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Protective effect of infliximab on ischemia/reperfusion injury in a rat ovary model: biochemical and histopathologic evaluation

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ABSTRACT

Objective: The aim of this study was to investigate the effect of infliximab on experimentally induced ovarian ischemia/reperfusion injury (IRi).
Study design: A total of 42 female rats were equally divided into 6 experimental groups; group 1: sham operation, group 2: 3-h ischemia, group 3 and 4: 3-h ischemia, 3-h reperfusion, group 5 and 6: 3-h ischemia, 24 h reperfusion. In group 4 and group 6, 30 min before reperfusion, infliximab was administered intraperitoneally at a dose of 5 mg/kg. Bilateral ovaries were removed for histopathologic and biochemical analysis. Serum MDA (sMDA), tissue MDA (tMDA), serum NO (sNO), tissue NO (tNO) and serum catalase concentrations were analyzed. Tissue damage of ovarian tissue was scored by histological examination.
Results: The infliximab administration significantly lowered the sNO, tNO and sMDA concentrations in group 4 compared to group 3 ($p = 0.041$, $p = 0.025$ and $p = 0.035$, respectively). sNO, tNO and sMDA concentrations were also lower in group 6 when compared to group 5, but this differences were not significant ($p > 0.05$). On the other hand, tMDA concentrations were lower in infliximab-applied groups when compared to ischemia/reperfusion groups (group 3 vs. 4 and 5 vs. 6) ($p = 0.045$ and $p = 0.048$, respectively). Moreover, histopathologic tissue damage scores in infliximab administration groups were significantly lower than in ischemia/reperfusion groups ($p < 0.001$).
Conclusion: Infliximab attenuates I/R-induced ovarian tissue injury in rats subjected to ischemia/reperfusion.

1. Introduction

Adnexal torsion is a gynecological emergency with a prevalence of 2.7% [1]. Early diagnosis and management are crucial for the preservation of ovarian function. In general, the management modality of patients, especially at younger ages, to preserve fertility is detorsion rather than removal of adnexa. Detorsion, however, leads to neutrophil infiltration and excessive production of reactive oxygen species [2]. The oxidative distress occurring with the reperfusion of the ischemic tissue is called "ischemia/reperfusion injury" (IRi) [2]. Inflammatory mechanisms are also important in the pathogenesis of IRi. Various inflammatory mediators were described contributing to IRi, such as leukocytes, adhesion molecules and cytokines [3]. Tumor necrosing factor alpha (TNFa) is the primary mediator of inflammation during IRi [4]. Thus, we hypothesized that the application of TNFa antagonists would result in decreased IRi. Infliximab (Remicade, Schering-Plough, Berlin) is a chimeric monoclonal antibody for TNFa, and binds both transmembrane and soluble forms of TNFa. It is approved for treatment of inflammatory diseases. To the best of our knowledge, this is the first study investigating possible effects of a TNFa antagonist on IRi of ovarian tissues due to ovarian torsion. We designed a rat model including various torsion and reperfusion models to test our hypothesis.

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2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats, 8–10 weeks old and weighing 220–265 g, were obtained from the Institute of Experimental Medicine, Istanbul University. The animals were maintained on a 12 h/12 h light/dark cycle with ad libitum access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Istanbul University.

A total of 42 female rats were divided equally into 6 experimental groups; group 1 (S): sham operation, group 2 (pure ischemia): 3 h ischemia, group 3 (ischemia–reperfusion 1): 3 h ischemia, 3 h reperfusion, group 4 (ischemia–reperfusion + infliximab-1): 3 h ischemia, 3 h reperfusion with infliximab administration before reperfusion, group 5 (ischemia–reperfusion 2): 3 h ischemia, 24 h reperfusion, group 6 (ischemia–reperfusion + infliximab-2): 3 h ischemia, 24 h reperfusion with infliximab administration before reperfusion.

2.2. Chemicals

Ketamine hydrochloride (Ketalar 50 mg/mL) was purchased from Pfizer, Istanbul, Turkey. Infliximab (Remicade®) was purchased from Schering Plough Co., Innihannon, County Cork, Ireland.

2.3. Surgical technique

Rats were anesthetized with ketamine hydrochloride (50 mg/kg, i.p.) under sterile conditions, and anesthesia was maintained by additional injections. Rats were placed in the dorsal recumbent position and covered with sterile drapes during surgery. A 2 cm longitudinal incision was made in the midline area of the lower abdomen. Then a small peritoneal incision was performed, and the uterine horns and adnexa were located. Vessel clips were used for bilateral ovarian torsion. In group 1, a sham operation (laparotomy only) was performed ($n = 7$). In the other 5 groups, bilateral ovarian ischemia was induced by application of vascular clips below the ovaries for 3 h. The incision was closed with 4/0 nylon sutures. In group 2, the bilateral ovaries were surgically removed, after a 3-h period of ischemia ($n = 7$).

At the end of a 3-h period of ischemia the bilateral vascular clips were removed. Afterwards, in group 3 ($n = 7$) and group 4 ($n = 7$) the 3-h period of ischemia was followed by 3 h of reperfusion. In group 5 ($n = 7$) and group 6 ($n = 7$) the 3-h ischemia was followed by 24 h of reperfusion. In group 4 and group 6, 30 min before reperfusion, infliximab was administered intraperitoneally at a dose of 5 mg/kg [5]. At the end of the these procedures, bilateral ovaries were removed and one of the ovaries was transferred to 10% formaldehyde solution for histopathological examination, while the other ovary was cleaned of adherent soft tissues and then stored in a freezer at −80 °C for biochemical analysis.

2.4. Histological analysis

For histopathologic evaluation, tissues were recovered, washed with 0.09% saline solution and fixed in 10% formalin solution. For conventional light microscopy follow up, tissues were embedded in paraffin blocks. The paraffin blocks were cut at a thickness of 4 μm and were stained with hematoxylin and eosin. The sections were examined for the presence or severity of tissue damage with a microscope (Olympus Clinical Microscope BX45). Whole ovarian section was examined for each slide. The scoring system used for histopathologic evaluation of ovarian tissues was as previously described by Sagsoz et al. [6]. Interstitial edema, congestion (vascular dilatation), hemorrhage and loss of cohesion (separation of parenchymal cells along with normal ovarian cortex and follicles) were scored from 0 to 3 for each parameter and the sum of the four parameters was the final tissue damage score of the inspected ovarian tissue. Scores represented the severity of the pathological finding; 0 represented no pathological finding, 1 represented pathological finding less then 33%, 2 represented pathological finding between 33–66%, and 3 represented pathological finding more than 66% of the ovarian section. The total tissue damage score was ranged from 0 to 12 for each ovarian section. One pathologist, blinded to study groups, performed the examination and scoring of all of the ovarian sections.

2.5. Biochemical analysis

For biochemical analysis, the ovarian tissues were removed and washed with physiological saline. They were then homogenized for 3 min in cold phosphate buffer. These homogenates were centrifuged at 2000 × g for 10 min to obtain supernatants. The levels of MDA and NO were determined in the supernatants. Protein content of homogenates was determined by the Lowry method [7].

2.5.1. Malondialdehyde (MDA) assay

Tissue and serum MDA levels were determined by the double heating method of Draper and Hadley [8]. The principle of the method was spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5\ cm^{-1}\ M^{-1}$ and expressed in μM.

2.5.2. Determination of serum catalase activity (CAT)

The spectrophotometric method of Goth was used for the determination of CAT activity in serum [9]. The yellow complex of molybdate and H_2O_2 was measured at 405 nm against a blank. One unit of CAT decomposes 1 μmol of H_2O_2/L min: results are expressed as kU/L.

2.5.3. Nitric oxide determination

Serum and tissue nitric oxide levels were measured as its stable metabolites; nitrate (NO_3) and nitrite (NO_2). Nitrate was first

Table 1
Biochemical test results of serum and tissue nitric oxide, serum and tissue malondialdehyde and serum catalase analysis.

	sNO (μM)	sMDA (μM)	sCAT (kU/L)	tNO (μM/g tissue)	tMDA (μM/g tissue)	Tissue damage score
Group 1	35.79 ± 4.76	3.67 ± 0.59	56.31 ± 5.82	60.75 ± 6.25	3.48 ± 0.43	1.00 ± 0.00
Group 2	57.33 ± 6.78	5.95 ± 0.58	48.76 ± 5.01	72.60 ± 4.46	6.47 ± 0.40	7.57 ± 0.20
Group 3	65.18 ± 3.84	6.77 ± 0.51	35.85 ± 2.86	88.52 ± 5.66	5.36 ± 0.43	8.29 ± 0.42
Group 4	45.50 ± 7.25	4.82 ± 0.63	53.41 ± 7.46	67.98 ± 5.76	4.33 ± 0.61	7.00 ± 0.31
Group 5	47.37 ± 3.69	4.99 ± 0.38	46.59 ± 3.26	70.72 ± 5.46	5.55 ± 0.49	7.86 ± 0.46
Group 6	36.22 ± 3.29	4.25 ± 0.54	53.64 ± 5.77	60.83 ± 5.22	4.04 ± 0.58	6.00 ± 0.37
p-Value	0.005	0.012	0.107	0.033	0.003	<0.001

Data are presented as mean ± SEM from 7 rats in each group. sNO, serum nitric oxide; tNO, ovarian tissue nitric oxide; sMDA, serum malondialdehyde; tMDA, ovarian tissue malondialdehyde; sCAT, serum catalase.