, the data were represented as median (IQR), [a]marked elevation (p=0.001) vs Sham group, [b]marked reduction (p=0.033) vs I group

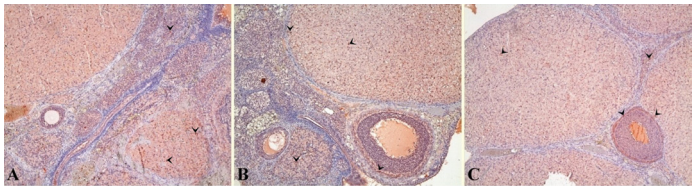

Figure 4. Caspase-3 immunoreactivity in Sham **4A.**, I **4B.**, and DEX+I **4C.**, groups. Arrowheads indicate caspase-3 positive cells. Caspase-3 x10

Biochemistry

The results are represented in detail in Table 2. The GSH level was remarkably lower in the I group versus the Sham group. Discordantly, GSH was slightly elevated in the DEX+I group versus the I group. MDA levels were increased in the I group contrary to the Sham group. This elevation was slightly reversed in the DEX+I group. In terms of TOS level, a slight increase was determined in the I group versus the Sham group. This increment was slightly reversed in the DEX+I group.

Table 2. The effect of DEX treatment on biochemical indicators analyzed in ovarian tissue

Parameters	Groups			p-value
	Sham	I	DEX+I	
TOS (µmol H2O2 Eqv/L)	14.34 (8.4)	23.92 (20.04)	12.97 (16.34)	0.48
TAS (mmol Trolox Eqv/L)	1.33 (0.28)	1.18 (0.17)	0.74 (0.73)	0.12
OSI (mmol Trolox/mmol H_2O_2)	11.25 (4.52)	20.95 (17.22)	21.03 (10.87)	0.12
MDA (nmol/g wet tissue)	38.02 (20.06)	39.78 (20.47)	32.64 (23.95)	0.78
GSH (nmol/g wet tissue)	705.64 (63.09)	638.48[a] (92.08)	659.34[a] (55.45)	0.04

TOS: total oxidant status, TAS: total antioxidant status, OSI: Oxidative Stress Index, MDA: malondialdehyde, GSH: reduced glutathione, [a]significant decrease ($p<0.05$) vs Sham group, the values were represented as median (IQR)

Discussion

The main findings of this study were that DEX prevents I-induced ovarian degeneration, slightly protects ovaries against I-induced oxidative stress, and provides a mild contribution to antioxidant response with a slight increase in GSH.

In this study, ovarian I caused congestion-hemorrhage, infiltration, and degeneration. Also, we observed a slight increase in oxidant markers including ovarian MDA and TOS, and a marked reduction in GSH, an antioxidant marker, due to I. This high production of MDA may be explained by the peroxidation of the cell membrane lipids resulting from reactive oxygen species. GSH is a crucial constituent of the antioxidant safeguard and is utilized by antioxidant enzymes including glutathione peroxidase (GPx) [27]. Therefore, GSH decline can be a consequence of its consumption while alleviating oxidative stress. These outcomes are in parallel with the earlier works. Afolabi et al. [2] observed moderate damage including follicular degeneration in the ischemic ovary of rats. Also, they reported congestion-hemorrhage, edema, and infiltration due to ovarian I. They showed a marked elevation in ovarian MDA and a marked reduction in antioxidant markers including ovarian GSH, superoxide dismutase (SOD), catalase (CAT), GPx, and glutathione-S-transferase (GST). Also, Turkler et al. [28] demonstrated an elevation of ovarian MDA and

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cyclooxygenase-2 levels in rats with ovarian I/R damage. These studies indicate that oxidative stress is a crucial constituent of ovarian ischemic damage.

DEX, a precursor of pantothenic acid, enhances antioxidant response by elevating cellular GSH [29]. Previous studies showed the antioxidant and anti-inflammatory effects of DEX. Ucar et al. [30] showed that DEX administration 30 min before the liver I/R increased the liver GSH and decreased the liver myeloperoxidase (MPO). Nonetheless, DEX caused no change in plasma levels of SOD, CAT, and MDA. Therewithal, DEX alleviated the liver damage caused by I/R in rats. Toplu et al. [8] stated that DEX reduced plasma MDA, TOS, and OSI levels and elevated plasma SOD, CAT, GPx, and TAS levels in cisplatin ototoxicity. Kalkan et al. [31] observed that DEX reduced heart TOS and OSI levels and alleviated cardiac damage. Cagin et al. [32] showed a decline in intestinal MDA, TOS, and OSI levels and an elevation in GSH and GPx levels due to DEX administration in intestinal damage induced by I/R. Recently, Uremis et al. [33] conducted an experimental study investigating the effects of DEX on a rat model of nicotine-induced kidney injury. In their study, DEX pretreatment (500 mg/kg/day, i.p. for 8 weeks) caused a significant increase in antioxidant parameters (CAT, GSH-Px, SOD, GST, GSH, TAS) and a significant decrease in oxidant parameters (TOS and OSI). Also, DEX prevented renal injury and improved renal functions. Considering the protein expression levels of protein kinase B (AKT), Nuclear factor erythroid 2-related factor 2 (Nrf2), and heme oxygenase-1 (HO-1) determined in the study, it can be said that, DEX provided protection against kidney injury and improved renal function by inhibiting oxidative stress and apoptosis through activation of the AKT/Nrf2/HO-1 pathway. Another experimental study showing the antioxidant effect of DEX was conducted by Bektasoglu et al. [34]. The study reported that a single i.p. dose of 500 mg/kg DEX administered immediately after traumatic brain injury (TBI), decreased the brain levels of MPO, MDA, luminol, and caspase 3 and increased the activities of the SOD and CAT in the brain. Also, a decrease in neuronal degeneration was observed in the DEX-treated group. These findings suggest that DEX exerts neuroprotective effects in a rat model of TBI by reducing oxidative damage and inhibiting apoptosis via its antioxidant effect. Soylu Karapinar et al. [3], assessed the impacts of DEX on ovarian I/R. However, they did not examine the effect of DEX on a pure ischemic ovary. They showed congestion-hemorrhage and edema induced by I/R. They observed that DEX alleviates edema and infiltration. They also reported that while DEX reduced the ovarian TOS, OSI, and MDA levels, it elevated the GPx activity. Similarly, in our study, DEX remarkably prevented degenerative changes. Also, it led to GSH elevation and MDA and TOS reduction in ovaries. These findings demonstrate that pre-ischemic application of 500 mg/kg DEX mitigated ovarian ischemic damage via the promotion of the antioxidant response and by reducing lipid peroxidation. Also, the reversal of follicular degeneration by DEX may help to maintain ovarian reserve and fertility.

Conclusion

Consequently, DEX prevented ovarian damage in the rat model of ovarian ischemia through its antioxidant properties. Accordingly, DEX may be a useful agent in the protection of the ovaries from ischemic damage. There is a need for well-designed experimental, translational, and clinical research for confirmation of the established outcomes.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical Approval

This study was approved by the Animal Ethics Committee of İnönü University (protocol number:2016/A-55).

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