

Original Article

Remifentanil protects the ovaries against ischaemia-reperfusion injury in rats

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Abstract: Ovarian ischaemia reperfusion (I/R) injury may affect fertility. Protecting ovaries against organ damage is therefore clinically important. In this study, we assessed the effects of remifentanil and investigated whether it has a protective effect. Eighteen female Wistar albino rats were assigned to three groups (n = 6): a sham group, an ischaemia reperfusion (I/R) group, and an ischaemia reperfusion and remifentanil (I/R+R) group. Rats exposed to experimentally induced I/R injury. Two-hour periods of ischaemia were followed by 1 h of reperfusion in the I/R and I/R+R groups. After ischaemia, no drug was administered in the sham and I/R groups. In the I/R+R group, remifentanil infusion (2 µg/kg/min) was started in the last 30 min of the ischaemia period, and continued until the end of reperfusion. After the ischaemic and reperfusion period, the ischaemic ovaries were surgically removed for biochemical and histopathologic examination. Tissue damage scores (dense vascular congestion, oedema, and loss of cohesion) were examined. Malondialdehyde levels and catalase enzyme activity in tissue were measured. We found significantly lower tissue damage scores in the I/R+R group than in the I/R group (P < 0.05). The administration of remifentanil significantly decreased oxidative stress (malondialdehyde level) (P < 0.05), and significantly increased antioxidant enzyme (catalase enzyme) activity compared with the ischaemia reperfusion group (P < 0.05). These findings suggest that remifentanil has a protective effect against ovarian ischaemia reperfusion injury and can be used safely in ovarian torsion surgery.

Keywords: Remifentanil, ovary, ischaemia, enzymes, rat

Introduction

Ischaemia plays a role in the pathophysiology of many clinical conditions [1]. Ovarian torsion is one of the pathologies faced by anesthesiologists as a gynaecologic emergency. This pathology is the rotation of the ovary and/or fallopian tube on its ligamentous support to such a degree that the ovarian artery and/or vein is occluded, causing ischaemia [2]. Ischaemia stimulates the production of reduced oxygen molecules that directly or indirectly damage the structural and genetic apparatus of cells [3]. Restoring circulation (reperfusion) to

prevent irreversible cellular injury leads to the formation of toxic reactive oxygen species (ROS), and this may increase tissue injury [1]. The main pathophysiology in ovarian torsion is ischaemia followed by reperfusion; therefore, ovarian torsion-detorsion is an ischaemia-reperfusion (I/R) injury to the ovaries [4]. Early diagnosis and treatment in ovarian torsion are essential to optimize ovary function and fertility. Determining the preventive effects of drugs on ovarian damage during torsion and detorsion is therefore clinically important. To date, numerous drugs have been investigated to protect the ovary against I/R injury [5]. The

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effects of intravenous anaesthetic agents used during surgical intervention in ovarian I/R injury have not been investigated.

Remifentanyl is an ultra-short-acting and widely used opioid analgesic that is usually administered as a continuous infusion in clinical settings [6]. It has protective effects on some organs, like the heart, liver, and kidney [6-8]. It can also protect ovaries. We therefore decided to perform an experimental study to investigate the possible effects of remifentanyl on I/R-induced ovarian damage. The results of this study may provide guidance for choosing the appropriate opioid in ovarian torsion surgery.

Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Marmara University, and was conducted according to the tenets of the Declaration of Helsinki. Assuming a statistical power of 92% and an alpha of 5%, six rats were required in each group to reach a level of significance [4]. We conducted the study with 18 female Wistar albino rats aged 8-10 weeks old and weighing 220-250 g in the Experimental Animals and Application Research Center of Marmara University, Istanbul, Turkey. The animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment ($22 \pm 20^\circ\text{C}$), and were exposed to a 12 h/12 h light/dark cycle. The animals were all in the estrous phase, which was determined with a vaginal smear. The rats were randomly allocated into three study groups: the sham group ($n = 6$), the I/R group ($n = 6$), and the I/R and remifentanyl group (I/R+R) ($n = 6$).

Each rat was weighed and anaesthetized with intramuscular ketamine hydrochloride (50 mg/kg Ketalar; Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (10 mg/kg Rompun; Bayer, Istanbul, Turkey), which were repeated as necessary to maintain anaesthesia during the experiment. Following the preoperative sterilization, a longitudinal incision (2.5 cm) was performed in the midline area of the lower abdomen. After a small peritoneal incision, the uterine horns and adnexa were located. Ovarian torsion was produced by applying atraumatic vascular clips (to prevent damage to the surrounding tissue) to the vascular pedicle 1 cm above and below the ovaries [9]. In the sham group, only laparotomy and bilateral oophorec-

tomy were performed. In the I/R group, a 2-h period of ischaemia was followed by 1 h of reperfusion. In the I/R+R group, ischaemia was performed as in the I/R group, followed by remifentanyl infusion ($2 \mu\text{g}/\text{kg}/\text{min}$) in the last 30 min of the ischaemia period via a previously cannulated tail-vein catheter, which continued until the end of the reperfusion [7]. At the end of reperfusion, bilateral ovaries were removed. The right ovaries were used for histopathologic examination, and the left ovaries were stored in a freezer at -80°C for the biochemical analysis. Following the oophorectomy, the rats were killed by decapitation.

For the histopathologic analysis, ovarian samples were fixed in 10% buffered formalin for 48 h, dehydrated in an ascending alcohol series, cleared in xylene, and embedded in paraffin. Tissue sections ($5 \mu\text{m}$ thick) were stained with hematoxylin-eosin and Masson's trichrome for general morphologic analysis. Histologic assessments were performed with a photomicroscope (Olympus BX-51; Olympus Co, Tokyo, Japan) by the same pathologist who was blinded to the experimental groups. At least 10 histologic sections from the ovary were assessed at a magnification of $10\times$ and $20\times$ objectives and then photographed. The slides were coded, and an evaluation was performed by a pathologist without knowledge of the treatment protocol. The scoring system defined by Sagsoz et al. was used [10]. Congestion, bleeding, oedema and loss of cohesion (separation of parenchymal cells along with normal ovarian cortex and follicles) were scored from 0 to +3 according to their severity (0 = no pathological finding; 1 = pathological findings in $< 33\%$ of the ovarian section; 2 = pathological findings in $33-66\%$ of the ovarian section; 3 = pathological findings in $> 66\%$ of the ovarian section). The scores for each parameter were summed and the total tissue damage scores were calculated.

The ovarian tissue samples were homogenized with ice-cold Tris-HCl buffer solution (0.2 mol/l), centrifuged at $3500\times g$ for 60 min, and the supernatant was collected. Catalase (CAT) activity was determined in the supernatant with an assessment of the rate constant of hydrogen peroxide decomposition at 240 nm [11]. The malondialdehyde (MDA) levels in the ovarian tissues were studied in the homogenate, and were analyzed according to the reaction with thiobarbituric acid at $90-100^\circ\text{C}$.

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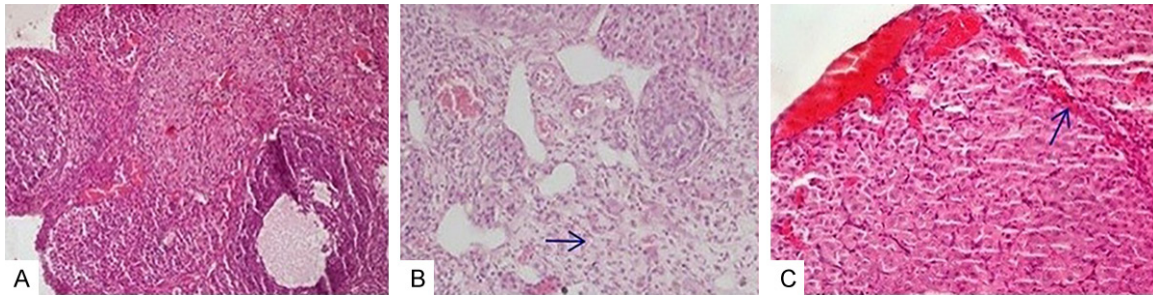


Figure 1. A. No pathological changes were detected in the sham group. B. Dense edema and loss of cohesion (arrow) were observed in the ischemia reperfusion group. C. Mild vascular congestion (arrow) was detected in the ischemia reperfusion and remifentanil group (H&E, original magnification $\times 200$).

Table 1. The tissue damage scores of the groups

Group	Median	Interquartile range (min-max)	P value
Sham	1	0 (1-1)	0.000
I/R	4	5 (2-7)	
I/R+R	1	0 (1-1)	

I/R: Ischemia reperfusion group. I/R+R: Ischemia reperfusion and remifentanil group.

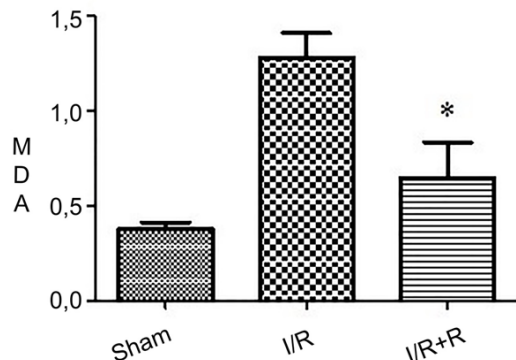


Figure 2. Effect of remifentanil on malondialdehyde (MDA) levels. Remifentanil infusion ($2 \mu\text{g}/\text{kg}/\text{min}$) was started in the last 30 min of the ischemia period, and was continued until the end of reperfusion. Data expressed as mean \pm SEM. $P < 0.01$ versus ischemia reperfusion group (I/R) group.

Malondialdehyde and thiobarbituric acid react together and produce a pink pigment with a maximum absorption of 532 nm in the thiobarbituric acid test reaction [12].

Statistical analysis

The statistical analysis was performed with the Kruskal-Wallis test for tissue scores, one-way analysis of variance (ANOVA), followed by

Duncan's multiple range test for biochemical results. We used SPSS version 15.0 (SPSS Inc. Chicago, IL) for Windows. The results are presented as means \pm standard error of the mean (SEM). The differences between groups were considered statistically significant at a P value of < 0.05 .

Results

On the hematoxylin-eosin-stained slides, no pathologic changes were detected in the sham group (**Figure 1A**), while dense oedema and loss of cohesion were observed in the I/R group (**Figure 1B**). In the I/R+R group, the ovarian tissue showed only mild vascular congestion (**Figure 1C**). The tissue damage scores were significantly higher in the I/R group than in the sham and I/R+R groups ($P = 0.000$) (**Table 1**). In the I/R+R group, the score was nearly the same as for the sham group, and there was no statistically significant difference compared with the sham group ($P > 0.05$).

The MDA levels increased significantly in the I/R group compared with the sham group ($P = 0.001$). Compared with the I/R group, administration of remifentanil significantly decreased MDA levels in the I/R+R group ($P = 0.001$) (**Figure 2**). Catalase activity decreased in the I/R group compared with the sham group ($P = 0.025$). However, administration of remifentanil significantly increased CAT activity in the I/R+R group compared with the I/R group ($P = 0.025$); see **Figure 3**.

Discussion

Results of the present study indicate that remifentanil administration decreased histopathologic injury levels and oxidative stress (MDA

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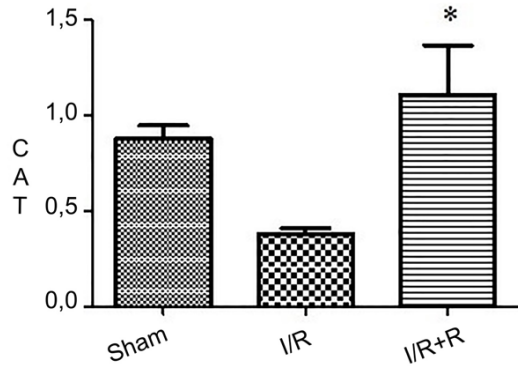


Figure 3. Effect of remifentanil on catalase (CAT) levels. Remifentanil infusion ($2 \mu\text{g}/\text{kg}/\text{min}$) was started in the last 30 min of the ischemia period, and was continued until the end of reperfusion. Data expressed as mean \pm SEM. $P < 0.05$ versus ischemia reperfusion group (I/R) group.

level) and increased antioxidant enzyme (CAT) activity after ovarian I/R injury. All histologic and biochemical results indicate that administering remifentanil has beneficial effects in preventing reperfusion injury of the ovaries.

Several experimental studies have shown that opioid agonists have a protective effect against I/R injury in a wide variety of tissues [13]. Many researchers have administered remifentanil during ischaemic preconditioning and postconditioning [14-16]. Preconditioning refers to the application of a protective intervention prior to ischaemia. This can be pharmacological, or it can be mechanical, which involves inducing brief periods of sublethal ischaemia separated by periods of reperfusion to increase the tolerance of the tissue to I/R [17, 18]. Remifentanil preconditioning has been used to decrease I/R injury in different organs, such as the liver, kidney, heart, brain and intestine. Researchers have reported remifentanil has a protective effect against ischaemic injury [7, 8, 14, 17, 19]. Ischaemic postconditioning initially refers to the application of a protective intervention after reperfusion. This technique is similar to preconditioning, only the timing of the application differs [20]. Postconditioning with remifentanil has also shown protection in I/R injury of the heart and brain [16, 21]. Chun et al. investigated whether the protective effects of remifentanil are present only during preconditioning and postconditioning or if it has a protective effect when given continuously. They reported

that continuous administration of remifentanil is also effective, and it remains protective against I/R injury [6]. Remifentanil is usually administered as a continuous infusion in current clinical practice; therefore, we chose to administer the agonist in this way.

Previous studies on ovarian I/R injury showed that biochemical and histopathologic changes can be observed 1, 2, 3, and 4 h after the injury [22]. Coskun et al. attempted to find the critical ischaemic duration in rat ovary. In their experimental study, complete ischaemia durations were 1, 2, and 3 h, and the duration of reperfusion was 1 h. The researchers demonstrated that the critical ischaemic duration for rat ovary was 2 h [23]. In light of this study, we induced 2 h of ischaemia and 1 h of reperfusion in our study.

Reperfusion activates the inflammatory signal transduction pathway initiated by the release of toxic ROS, proteases and elastases, resulting in increased microvascular permeability, oedema, thrombosis and parenchymal cell death [3]. In a study that investigated pharmacological preconditioning with remifentanil in intestinal I/R injury, the authors reported that remifentanil conveys a protective effect on intestinal mucosa by decreasing apoptosis [17]. In another study, remifentanil preconditioning in liver I/R significantly decreased apoptosis and showed less tissue damage, which is in agreement with our findings [7].

When oxygen is reintroduced during reperfusion, xanthine oxidase converts the excess hypoxanthine to toxic ROS, which initiates lipid peroxidation and results in cellular damage [24]. Lipid peroxidation leads to the formation of lipid radicals, lipid hydroxyl radicals and hydroperoxides. Malondialdehyde serves as a sign of lipid peroxidation in I/R damage [9]. Malondialdehyde disorganizes ionic transport and enzymatic activity, leads to increased membrane permeability and causes breaks in cell content [25]. Zhao et al. reported a decrease in MDA levels with remifentanil preconditioning in I/R injured livers [7]. Similarly, Cho et al. showed decreased MDA levels with remifentanil in an experimental model of intestinal ischaemia [17]. In our study, we also found a significant decrease in MDA levels with remifentanil infusion.

Antioxidants are among the defense mechanisms against free radical-induced oxidative stress. Enzymatic antioxidant defenses include CAT, superoxide dismutase and glutathione peroxidase [26]. In the present study, CAT, which decays hydrogen peroxide and limits accretions of toxic compounds, was used to determine the antioxidant levels. We found that remifentanil administration significantly increased CAT activity. We failed to find any published reports on the effect of remifentanil on CAT activity.

Limitations

The present study has some limitations. First, we assessed ovarian function using only tissue damage scores and biochemical changes; however, the quality of the oocytes in the ovary and the size of the primordial follicle pool are also important. The anti-Müllerian hormone and the serum levels of the ovarian hormones (inhibin B, estradiol) can be used as a marker for the quantitative aspects of ovarian reserves when assessing follicles [22]. Therefore, we should evaluate not only the tissue damage and biochemical changes, but also the follicle count and serum levels of anti-Müllerian hormone, inhibin B and estradiol in the assessment of the ovarian reserve. Second, we observed protective effects in rats-not in humans. Hence, additional experimental and clinical studies are required to assess the protective effect of remifentanil on ovarian reserves.

In summary, as an important gynaecologic emergency, ovarian torsion must be managed immediately to preserve fertility. The agents used during the surgical period must be chosen carefully to protect the ovarian tissue from reperfusion damage. Data from this study confirmed remifentanil can confer protection against ovarian I/R in rats. The next challenge is to investigate the effect of remifentanil on ovarian oocytes and follicles.

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Disclosure of conflict of interest

None.

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