

The effect of metamizole on ischemia/reperfusion injury in the rat ovary: An analysis of biochemistry, molecular gene expression, and histopathology

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ABSTRACT

Objectives: In this study, we investigated the effect of metamizole on ischemia/reperfusion (I/R) injury an analysis of biochemistry, molecular gene expression, and histopathology in the rat ovary of female albino Wistar rats.

Materials and Methods: Animals were divided into four groups; control group with induced ischemia-reperfusion (IRC), ischemia-reperfusion +100 mg/kg metamizole sodium (MS) (IRM-100), ischemia-reperfusion +200 mg/kg MS (IRM-200), and healthy group applied sham operation (SG).

Results: Myeloperoxidase (MPO) activity and gene expression increased significantly in IRC and IRM-100 group rat ovarian tissue compared with the SG group ($P < 0.0001$). However, MPO activity and gene expression in IRM-200 group ovarian tissue decreased significantly compared with the IRC and IRM-100 groups ($P < 0.0001$). Histopathologically, pronounced congestion, dilated vessels, hemorrhage, edema, degenerative cells, and neutrophil migration and adhesion to the endothelium were observed in the IRC and IRM-100 group ovarian tissues. A small number of congested dilated vessels, mild congestion, and edema were observed in the IRM-200 group, but no neutrophil migration and adhesion to the endothelium or degenerative cells.

Conclusions: At 200 mg/kg dose metamizole prevented ovarian injury induced with I/R. This data show that metamizole can be used in the ovarian I/R injury treatment.

KEY WORDS: Gene expression, metamizole, myeloperoxidase, ovary, rat

Introduction

Ischemia describes tissue oxygen deprivation as a result of stoppage of blood flow to the tissue.^[1] Ovarian torsion is one cause of ovarian ischemia.^[2] It is reported primary pathology is rarely

determined in ovarian torsion. Ovarian torsion can be seen in normal ovaries, as well as ovarian cyst and tumor. Abdominal pain is one important sign of ovarian torsion. Ovarian torsion-related

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pelvic pain is more common in adolescent girls. Generally, pain is seen colic and febrile. If left untreated, ovarian torsion causes necrosis of the ovaries and result in ovariectomy.^[3] Therefore, for the protection of ovaries, ovarian torsion is provided reperfusion by detorsion.^[4] However, reperfusion of ischemic tissue has been shown to lead to more severe injury than that caused by the ischemia.^[5] Increased polymorphonuclear leukocytes (PNL) activity reaching the ischemic region in abundant quantities is known to play an important role in reperfusion injury.^[6] PNLs release proinflammatory agents in tissues applied to ischemia/reperfusion (I/R). Isaoglu *et al.* reported that inflammation symptoms in ischemic ovarian tissue become more severe with reperfusion.^[7] Increased migration and adhesion to the epithelium of PNLs, an inflammation marker, has been observed in rat ovarian tissue with induced I/R. An increase in myeloperoxidase (MPO) activity has also been determined in parallel to an increase in PNLs in rat ovarian tissue in an experimental I/R model induced with torsion and detorsion.^[8] As is known, MPO is released from activated PNLs: Excessive production of MPO from activated PNLs increases the formation of hypochlorous acid (HOCl), a toxic oxidant, by oxidizing chloride ions with hydrogen peroxide.^[9] This HOCl protein of PNL origin can cause tissue damage by reacting with amino acids, lipids, and nucleic acids. It can occur in any tissue in the body, with or without neutrophilic inflammation. This literature information indicates that I/R damage will decrease with inhibition of MPO production from PNLs. In this study, the metamizole sodium (MS) is a drug with powerful analgesic, antipyretic effects and a weak anti-inflammatory effect with a N-(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl) - N-methyl amino methanesulfonate structure that tested against ovarian I/R injury.^[10] Metamizole has been shown to suppress production of MPO and its toxic products.^[11] Metamizole's inhibitor effect on MPO suggests to be effective against ovarian I/R injury. In literature search found no information concerning the protective effect of metamizole against injury induced with I/R in the rat ovary. Therefore, the purpose of this study to investigate the relation between MPO of neutrophil origin and ovarian I/R injury in rats, and to examine the protective effect of metamizole be or not against I/R injury at the biochemical, molecular gene expression, and histopathological levels.

Materials and Methods

Animals

In this study was used a total of 24 albino Wistar type of female rats, the weights of which varied between 210 and 220 g, animals were obtained from the Recep Tayyip Erdogan University, Medical Experimental Practice and Research Center, Turkey. Animals were kept and fed for a period in the Pharmacology Laboratory where the experiment was to be performed at normal room temperature (22°C) to acclimatize to their surroundings.

Chemical Substances

Of the chemical substances used for the experiments, thiopental sodium was provided by IE Ulagay-Turkey, and MS (Novalgine) by Sanofi-Aventis (Turkey).

Experimental Groups

The female rats to be used in the experiment were divided into four groups; control group with induced ischemia-reperfusion (IRC), ischemia-reperfusion + 100 mg/kg

MS (IRM-100), ischemia-reperfusion + 200 mg/kg MS (IRM-200), and healthy group applied sham operation (SG).

Surgical Procedures

Thirty minutes before application of the I/R procedure to rat ovaries, MS was injected intraperitoneally (i.p.) in doses of 100 and 200 mg/kg into the rats in the IRM-100 and IRM-200 groups, respectively. The SG group rats were administered distilled water by the same way. Thirty minutes after drug administration, anesthesia was induced in all rats (IRC, IRM-100, IRM-200, and SG) with the i.p. administration 25 mg/kg doses of thiopental sodium. The period of time the animals remained inactive in a supine position is regarded as an appropriate time span for the surgical attempt.^[12] During this period, the ovaries were accessed through a 2–2.5 cm vertical incision in the lower abdomen. A vascular clip was applied to the inferior part (the region where the ovary joins the uterus) of the right ovaries of rats in the IRC, IRM-100, and IRM-200 groups. Two-hour-ischemia was applied, and 2-h-reperfusion was provided. The SG group ovaries were closed with no procedure. Following reperfusion, all animals were killed by an overdose of anesthesia. The rats were killed were extracted right ovaries, and biochemical, histopathological, and molecular gene expression examinations were performed on the excised ovaries. Results from the IRM-100 and IRM-200 groups were compared with those from the IRC and SG groups.

Biochemical Procedures

Myeloperoxidase activity assay

At this stage of the study, 0.2 g was weighed from each ovary. Homogenates were prepared from ovarian tissues to measure MPO activities. Potassium sulfate buffer at pH 6 containing 0.5% hexadecyltrimethylammonium bromide was prepared. These were then centrifuged at 10,000 rpm at +4°C for 15 min. The supernatant part was used as the analysis specimen. MPO-mediated oxygen reaction performed with H₂O₂ containing 4-aminoantipyrine/phenol solution was used to determine MPO enzyme activity.^[13]

Determination of myeloperoxidase gene expression

Two hundred microliters of the extract obtained from the fragmented ovarian tissue were placed in a ROCHE MagNA Pure Compact automatic RNA Isolation Device. Next, a 50 µl sample was obtained by RNA isolation using the Roche MagNA Pure Compact RNA isolation kit.

Complementary DNA synthesis

Concentration of the RNA obtained was measured. Based on that DNA concentration, the DNA was either diluted or undiluted so as to yield 15–20 nanograms complementary DNA (cDNA). Ten microliters of each calibrated sample, 2 µl random primer and 1 µl distilled water from a Transcriptor First Strand cDNA Synthesis Kit (tube no. 6) were transferred into a 0.2-polymerase chain reaction (PCR) tube. Next, denaturation was carried out in the reverse transcriptor PCR device at 65°C for 10 min. In the meantime, the mixture to be added to denatured RNA for the formation of cRNA was prepared. The substances and quantities thereof included in the mixture used for each sample were as follows (from the Transcriptor First Strand cDNA Synthesis Kit): Reaction buffer (no. 2) 4 µl, RNAase (no. 3) 0.5 µl, deoxynucleotide mix (no. 4) 2 µl, and

reverse transcriptase (no. 1) 0.5 μ l. After adding 7 μ l of the mixture to denatured RNA, the tube was placed in the reverse transcriptase PCR device adjusted to an appropriate PCR program.

Loading real-time polymerase chain reaction device

First, the mixture was prepared; 8 μ l distilled water, 5 μ l probe master mix, 2 μ l primer, and 5 μ l cDNA were used for each sample.

Real-time polymerase chain reaction program

Denaturation program:	At 95°C for 10 min	} 45 cycles
Amplification program:	At 95°C for 10 s	
	At 60°C for 30 s	
	At 72°C for 60 s	
Cooling:	At 40°C for 30 s	

Histopathological Procedures

Extracted rat ovaries were fixed in 10% formalin. Following routine procedures, 4 μ m-thick sections were obtained from the resulting paraffin blocks. After deparaffinization and rehydration, sections were stained with hematoxylin and eosin. All sections were examined under a light microscope (Olympus CX 51, Tokyo, Japan).

Statistical Analysis

All data were subjected to one-way analysis of variance using The Statistical Package for the Social Sciences (SPSS, Inc., v. 18.0, IBM Corporation, Armonk, NY, USA) software. Differences among groups were obtained using the least significant difference option, and significance was set at $P \leq 0.05$. Results are expressed as a mean $\pm$ standard error of the mean.

Human and Animal Rights

This study is suitable for human and animal rights.

Results

Myeloperoxidase Activity Results

As shown in Figure 1, MPO activity was significantly higher than the healthy group in the rat ovarian tissue exposed to I/R ($P < 0.0001$). 100 mg/kg dose of metamizole was unable to significantly suppress MPO activity increasing with I/R ($P > 0.05$). However, 200 mg/kg dose of metamizole suppressed MPO activity in a pronounced and significant ($P < 0.0001$).

Myeloperoxidase Gene Expression Results

MPO gene expression in IRC group ovarian tissue increased significantly compared with the IRM-200 and SG groups. 200 mg/kg dose of metamizole significantly reduced the increase in MPO gene expression in rat ovarian tissue with induced I/R ($P < 0.0001$), while at a dose of 100 mg/kg its effect was insignificant ($P > 0.05$) [Figure 2].

Histopathological Results

A normal appearance was seen at light microscopic evaluation of SG group ovarian tissue [Figure 3]. A large number of dilated vessels (arrow), severe hemorrhage, and stromal edema (star) were observed at low magnification in IRC group ovarian tissue [Figure 4a]. At high magnification, pronounced neutrophil migration and adhesion to the endothelium (arrow) and large numbers of degenerative cells were observed [Figure 4b]. Figure 4c shows a large number of congested dilated vessels (arrow), congestion (arrow), and edema at low magnification in the IRM-100 ovarian

Figure 1: The effect of metamizole on myeloperoxidase levels in rat ovarian tissue after induction of ischemia/reperfusion (used analysis of variance + least significant difference tests, compared with the ischemia reperfusion group). * $P < 0.0001$

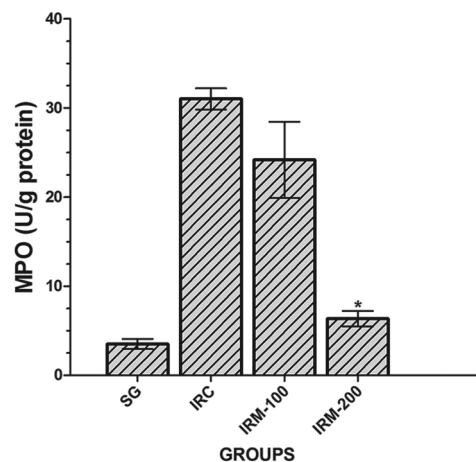


Figure 2: Myeloperoxidase gene expression levels in rat ovarian tissue (used analysis of variance + least significant difference tests, compared with the ischemia reperfusion group). * $P < 0.0001$

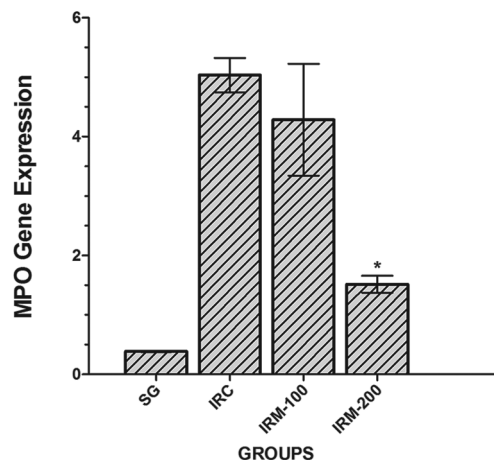


Figure 3: In the histopathological evaluation of the ovarian tissue of sham operation group

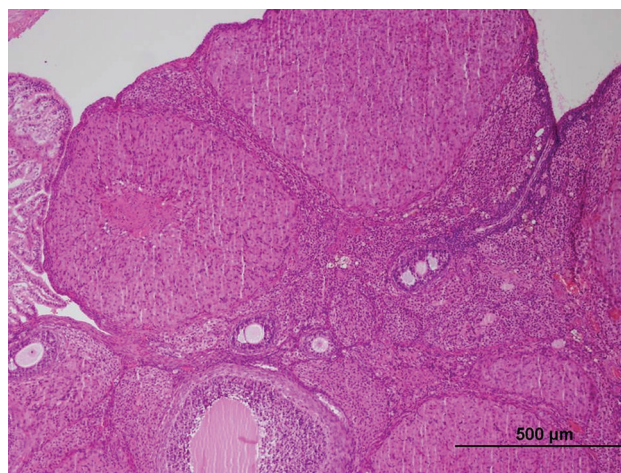
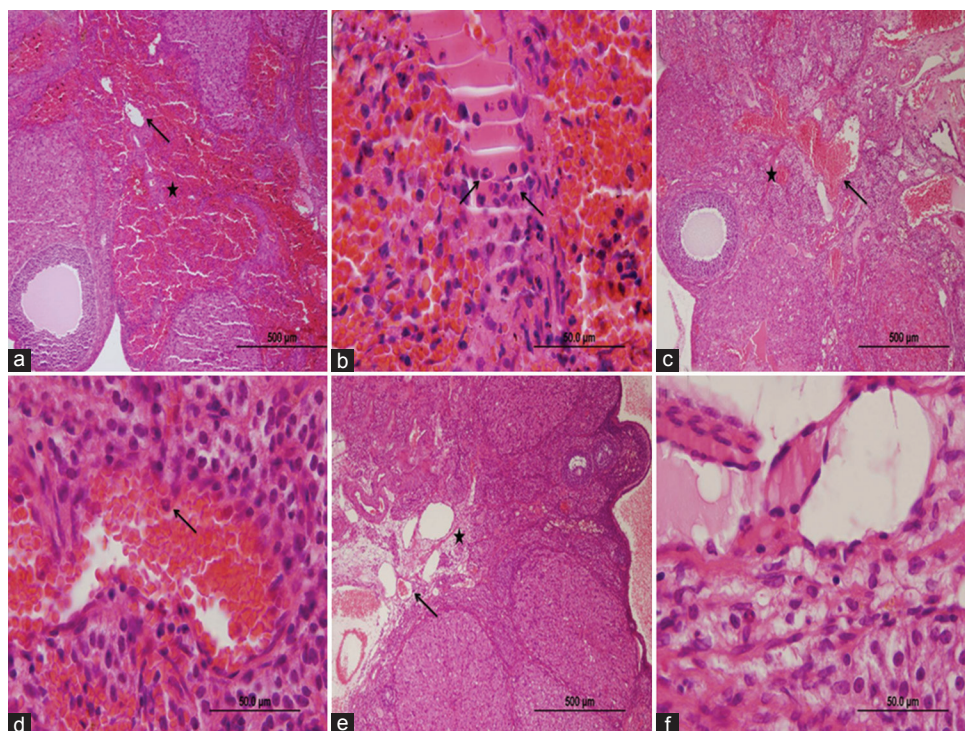


Figure 4: (a and b) In the histopathological evaluation of the ovarian tissue of control group with induced ischemia-reperfusion. (c and d) In the histopathological evaluation of the ovarian tissue of ischemia-reperfusion +100 mg/kg metamizole sodium group. (e and f) In the histopathological evaluation of the ovarian tissue of ischemia-reperfusion +200 mg/kg metamizole sodium group



tissue. Figure 4d shows degenerative cells with pronounced neutrophil migration and adhesion to the endothelium (arrow) at high magnification. A small number of congested dilated vessels (arrow) and mild congestion and edema (star) were observed at low magnification in IRM-200 group ovarian tissue [Figure 4e], while no neutrophil migration and adhesion to endothelial cells or degenerative cells were observed at high magnification [Figure 4f].

Discussion

This study investigated the relation between ovarian I/R injury and MPO of neutrophil origin and examined whether metamizole has a protective effect against ovarian I/R injury at the biochemical, molecular gene expression, and histopathological levels. The experimental results show that I/R caused a significant increase in MPO activity and gene expression in ovarian tissue compared to the healthy group. MPO is known to be produced at physiological levels by PNLs in healthy tissue. However, excessive production of MPO can result in tissue damage.^[14] A rise in MPO activity parallel to an increase in PNL has been determined in rat ovarian tissue with induced I/R injury.^[8] In addition, tissue damage originating from neutrophils has been reported to be inflammatory damage associated with excessive MPO production.^[14] It has been suggested in the literature that I/R leads to tissue damage by causing neutrophil activation and adhesion; studies involving antineutrophil serum have shown that neutrophils are particularly responsible for an increase in microvascular permeability with reperfusion.^[12] In our study, inflammatory signs such as pronounced vasodilation, hemorrhage, edema,

and migration and adhesion of neutrophils to the endothelium were also observed histopathologically in IRC rat group ovarian tissue with high MPO activity and gene expression. Migration and adhesion of PNLs to endothelium, an inflammatory marker, have also been observed to increase in rat ovarian tissue with induced I/R injury in previous studies. Demiryilmaz *et al.* obtained similar histopathological findings to ours in rat ovarian tissue applied to I/R.^[15] Zimmerman and Granger reported that degree of reperfusion injury was associated with the degree of neutrophil activation and tissue infiltration.^[16] This information from the literature supports the findings of this study. 100 mg/kg dose of metamizole, the effect of which against ovarian I/R injury was tested in this study, was unable to prevent a significant rise in MPO activity and gene expression in ovarian tissue subjected to I/R. Histopathological findings in the IRM-100 group were almost identical to those in the IRC group. Metamizole has been shown to inhibit MPO weakly at a dose of 120 mg/kg but powerfully at 500 mg/kg.^[11] This information from the literature and our own experimental results show that inhibition of MPO activity is possible with metamizole at doses above 100 mg/kg. In the IRM-200 group, MPO activity and MPO gene expression were significantly lower and pathological findings milder compared to the IRC group. This also indicates that our results are compatible with the literature.

Free oxygen radicals produced and released with PNLs are implicated in the pathogenesis of PNL-related injury in tissue exposed to I/R.^[17] This also shows that I/R leads to oxidative stress. The mechanisms involved in damage caused by I/R in numerous organs, such as the brain, heart, lung, liver, and intestines, have been investigated, and free oxygen has been

shown to be one of the major components of I/R injury.^[18] Pathological findings being mild in IRM-200 group ovarian tissue, and the suppression of MPO activity and gene expression suggest that metamizole may have reduced oxidative stress caused by I/R by reducing MPO gene expression. It has also been reported that metamizole inhibits the formation of MPO and MPO products and possesses antioxidant activity.^[19]

Conclusions

I/R leads to an increase in MPO activity and gene expression in ovarian tissue. In addition, a pathological picture of pronounced congested dilated vessels, hemorrhage, edema, and migration and adhesion of neutrophils to the endothelium and a large number of degenerative cells was observed in ovarian tissue exposed to I/R with increased MPO activity and gene expression. 100 mg/kg metamizole was unable to prevent the pathological process caused by I/R. At 200 mg/kg, however, it reduced I/R injury-related oxidative stress and pathological signs to a minimum. The fact that metamizole has no side effects such as gastrointestinal, renal, and thrombocyte inhibition or hemorrhage^[20] shows that it can be preferred to other nonsteroidal anti-inflammatory drugs in the treatment of I/R injury.

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Conflicts of Interest

There are no conflicts of interest.

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