The effect of nizatidine on ovarian ischaemia/reperfusion injury in rats

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ABSTRACT

An ischaemia/reperfusion (I/R) injury in an organ can also cause damage to other organs. In this study, the effects of nizatidine were investigated on ovarian I/R injury and subsequent heart damage in rats. Rats were divided into groups; ovarian I/R (IRG), nizatidine 25 mg/kg+ ovarian I/R (NIR), and control (CG) groups. The NIR and IRG groups were given 2 hours of ischaemia followed by 2 hours of reperfusion by applying, and subsequently releasing, a vascular clip to the right ovarian artery. While oxidant levels in the IRG group were higher than NIR and CG groups, antioxidant levels were lower. Necrosis, congested blood vessels, haemorrhage, leukocyte infiltration and oedema were observed in the ovarian and heart tissues of the IRG group. These findings were reduced in the NIR group. Nizatidine suppressed oxidative stress in ovarian and heart tissues and may be useful in prevention of I/R–induced ovarian and cardiac injury.

Key words: Ischaemia, Nizatidine, Ovary, Reperfusion, Rat.

INTRODUCTION

Ovarian ischaemia usually results from the rotation of the ovaries around their vascular axis (Huchon and Fauconnier, 2010). Ovarian torsion causes necrosis of the ovaries and may result in an ovariectomy (Poonai et al., 2013). A quick detorsion provides immediate reperfusion to the tissue (Dikensoy et al., 2007). However, the reperfusion process can cause more damage more than the ischaemia (Rock, 2003.). An I/R injury can cause serious damage to ischaemic tissue as well as other sensitive remote organs. Distant organ effects of I/R are frequently seen in the cardiovascular system, and these can be fatal (Carden and Granger, 2000).

Reactive oxygen species (ROS) and myeloperoxidase (MPO), secreted by activated polymorphonuclear leukocytes (PMNLs), are implicated in the pathogenesis of distant organ damage in I/R (Carden and Granger, 2000). This may imply that drugs inhibiting oxidants and PMNL–mediated MPO production would be useful in I/R damage.

Nizatidine has antioxidant properties and prevents oxidative damage to the stomach. Nizatidine inhibits the increase of oxidants such as malondialdehyde (MDA) and MPO in the stomach, and the decrease of antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPO). Hence, the gastro-protective effect of nizatidine is due largely to the inhibition of ROS rather than the inhibition of acid production (Liu et al., 2016).

The antioxidant property of nizatidine (van Zyl et al., 1993) may be useful for the prevention of ovarian I/R damage and possible cardiac complications. We aimed to investigate the effect of nizatidine on ovary I/R and its effect on cardiac complications.

MATERIALS AND METHODS

Animals: This experiment was carried out on 18 albino Wistar male rats weighing 235–250 grams. The rats were maintained in groups of six at room temperature (22 °C) in the laboratory and fed ad libitum for 7 days.

Experimental procedures: Rats were divided into three groups: ovarian I/R (IRG), ovarian I/R+ nizatidine 25 mg/kg (NIR), and the control group (CG). Surgical procedures were performed under sterile conditions with 25 mg/kg intraperitoneal (IP) thiopental sodium anaesthesia in an appropriate laboratory setting. In addition, the rats were administered 20 mg/kg meperidine IP as a pain killer.

Nizatidine (25 mg/kg) was injected IP to rats in the NIR group one hour prior to anaesthesia. Similarly, distilled water was injected into rats in the IRG and CG groups. One hour later, the rats were anesthetized by thiopental sodium. The ovaries were then reached through 2–2.5-cm vertical abdominal cuts.
Two hours of ischaemia were applied to the NIR and IRG groups and two hours of reperfusion were applied using a vascular clip on the distal part of the right ovarian artery. This was followed by two hours of reperfusion in which the vascular clip was released. (Kurt et al., 2011). At the end of this period, all animals were killed by a high dose of anaesthesia. Biochemical and histopathological examinations of the ovary and heart were made after the removal of these tissues from the sacrificed animals, including those of the CG group. The results of the NIR group were compared with the IRG and CG groups.

**Biochemical analysis:** The homogenates were prepared to measure the enzymatic activity in the ovarian and heart tissues. The xanthine oxidase (XO) activity was measured spectrophotometrically (Rowe and Wyngaarden, 1966), MDA activity was determined using the thiobarbituric acid test (Ohkawa et al., 1979), and MPO activity (Bradley et al., 1982), total glutathione (tGSH) levels (Sedlak and Lindsay, 1968) and GPO activity (Lawrence and Burk, 1976) were measured using standard procedures.

**Histopathological examinations:** Ovarian and heart tissues were fixed in 10% formalin for 24 hours. Tissue sections (4-μm thick) were obtained from the paraffin blocks after routine tissue application and stained with haematoxylin and eosin. All sections were evaluated under a light microscope (Olympus BX 52, Tokyo, Japan) by a pathologist following a blind allocation of samples.

**Statistical analysis:** Results were calculated as mean ± standard error of the mean (x ± SEM). The significance level between the groups was determined using a one-way analysis of variance (ANOVA). The least significant difference (LSD) test was also performed. All statistical procedures were performed in SPSS Statistics version 18, and p < 0.05 value was accepted as significant.

### RESULTS AND DISCUSSION

Tissue injury can lead to biochemical or structural changes (Salman et al., 2011). While histopathological methods are used to detect structural damage, biochemical damage is assessed by measuring oxidants (XO, MDA, MPO) and antioxidants (tGSH, GPO, SOD) (Kunak et al., 2015).

In this study, we investigated the protective effect of nizatidine on the biochemical and histopathological changes in ovarian and cardiac tissues following I/R in rats. As is depicted in Figure 1 the XO activity in the rat ovaries of the IRG group was higher than in the NIR and CG groups (p < 0.0001 for both). Nizatidine significantly reduced XO activity, which is increased by I/R. XO activity in the NIR group was higher than that of the CG group. However, the difference in XO activity between NIR and CG groups was not statistically significant (p > 0.05). Similarly, an elevated XO during ischaemia has been reported to decrease significantly after reperfusion (Kumbasar et al., 2014). As a consequence of the reduction of XO after reperfusion, XO has been shown to be consumed in the production of oxidants during reperfusion (Cetin et al., 2014).

XO has been shown to render hypoxanthine to xanthine during reoxygenation during reperfusion, thus leading to excessive ROS production (Liu et al., 2016). However, during reperfusion, XO is found to be higher than in healthy tissues (Kumbasar et al., 2014). ROS, which is increasingly produced by reperfusion and is therefore called a reperfusion mediator, leads to the oxidation of cell membrane lipids. MDA, which results from the oxidation of lipids, increases the severity of cell damage and causes cell death (Ayaz et al., 2017; Yapca et al., 2013). In the current study, I/R resulted in an increase in MDA levels in ovarian tissue (p < 0.0001). However, the amount of MDA in the NIR group was almost the same as that of the CG. The high

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**Figure 1:** XO, MDA, MPO, GPO, GST and SOD levels in ovarian tissues of the IRG, NIR and CG groups. The NIR and CG groups were compared with the IRG group. (n = 6), * = p < 0.0001.
amount of MDA in the IRG group suggests that the I/R process is damaging to ovarian cell membrane lipids. Production of MDA is increased in oxidative ovarian damage due to I/R (Demiryilmaz et al., 2013). In addition, MPO is also increased in an I/R injury (Kisaoglu et al., 2013).

In the present experiment, MPO showed a significant increase in ovarian tissue in the IRG group. Nizatidine significantly suppressed the increasing MPO activity with I/R \( (p < 0.0001) \). The MPO difference between the NIR and CG groups was statistically insignificant. In the literature, the increased MPO activity in the ovaries has been suggested to be associated with an inflammatory injury (Yapca et al., 2014). As a result, excessive production of hydrogen peroxide catalyses the chloride ions to form hypochlorous acid (HClO). It has been also suggested that MPO’s inflammatory effect is caused by HClO formation in the tissues (Lavelli et al., 2000).

The decrease in antioxidants is responsible for damage in the ovaries (Kisaoglu et al., 2013). The amount of tGSH in ovarian tissue of the IRG group was decreased compared to that of the NIR and CG groups. The amount of tGSH was measured at almost the same level in the NIR and CG groups. In this study, the I/R process also caused a decrease in GPO activity in ovarian tissue. GPO activity in the IRG group showed a decrease compared to the NIR and CG groups. In the IRG group, the SOD activity was reduced compared to the NIR and CG groups. The decrease of tGSH in ovarian tissue is responsible for the histopathological damage (Ingec et al., 2012). Normally, tGSH maintains the sulphhydryl groups of the molecules in a reduced state, thereby protecting them from oxidative damage (Urso and Clarkson, 2003). The GPO activity protects the cellular environment from oxidative damage by reducing the formation of hydrogen peroxide in damaged tissue (Melchiorri et al., 1997).

Another indicator of oxidative tissue damage is a decreased SOD activity. SOD is an enzymatic antioxidant molecule that protects cells against ROS damage (Fang et al., 2002; Lohiya et al., 2017). Because of this, SOD activity is reduced in tissues exposed to oxidative stress (Cadirci et al., 2010).

The damage caused by the I/R process in any tissue also affects the cardiovascular system (Neary and Redmond, 1999). The biochemical results showed that the I/R procedure applied to the ovarian tissue also led to oxidative stress in the heart tissue. As shown in Fig 2, the I/R process increased XO activity in the heart. However, XO activity in rats treated with nizatidine did not show a significant increase.

Nizatidine also suppressed the MDA in the heart tissue as it did in the ovarian tissue. The I/R process increased MPO activity in the heart tissue. However, nizatidine decreased the MPO activity to the same level as the CG group. The I/R process decreased the tGSH level in the heart tissue. However, the administration of nizatidine increased tGSH levels. In addition, nizatidine also decreased the activities of endogenous antioxidants such as GPO and SOD in heart tissues in animals where I/R had been applied. Nizatidine ensured the same levels of XO, MDA, MPO, tGSH, GPO and SOD in I/R-applied tissues as in healthy tissue.

Inflammation plays an important role in distant organ tissue damage, as do oxidants. Inflammation can occur in a post-ischaemic period in the affected tissue as well as distant organ tissues. It is suggested that inflammatory damage seen in distant organs is caused by involvement of

![Fig 2: XO, MDA, MPO, GPO, GST and SOD levels in heart tissues of NIR and CG groups compared with the IRG group. \((n = 6)\), * \(p < 0.0001\).](image)
blood circulation of activated PMLs in the ischemic area during reperfusion. Furthermore, MPO secreted from PMNL catalyses the formation of HClO from $\text{H}_2\text{O}_2$ and chloride ions (Carden and Granger, 2000; Howell et al., 2000).

In this study, it was observed that nizatidine inhibits the increase of MDA and MPO production in ovarian and heart tissues. It is thought that this effect of nizatidine on MDA and MPO is through an inhibition of XO. In the literature, it is argued that nizatidine inhibits lipid peroxidation by reducing the synthesis of MDA (Liu et al., 2016).

The inhibitory effect of nizatidine on MPO has been shown to be due to clearing of HClO, which is a strong chlorinating oxidant produced in the MPO–$\text{H}_2\text{O}_2$–Cl system. In addition, H2 receptor antagonists have also been shown to be hydroxyl radical inhibitors produced in the Fe$^{2+}$–$\text{H}_2\text{O}_2$ reaction (van Zyl et al., 1993). It has been shown that H2 receptor antagonists alter the oxidant/antioxidant balance in favour of antioxidants in ovarian tissue (Kurt et al., 2011). In our study, tGSH, GPO and SOD levels in ovarian and heart tissues were preserved at normal levels in the nizatidine group and were not significantly changed.

Fig 3A: In the CG group, the normal primordial follicle (plain arrow), primary follicle (striated arrow), secondary follicle (squared arrow) and corpus luteum (circle arrow) were observed microscopically (HEX100). 3B. Histopathological examination of the IRG group revealed secondary folliculitis (smooth arrow) and dilated and congested blood vessels (striated arrow) (HEX400). 3C. The haemorrhagic area (straight arrow), PMNL infiltration and oedema (striated arrow) were seen in the ovarian tissue of the IRG group (HEX400). 3D. Only dilated and congested blood vessels (arrows) were found in the NIR group (HEX100).

Fig 4A: In the heart tissue of the CG group, a normal epicardium (plain arrow), endocardium (double sided arrow) and myocardium (striated arrow) were observed histopathologically (HEX400). 4B. PMNL infiltration (plain arrow), a haemorrhagic area (circle arrow), oedema (squared arrow) and muscle necrosis (striated arrow) were seen intensely in the heart tissue of the IRG group (HEX400). 4C. Only dilated and congested blood vessels (arrows) were found in the NIR group (HEX200).
Pathological manifestations, such as necrosis, congested blood vessels, haemorrhage, PMNL infiltration and oedema, were seen in ovarian and heart tissues with high oxidant and low antioxidant levels. I/R causes some pathological symptoms in the ovary (Kumbasar et al., 2014).

The results suggest that nizatidine has both antioxidant and anti-inflammatory effects, in accordance with the literature (Lavelli et al., 2000). In the CG group, microscopically normal ovarian structures (primordial follicle, primary follicle, secondary follicle, corpus luteum) were observed (Fig. 3A). However, ovarian tissues in the I/R group showed secondary folliculitis, dilated and congested blood vessels (Fig. 3B) and PMNL infiltration in the haemorrhagic area (Fig. 3C). Only dilated and congested blood vessels were seen in the NIR group (Fig. 3D).

The microscopic examination of the epicardium, endocardium and myocardial structures from the CG group revealed no pathological findings (Fig. 4A). Intensive PMNL infiltration, haemorrhage, oedema and muscle necrosis were observed in the I/R group (Fig. 4B). However, no histopathological findings, other than dilated and congested blood vessels, were detected in the NIR group (Fig. 4C).

In conclusion, the process of I/R in rat ovarian tissue is responsible for oxidative stress in heart tissue. The reperfusion procedure applied to the ovarian tissue can cause an oxidative stress in not only the ovaries but also in heart tissue. Nizatidine reduced oxidative stress in both the ovary and heart tissues. This information suggests that nizatidine may be useful in the treatment of ovarian I/R damage and its subsequent cardiac complications.

Conflicts of interest

The authors have no conflicts of interest.

REFERENCES


