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Adalimumab mitigates ovarian ischemia–reperfusion injury in rats by regulating oxidative stress, apoptosis and resolution of inflammation

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Abstract

Aim: Ovarian torsion is a rare but an important reason of acute lower abdominal pain in women and associated with serious morbidity and mortality, if not treated promptly. The aim of this study was to evaluate the effects of an antitumor necrosis factor-α antibody on ovarian torsion in a rat model of ischemia–reperfusion (I/R) injury.

Methods: Forty female Wistar Albino rats were used in the present study. The rats were randomly divided into four groups: group I (sham), group II (I/R), group III (I/R + isotonic saline) and group IV (I/R + adalimumab). The I/R model was induced by torsion of both ovaries. Immunohistochemical staining for interleukin-1β (IL-1β), nuclear factor-κB (NF-κB), and inducible nitric oxide synthase was performed. Tissue and serum oxidative stress markers in conjunction with apoptotic index (AI) with the terminal deoxynucleotidyl transferase dUTP nick end labeling method were also calculated.

Results: Tissue total oxidant status, oxidative stress index and nitric oxide values were significantly decreased, and tissue total antioxidant status was found to be increased in group IV. Inflammation, vascular congestion and hemorrhagia were significantly lower in adalimumab-treated group. Serum oxidative stress markers and tissue malondialdehyde levels did not differ in study groups. The AI was significantly increased in groups 2 and 3. Adalimumab treatment significantly decreased the AI.

Conclusion: Adalimumab therapy in rats attenuated I/R induced ovarian injury, possibly suppressing inflammation, inhibiting oxidative stress, and altering apoptotic pathways.

Key words: adalimumab, apoptosis, immunohistochemistry, ovarian torsion, oxidative stress.

Introduction

The twisting or torsion of the ovary is a relatively common gynecological emergency especially in 1st three decades of life, which denotes the bending of the ovary and fallopian tube around the broad ligament.[1] Clinical presentation is sometimes confounded by non-specific symptoms such as abdominal pain, nausea and vomiting that leads to late or missed diagnosis.[2] In order to prevent potential necrosis, infertility and life-threatening sequels of this entity, early diagnosis and prompt institution of adequate treatment may be life-saving. For this reason, in patients with suspected ovarian torsion, gynecologic consultation with subsequent surgical procedures are critical, irrespective of whether laboratory examinations and radiologic imaging yield normal results. Surgical intervention, either laparoscopic or laparotomy commonly re-establish blood circulation nevertheless, tissue atrophy or necrosis, may still occur and

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is a common consequence.[3,4] Thus, it is important to develop alternative nonsurgical and specific therapeutic strategies against ovarian torsion in order to achieve short- and long-term preservation of ovarian function at a cellular level including protection from tissue ischemia caused by reperfusion damage from free radicals that are released after detorsion.

Tumor necrosis factor-α (TNF-α) is one of the main mediators of inflammation and has been recognized as a target of therapeutic intervention in many inflammatory disease states.[5] Clinical data advocate that TNF-α is released at the beginning of reperfusion, and its level increases during the early stages of ischemia and reperfusion (I/R).[6] Being a cell signaling circulating protein, TNF-α is mainly involved in many harmful biological processes and diseases in the body during the course of I/R.[7,8] Adalimumab (ADA) is a recombinant human IgG1 monoclonal antibody specific for human TNF-α and is traditionally used to treat psoriasis, psoriatic arthritis, and several other inflammatory conditions such as rheumatoid arthritis and inflammatory bowel diseases. The inhibition of TNF-α with ADA has been shown to decrease the severity of liver and intestinal I/R injuries in several animal models.[9,10] However, there have been no studies of the protective effects of ADA against ovarian I/R injury.

In this study, we aimed to evaluate the therapeutic effect of ADA on ovarian injury that is induced by I/R in an experimental rat model. Furthermore, we aimed to shed light on the possible mechanisms by which ADA could protect rat against I/R induced ovarian injury.

Materials and Methods

Materials and experimental design

This experimental protocol was approved by the Institutional Animal Use and Care Committee of Çanakkale Onsekiz Mart University (COMU) (Approval No: 2017/06–15) and performed in accordance with the Helsinki Declaration of World Medical Association recommendations on animal studies. A total of 40 Wistar albino adult female rats were obtained from COMU Experimental Research Application and Research Center with a mean age of 4 months and a mean weight of 250–300 g. The rats were housed in stainless steel cages in an animal room maintained at a standard humidity (50–55%) and temperature 22 ± 2°C with 12-h light/dark cycles. All animals were fed standard food and water. Twelve hours before the study procedure feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

Experimental protocol

Anesthesia

Rats were anesthetized with intraperitoneal (i.p.) ketamine hydrochloride (75 mg/kg) and xylazine (5–10 mg/kg), and if required anesthesia was maintained with additional injections of ketamine hydrochloride.

Ovarian I/R

The ovarian I/R model was designed in a way parallel to previous trials.[11] In brief, the rats were supine positioned on a heated platform. The skin was prepared under aseptic conditions, and an approximately 2.5-cm longitudinal incision was performed in the midline area of the lower abdomen after shaving of abdominal area. Afterward peritoneal incision was done in order to locate the uterine horns and adnexa. After finding the adnexes, both ovaries were twisted and rotated 720° clockwise and fixed to the abdominal wall with a non-absorbable suture. Then abdomen wall was closed with continuous 1/0 silk suture in one layer. After 3 h, the fixed ovaries were freed by cutting the suture and both ovaries were detorsioned with a relaparotomy procedure and the abdomen was surgically closed again.

Groups

Wistar albino rats were randomly divided into four groups as follows:

Group I (sham, $n = 10$): After sham operation at zero and 3-h point, both ovaries were surgically removed for assessment at 6-h point.

Group II (torsion-detorsion group, $n = 10$): After torsion (at zero-point) and detorsion (at 3-h point), both ovaries surgically removed at the end of the study (6-h point).

Group III (torsion-detorsion + isotonic saline group, $n = 10$): Rats underwent torsion and detorsion operation. Twenty minutes prior to the detorsion, 0.3-mL i.p. isotonic saline was applied. Both ovaries removed at the end of the study (6-h point).

Group IV (torsion-detorsion + ADA group, $n = 10$): Rats underwent torsion and detorsion operation. Twenty minutes prior to detorsion, 50-mg/kg ADA (Humira; Abbott Laboratories) applied

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intraperitoneally. Both ovaries removed at the end of the study (6-h point).

Evaluation

Serum and tissue samples were used for evaluation purposes. Blood samples were collected at the beginning and at the end of the study (at 6-h point). In order to evaluate tissue samples, both of the ovaries were removed in all subjects. One of the ovaries was used for biochemical analysis, while the other was used for histopathological evaluation.

Histopathological examinations

In order to investigate histopathologic changes, ovarian tissue samples were consecutively numbered and fixated in 10% neutral buffered formalin for 48 h, dehydrated, cleared in xylene and embedded in paraffin and sent to the histology department of COMU. Evaluation of the pathology specimens was done by a histology specialist, who was blind to the four study groups. The paraffin blocks were cut in 5-μm thickness on Rotary Microtome (Leica RM2125 RTS), and the sections were stained with hematoxylin and eosin (H&E). The histopathologic sections were evaluated under a light microscope (Zeiss AxioScope A1) for the presence of hemorrhagia, vascular congestion, edema and inflammation (neutrophil infiltration) and rated on a modified semiquantitative scale of 0–3 as also stated by Bozkurt *et al.*[12] Scoring scale was as follow; 0, no findings; 1, findings of <33%; 2, findings of 33–66%; 3, findings of >66%.

Immunohistochemical staining

Immunohistochemistry for IL-1β (Cat #12242S, Cell Signaling Technology), NF-κB (Cat #RB-9034, Thermo Scientific, Lab vision) and inducible nitric oxide synthase (iNOS) (Cat #RB-9242, Thermo Scientific) was carried out with commercial kits according to the manufacturers' instructions.

Immunohistochemical (IHC) evaluation and scoring were done according to Jiang *et al.*[13] According to this scoring, IL-1β, NF-κB, and iNOS immunostaining scores were calculated by staining intensity (0, no staining; 1, weak but detectable staining; 2, moderate; and 3, strong staining).

Apoptosis assessment with the TUNEL method

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was used to detect apoptosis of the ovarian tissue. Prepared tissue was fixed with 4% neutral formaldehyde, embedded in paraffin, and 4-μm-thick sections were cut from each paraffin block. After dewaxing, hydration, and serum blocking, ApopTag Peroxidase in situ Apoptosis Detection Kit (S7100, Millipore) was used according to the manufacturer's protocol. The cells were observed and photographed under an optical microscope, and five high image fields of each sample were randomly selected, and 500 cells were counted in each field. Cells stained brown or black were judged TUNEL-positive apoptotic cells and presented in the form of apoptotic index (AI).

Biochemical evaluation

Ovarian tissue and blood samples were obtained from each animal at the end of the study. Blood samples were centrifuged at 3000 rpm for 15 min. The resultant serum samples were aliquoted into polypropylene tubes and stored at −80°C until the biochemical analysis. The tissues were prepared at +4°C in order to measure malondialdehyde (MDA), nitric oxide (NO), total antioxidant status (TAS) and total oxidant status (TOS) levels. After washing with phosphate buffer solution (PBS), ovarian tissue samples were weighed and cut into small pieces and homogenized in radio immunoprecipitation assay (RIPA) (for measuring MDA) buffer and PBS (for measuring NO, TAS and TOS). Tissues were homogenized in all groups by using Mixer Mill MM 400 (Retsch, Haan, Germany). Assays were performed on the supernatant of the homogenate that was prepared by centrifugation at +4°C.

Blood and tissue biochemical markers were calculated by spectrophotometric measurements. The protein contents of the ovarian tissues were calculated according to the method described by Lowry *et al.*[14] Tissue MDA levels were determined by using spectrophotometric kit (Cat. No.:10009055, Cayman). The results are expressed as micromolar per gram protein. Tissue NO assay was determined via spectrophotometry at 540 nm by using a nitrate/nitrite colorimetric assay kit (Cat. No. 780001, Cayman). The results are expressed as micromolar per gram protein.

Tissue TAS and TOS levels were determined at the end of the study, whereas serum TOS (Product Code: RL0024) and TAS (Product Code: RL0017) levels and oxidative stress index (OSI) measurement were carried out both at the beginning (at zero-point) and at the end of the study (at 6-h point). As described previously by Jansen and Ruskovska,[15] serum TOS and TAS levels were calculated by using spectrophotometric kits (Rel Assay Diagnostics, Gaziantep, Turkey). The results for TAS and TOS are expressed as μmol

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Trolox equivalent/L and μmol H2O2 equivalent/L, respectively. The ratio percentage of TOS to TAS was used to calculate the OSI. Specifically, OSI (arbitrary unit) = ([TOS, μmol H2O2 equivalent/L]/[TAS, μmol Trolox equivalent/L]).

Statistical analysis

The SPSS 21.0 software (SPSS for Windows) program was used for the statistical analysis. Both the histopathological and tissue IHC staining results were presented as mean ± standard deviation (SD) and median (min–max). Nonparametric tests were performed because the distribution of the data was not normal. The comparison of all groups was done using the Kruskal–Wallis test, and the Mann–Whitney *U*-test was used as the post-hoc test. Bonferroni correction was carried out for the prevention of significance inflation (0.05/total comparison number; $P < 0.0083$ was accepted as significant). A P value <0.05 was accepted as statistically significant.

Results

All rats survived and were included in the final analysis.

Histopathologic findings

All rats were evaluated for tissue damage by assessing related parameters, such as hemorrhagia, congestion, edema and polymorphonuclear leukocyte (PMNL) infiltration. Table 1 shows total histopathologic scores of these parameters expressed as mean (SD) and median (min-max). There were no histopathological changes in the sham group (Group I). The tissues in the I/R and I/R + saline group showed histopathological changes of condensed hemorrhagia, increased edema, signs of vascular congestion and infiltration of inflammatory cells along with degenerative and apoptotic cells. Histopathologic changes were similar in both I/R group and I/R+ isotonic saline group, whereas after i.p. ADA application tissue injury scores were reduced, and comparable with those in the sham group: Figure 1 shows histopathological findings for each group using H&E.

Effect of ADA on apoptosis induced by I/R

In order to evaluate the apoptotic cells in the ovarian tissue, we analyzed the AI of ovarian cells by using the TUNEL technique. Quantitative assessment of AI in ovarian tissue using TUNEL technique also confirmed histopathological findings. Ovarian tissue in the sham-operated group showed less TUNEL-positive cells (21.8 ± 7.85), whereas I/R (53.8 ± 17.13) and I/R + saline (58.1 ± 17.41) groups had significantly elevated TUNEL-positive cells ($P = 0.05$ for both). Intraperitoneal ADA application significantly decreased the AI (33.0 ± 11.94) compared with I/R and I/R + saline groups ($P < 0.05$ for both) (Fig. 2).

Table 1 Comparison of ovarian damage scores between all groups

	Hemorrhagia	Congestion	Edema	Inflammation
Group I ($n = 10$)				
Mean (±SD)	0.1 ± 0.10	0.3 ± 0.48	0.10 ± 0.31	0.1 ± 0.10
Median (min-max)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Group II ($n = 10$)				
Mean (±SD)	2.8 ± 0.42	1.6 ± 1.07	2.70 ± 0.48	2.8 ± 0.42
Median (min-max)	3 (2–3)	1 (0–3)	3 (2–3)	3 (2–3)
Group III ($n = 10$)				
Mean (±SD)	2.8 ± 0.42	2.8 ± 0.42	2.7 ± 0.48	2.8 ± 0.42
Median (min-max)	3 (2–3)	2 (2–3)	3 (2–3)	3 (2–3)
Group IV ($n = 10$)				
Mean (±SD)	1.7 ± 0.94	1.6 ± 1.07	0.6 ± 1.07	1.3 ± 1.25
Median (min-max)	1 (1–3)	1 (1–3)	0 (0–3)	1 (0–3)
P	0.001*	0.019†	0.007‡	0.015§

*Comparison of Group 1/Group 2, Group 1/Group 3, Group 1/Group 4, Group 2/Group 4, Group 3/Group 4 ($P = 0.001$, $P = 0.001$, $P = 0.001$, $P = 0.004$ and $P = 0.007$§, respectively); †Comparison of Group 1/Group 2, Group 1/Group 3, Group 1/Group 4, Group 2/Group 4, Group 3/Group 4 ($P = 0.001$, $P = 0.001$, $P = 0.004$, $P = 0.002$ and $P = 0.002$, respectively); ‡Comparison of Group 1/Group 2, Group 1/Group 3, Group 2/Group 4, Group 3/Group 4 ($P = 0.001$, $P = 0.001$, $P = 0.001$ and $P = 0.001$, respectively); §Comparison of Group 1/Group 2, Group 1/Group 3, Group 1/Group 4, Group 2/Group 4, Group 3/Group 4 ($P = 0.001$, $P = 0.001$, $P = 0.002$, $P = 0.008$ and $P = 0.008$, respectively). SD, standard deviation.

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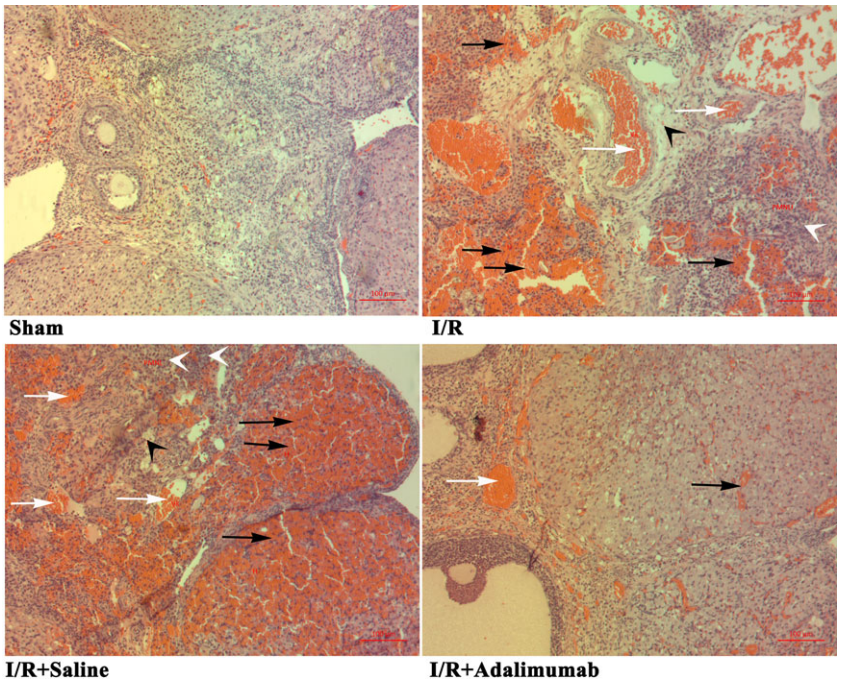


Figure 1 Microscopic comparison of inflammation, vascular congestion, and edema in all four groups. Ischemia–reperfusion (I/R) and I/R + saline-treated group showed significantly greater histological damage including increased cellular inflammation (black arrow), vascular congestion (white arrow), hemorrhage (white arrowhead) and edema (black arrowhead) compared to adalimumab (ADA)-treated group and sham.

IHC findings

I/R (group II) caused a significant increase in ovarian expression of IL-1β (2.40 ± 0.69), NF-κB (2.40 ± 0.69), and iNOS (2.80 ± 0.42) compared with the sham group. Significant reductions in these parameters were observed in the ADA-treated group in comparison with both group II (I/R) and group III (I/R + saline group) ($P < 0.05$) (Table 2).

Biochemical findings in ovarian tissue

Ovarian tissue oxidative stress markers were measured after the study. Tissue NO levels were similar in groups I (5.87 ± 2.16) and IV (5.46 ± 1.77), whereas tissue NO levels were found to be statistically increased in groups II (10.03 ± 4.12) and III (9.78 ± 3.68). Tissue MDA levels were found to be similar among all four groups ($P = 0.447$). Tissue TOS (1.14 ± 0.57) and OSI (6.67 ± 5.41) levels were found to be decreased in group IV compared with group II (2.20 ± 1.09 for TOS and 19.87 ± 7.0 for OSI) and group III (2.18 ± 0.76 for TOS and 22.37 ± 5.85 for OSI) ($P < 0.05$ for both). Total antioxidant status levels in the ADA group were found to be comparable with the sham group, whereas tissue TAS levels were found to be decreased in groups II and III (Fig. 3).

Oxidative stress markers in serum

Serum oxidative stress markers were measured both before and after the experiment. There were no significant relationships among groups for TOS, TAS and OSI. Although serum TOS levels were in a decreasing trend after ADA application (from 12.72 ± 4.11 to 10.65 ± 4.07), this was found to be statistically insignificant ($P = 0.112$) (Table 3).

Discussion

In this experimental model of ovarian I/R, we aimed to evaluate the antioxidant, anti-inflammatory and antiapoptotic effects of ADA with both histopathologic and biochemical examinations. And as a result, we demonstrated that treatment with ADA reduced I/R injury via anti-inflammatory and antioxidant pathways at cellular tissue level. These findings suggest that ADA with its immunomodulatory properties could be regarded as an excellent bridge to surgical intervention in selected cases not immediately suitable for definitive therapy.

The present study demonstrated that IL-1β expressions are significantly down-regulated in the ADA group compared with the I/R group and the I/R + saline group. Although, our study is the first that

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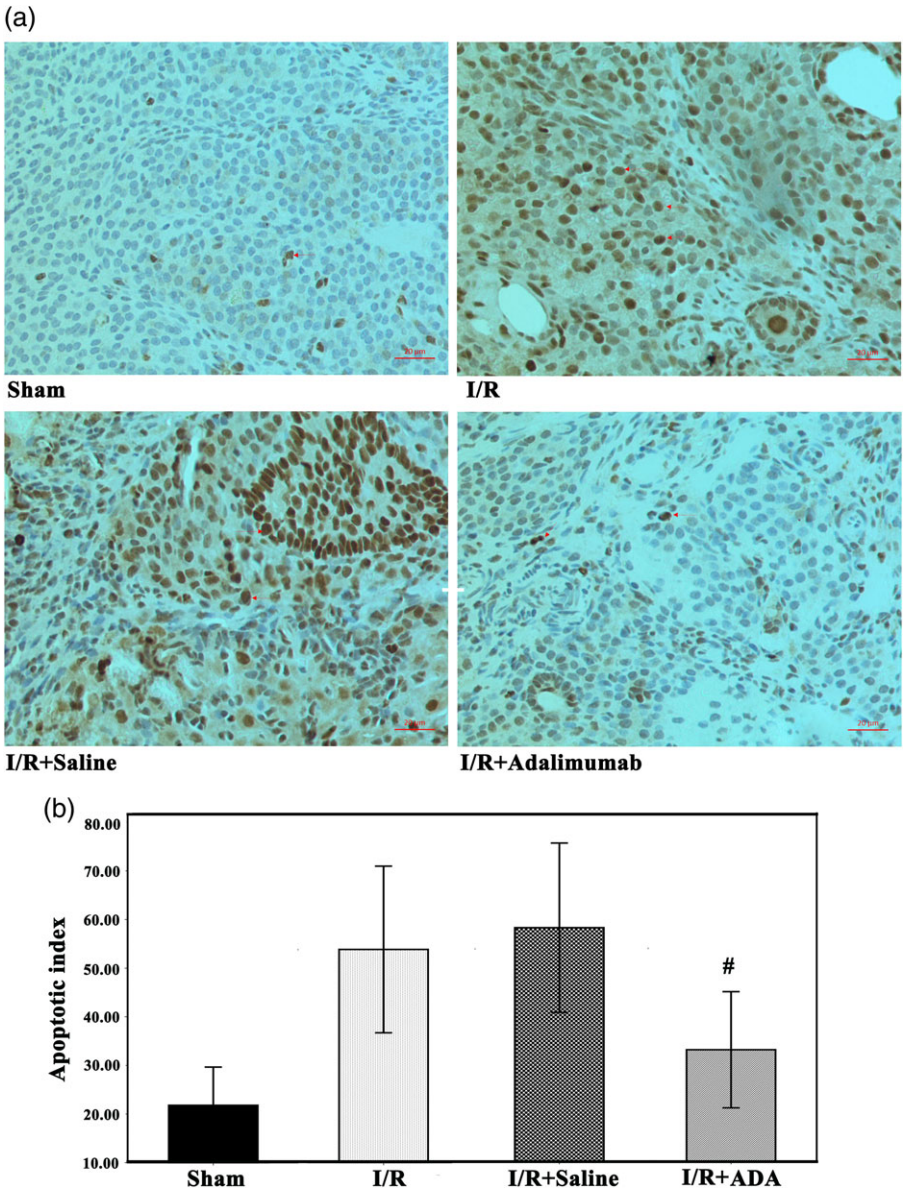


Figure 2 (a) Apoptosis assessment by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique within each group. Ischemia–reperfusion (I/R) and I/R + saline groups exhibited significantly elevated TUNEL -positive cells (brown stained cells) compared with the sham and adalimumab (ADA)-treated group (#$P < 0.05$ between ADA vs I/R and I/R + saline groups). (b) Apoptotic index in each group.

investigated the expression of IL-1β in ADA-treated ovarian I/R, in a study by Cure *et al*.[16] IL-1β, IL-6 and TNF-α expressions were analyzed after ADA treatment in a rat model of abdominal aorta cross-clamping. IL-1β and TNF-α levels were found to be decreased in the ADA-treated group compared with the I/R and control group. Because IL-1β is such a prominent proinflammatory cytokine in a multitude of systemic inflammatory states, prolonged IL-1β overproduction in the inflammatory response, may result in enhanced tissue damage due to the immune cells overactivation and the production of proteases.[17,18] Moreover, in some certain instances, IL-1β overproduction may be the cause, or be linked with more or less severe inflammatory conditions several of which have recently been classified as autoinflammatory diseases.[19] In this context, ADA could exert its beneficial effects in I/R injury by regulating IL-1β-induced vascular permeability, neutrophil recruitment and maturation in the early phases of inflammation. Therefore, histopathologic improvements including marked decreases in inflammatory cell infiltrate,

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Table 2 Comparison of ovarian immunohischemistochemical staining results between groups

	IL-1β	NF-κB	iNOS
Group I (n = 10)			
Mean (±SD)	0.70 ± 0.48	1.20 ± 0.78	2.20 ± 0.63
Median (min-max)	0 (0–1)	1 (0–2)	2 (1–3)
Group II (n = 10)			
Mean (±SD)	2.40 ± 0.69	2.40 ± 0.69	2.80 ± 0.42
Median (min-max)	2.5 (1–3)	2.5 (1–3)	3 (2–3)
Group III (n = 10)			
Mean (±SD)	2.20 ± 0.63	2.60 ± 0.69	2.70 ± 0.49
Median (min-max)	2 (1–3)	3 (1–3)	3 (1–3)
Group IV (n = 10)			
Mean (±SD)	1.40 ± 0.51	1.60 ± 0.69	2.40 ± 0.51
Median (min-max)	1 (1–2)	1.5 (1–3)	2 (2–3)
P	0.000*	0.001†	0.003‡

*Comparison of Group 1/Group 2, Group 1/Group 3, Group 2/Group 4 (P = 0.001, P = 0.001 and P = 0.005, respectively); †Comparison of Group 1/Group 2, Group 1/Group 3, Group 2/Group 4, Group 3/Group 4 (P = 0.004, P = 0.002, 0.004 and P = 0.008, respectively); ‡Comparison of Group 1/Group 2, Group 1/Group 3, Group 2/Group 4 (P = 0.002, P = 0.002 and P = 0.004, respectively). iNOS, inducible nitric oxide synthase; SD, standard deviation.

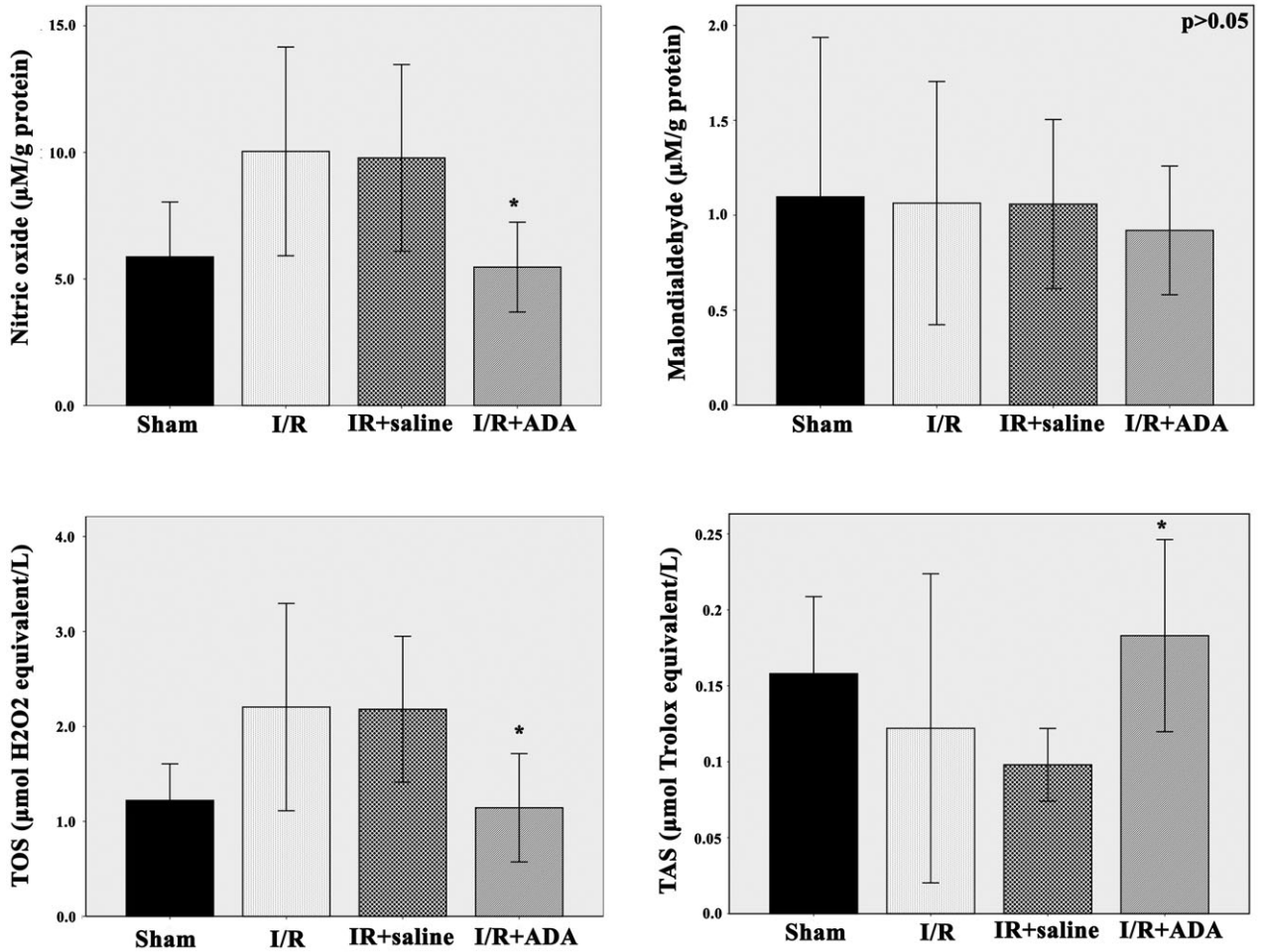


Figure 3 Expressions of oxidative stress markers and antioxidant enzymes in the ovarian tissue (*P < 0.05 between adalimumab [ADA] vs ischemia–reperfusion [I/R] and I/R + saline groups).

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Table 3 Serum TOS, TAS and OSI measurements among groups before and after the study

	Serum TOS			Serum TAS			Serum OSI		
	Before	After	*P*	Before	After	*P*	Before	After	*P*
Group I (n = 10)	11.8 ± 1.5	10.1 ± 1.7	0.981	1.1 ± 0.2	0.8 ± 0.2	0.051	11.2 ± 3.2	13.9 ± 3.7	0.051
Group II (n = 10)	12.4 ± 4.2	10.7 ± 2.3	0.299	1.1 ± 0.1	0.9 ± 0.3	0.107	11.35 ± 3.6	12.6 ± 3.9	0.455
Group III (n = 10)	12.9 ± 2.3	12.5 ± 2.5	0.216	1.1 ± 0.2	1.1 ± 0.3	0.848	12.1 ± 2.9	10.6 ± 3.4	0.327
Group IV (n = 10)	12.7 ± 4.1	10.6 ± 4.1	0.112	1.0 ± 0.3	0.8 ± 0.1	0.106	15.2 ± 5.2	12.7 ± 5.2	0.292
P	NS	NS	—	NS	N S	—	NS	NS	—

NS, not significant; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.

along with decreased vascular congestion and edema in the ADA-treated experimental group are an important finding of the present study.

Various physiopathological mechanisms have been put forward to explain the tissue damage that occurs during ovarian torsion and detorsion including ovarian lipid peroxidation, vascular leucocyte margination and apoptosis.[20] The detorsion involves the production of toxic reactive oxygen species (ROS) with the return of blood flow following ischemia.[21] Overproduction of ROS could lead to a significant elevation in lipid peroxidation, thereby generating MDA and destroying the antioxidant defense systems of the human body.[16,22] It has been demonstrated that I/R of a tissue is strictly linked with lipid peroxidation which causes oxidative demolition of the cellular membranes by autocatalytic processes leading to harmful accumulation of toxic metabolites and cellular death[23,24] In this context, MDA, being an end product of peroxidative decomposition of polyenoic fatty acids, is generally used as a reliable indicator of tissue damage.[25] In the present study, we found no significant alterations after i.p. ADA injection in tissue MDA levels indicating lack of lipid peroxidation in our study groups. The most logical explanation for the failure to document unaltered MDA levels is that the concentration of free radicals generated is not enough to activate lipid peroxidation or other antioxidant systems were able to compensate ongoing oxidative state.

Besides MDA, assessment of NO, TOS, TAS and OSI are also reliable indicators of oxidative stress caused by insufficient blood flow and possibly as predictive or prognostic markers in I/R conditions.[26] We assayed oxidative status as TOS, TAS and OSI in both tissue and serum samples. In addition, tissue NO measurement was done in order to provide a comprehensive suite of oxidative status indicators and markers of reperfusion injury. The present study is therefore considered to give a more complete overview of the effects of ADA in an I/R injury. We found increased TAS and decreased TOS, OSI and NO levels in ovarian tissue samples after i.p. ADA injection. Due to an increase in these oxidants and a decrease in total antioxidants, the oxidant/antioxidant balance shifted toward oxidative stress in the ovarian tissues of the I/R rats. Adalimumab treatment in these rats significantly decreased TOS and NO and increased TAS levels compared to untreated and isotonic saline applied groups. Although there is no study in the literature exploring the effects of ADA in an ovarian I/R injury, the salubrious effect of ADA in I/R injuries have also been demonstrated in several other studies. In an experimental model of liver injury by Cure *et al.*,[16] it has been demonstrated that during I/R injury ADA decreases cytokines, prevents the increase of NO by maintaining the balance among arginase, carbamoyl phosphate synthetase-1, and NOS, and consequently protects the cells from death. Similarly, in a study by Kurt *et al.*,[27] it has been reported that with decreasing the release of cytokines and preventing the generation of ROS via blocking TNF-α, ADA could diminish lung damage during I/R process.

In our study, oxidative stress was only present in ovarian tissues, and no difference was found between groups in terms of serum oxidant/antioxidant parameters before and after the study. As we reported no effects of I/R on serum oxidative stress markers irrespective from the treatment protocols, this may be attributed to the short treatment time and I/R periods. The importance of treatment intervals on tissue and plasma oxidative stress levels was emphasized in several studies. Auberval *et al.*[28] demonstrated that although short-term high-fat diet (HFD) exhibits oxidative stress on pancreatic and hepatic tissues, this effect was not valid for plasma oxidative stress markers. However, longer period of treatment with HFD led to the detection of oxidative stress in plasma due to increases in the levels of oxidized proteins and lipids.

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A number of studies have confirmed the pivotal role of NF-κB in I/R injury cases.[29,30] NF-κB, which is a transcription factor that regulates the expression of multiple inflammatory and immune genes, plays a crucial role in host defense, chronic inflammatory diseases and even tumoural conditions.[31] It is activated through phosphorylation and subsequent degradation of IκB. Several agents including ROS, IL-1β and TNF-α are capable to phosphorylate IκB.[32] After activation of IκB, NF-κB triggers key mediators of inflammation responsible from I/R injury, including intercellular adhesion molecule 1, iNOS, cyclooxygenase-2, interleukin-1ß, interleukin-6, and vascular cellular adhesion molecule 1 that leads to the development of apoptosis.[33,34] In the present study, we found that ADA partially rescued the effects of I/R injury via multiple mechanisms, including the down-expression of NF-κB and inhibiting ovarian cell apoptosis. Adalimumab has significantly improved the postischemic recovery of rat ovaries, which was paralleled by significant alterations of ovarian cell apoptosis. Terminal deoxynucleotidyl transferase dUTP nick end labeling assay demonstrated that rats in our I/R and I/R plus saline groups showed a significantly increased number of TUNEL-positive cells compared to that in sham-operated and ADA applied rats. This finding of the study with down-expression of NF-κB suggests that NF-κB inhibiton is important in the antiapoptotic process during I/R injury.

Increased tissue NO levels through activation of iNOS is one of the key mechanisms involved in I/R injuries.[35] Overproduction of NO by iNOS interacts quickly with the superoxide radicals that are produced during reperfusion injuries to form peroxynitrite, which induces protein damage by forming nitrotyrosine.[34,36] In a rat model of experimental kidney injury, Wang *et al*.[36] demonstrated elevated levels of NO metabolites and the mRNA of iNOS in ischemia-reperfused kidneys in which tyrosol treatment attenuated iNOS-mediated NO production, which in turn reduced oxidative stress and minimized the extent of renal injury induced by I/R. Similarly, in a study by Ozcan and colleagues,[35] authors determined the efficacy of a selective NF-κB inhibitor, pyrrolidium dithiocarbamate (PDTC), on long-term histological damage in testicular I/R injuries. In rats administered PDTC, iNOS and p65 expressions were found to be significantly reduced compared with the torsion group suggesting a major role of iNOS and NF-κB in reperfusion injuries. Immunohistochemical analysis of the present study revealed severe staining in iNOS and the NF-κB in the torsion and torsion +saline group, whereas a weak staining in the sham and ADA-treated group suggesting an activation of NF-κB and iNOS by I/R and resulting with increased intraovarian NO levels and subsequent ovarian injury.

In conclusion, ADA therapy attenuated I/R induced ovarian injury, possibly due to suppression of inflammation, blockade of oxidative stress, and alteration of apoptotic pathways. Our results indicate substantial new aspects of this field and highlight the therapeutic potential of ADA for treating ovarian damage induced by I/R injury with providing the rationale for its use.

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Disclosure

All of the authors declare that there is no conflict of interest regarding to this article.

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