



Protective effects of intraperitoneal administration of nimodipine on ischemia–reperfusion injury in ovaries: Histological and biochemical assessments in a rat model



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ABSTRACT

Purpose: Ovarian torsion must be diagnosed and treated as much early as possible. The aim of the present study was to investigate effects of intraperitoneal administration of nimodipine on ischemia–reperfusion injury in ovaries. **Methods:** Thirty healthy male Wistar rats weighing approximately 250 g were randomized into six experimental groups ($n = 5$): Group Sham: The rats underwent only laparotomy. Group I: A 3-h ischemia only. Group I/R: A 3-h ischemia and a 3-h reperfusion. Group I/Nimodipine: A 3-h ischemia only and 1 mg/kg intraperitoneal administration of nimodipine 2.5 h after induction of ischemia. Group I/R/Nimodipine: A 3-h ischemia, a 3-h reperfusion and 1 mg/kg intraperitoneal administration of nimodipine 2.5 h after induction of ischemia.

Results: Nimodipine treated animals showed significantly ameliorated development of ischemia and reperfusion tissue injury compared to those of other groups ($P < 0.05$). The significant higher values of SOD, tGSH, GPO, GSHRd and GST were observed in I/R/Nimodipine animals compared to those of other groups ($P < 0.05$). The damage indicators (NOS, MDA, MPO and DNA damage level) were significantly lower in I/R/Nimodipine animal compared to those of other groups ($P < 0.05$).

Conclusions: Intraperitoneal administration of nimodipine could be helpful in minimizing ischemia–reperfusion injury in ovarian tissue exposed to ischemia.

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There are various conditions like long mesovarium and adnexal venous congestion that could result in torsion of ovary and subsequently obstruction of the ovarian vessels. This causes a life-threatening reduction in tissue blood flow and permanent tissue damage [1]. Therefore, ovarian torsion must be diagnosed and treated as much early as possible to preserve ovarian functions and prevent future infertility [2]. Upon detection of ovarian torsion, detorsion of the twisted adnexa and evaluation the tissue reperfusion are proposed to prevent future infertility even in case of cyanotic tissues [2,3]. This ovarian torsion–detorsion process is named as ischemia–reperfusion injury [4].

Reperfusion of the ischemic tissue leads to much more serious damage to the tissue than the damage caused by ischemia [5]. Reperfusion-related damage in the cell is created by many factors, mostly including oxygen-derived free radicals, which are rapidly generated in the tissue as a result of reperfusion [6]. Owing to physiological or pathological alterations, oxidative damage takes place with changes in favor of the oxidation process [7]. Prompt diagnosis to reduce ischemic and

reperfusion injury, and its consequents is still inevitable with this approach. Therefore, studies on preventing reperfusion injury seem very important [8].

A proposed pathogenesis of tissue injury during reperfusion is accumulation of the activated neutrophils that release reactive oxygen species [9]. Lipid peroxidation in the cell is the most deleterious effects of free radicals that end up reduction in the membrane potential and subsequently, cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in serious cell damage through induction of polymerization and cross linking in membrane components [10]. Free oxygen radicals react with DNA and form 8-hydroxyguanine (8-OHGua) that is one of the damage products of DNA [11]. In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals [12].

Various agents, anti-inflammatory and antioxidant free radical scavengers have been reported with promising beneficial effects on prevention of ischemic/reperfusion injuries in tissues [13–15]. In most of these studies the protective agents have been administered orally. In search of

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an alternative method, the authors were encouraged to evaluate intraperitoneal administration of nimodipine in prevention of ischemia reperfusion injury in ovary in rats. Nimodipine, a calcium channel blocker, is a US Food and Drug Administration approved drug used to reduce the morbidity and mortality associated with delayed ischemic deficits in patients with subarachnoid hemorrhage. Nimodipine inhibits calcium ion transfer into these cells and thus inhibits contractions of vascular smooth muscle [16]. The major tissue damage that occurs during ischemia–reperfusion injury is secondary to calcium influx into the cell. Hence, a calcium channel blocker might protect tissues against I–R injury by reducing calcium influx into the cell [17].

The physiologic characteristic of the peritoneal cavity which helps remove toxic metabolites from the body has been successfully exploited to provide peritoneal dialysis in end stage renal disease patients [18]. The same characteristics of the peritoneal membrane also provide a useful portal of entry in the body for several pharmacological agents. One advantage would be that the drug achieves therapeutic efficacy in the region of interest while minimizing the systemic toxicities. Intraperitoneal administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that transperitoneal absorption of the agent is far faster than oral administration [19].

The present study was different from the other studies in the literature for using a calcium channel blocker (nimodipine) on ischemia/reperfusion injury. Aimed to study peritoneal effects of nimodipine on ischemia/reperfusion injury, a study was designed to determine if nimodipine could in fact protect against ischemia/reperfusion induced ovarian damage. The assessments were based on histopathological and biochemical parameters.

1. Materials and methods

1.1. Study design and animals

Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of $23\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups. The present study was designed and modified based on a method described by Oral et al., 2011. Thirty healthy male Wistar rats weighing approximately 250 g were randomized into six experimental groups ($n = 5$): Group Sham: The rats underwent only laparotomy. Group I: A 3-h ischemia only. Group I/R: A 3-h ischemia and a 3-h reperfusion. Group I/Nimodipine: A 3-h ischemia only and 1 mg/kg intraperitoneal administration of nimodipine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) 2.5 h after induction of ischemia. Group I/R/Nimodipine: A 3-h ischemia, a 3-h reperfusion and 1 mg/kg intraperitoneal administration of nimodipine 2.5 h after induction of ischemia.

The right ovaries were transferred to a 10% formaldehyde solution for histopathological assessments and the left ovaries were cleaned of surrounding soft tissues and then stored in a freezer at $-80\text{ }^{\circ}\text{C}$ for biochemical assessments.

1.2. Surgical procedure

Animals were anesthetized by intraperitoneal administration of ketamine–xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain [20]. The ethical Committee of the Urmia University of Medical Sciences approved all the experiments.

A longitudinal midline incision was made in the lower abdomen and the uterine horns and adnexa were exposed. For induction of ischemia, a vascular clamp was applied on vessels of the ovaries in rats. After a 3-h

period of ischemia, both ovaries were surgically dissected out for histopathological and biochemical assessments. For induction of ischemia/reperfusion, both ovaries underwent ischemia the same way and at the end of a 3-h period, the vascular clamps were chosen, removed and a 3-h reperfusion was continued. Then, the ovaries were dissected out for histopathological and biochemical assessments.

1.3. Histopathological assessments

Ovaries were fixed in 10% buffered formalin for 24 h. The tissue samples were then processed and embedded in paraffin. A 5- μm semithin section was paraffin-embedded. The samples were then dewaxed, rehydrated and stained routinely with hematoxylin and eosin. The sections were then observed under a light photomicroscope. For semithin sections, ovaries were fixed in 2.5% buffered glutaraldehyde and postfixed in 2% OsO_4 for 2 h, dehydrated through an ethanol series and embedded in Epon. Semithin transverse sections (5 μm) were next stained with toluidine blue and examined under a light microscope.

1.4. Biochemical assessments

1.4.1. Tissue processing for biochemical assessments of ovary

The tissue samples of ovaries were kept at $-80\text{ }^{\circ}\text{C}$ for 3 days, and then enzyme activities were determined in rat ovary tissues. The ovary tissues were ground with liquid nitrogen in a mortar. One half gram was weighed for each group and then treated with 4.5 mL of an appropriate buffer. This mixture was homogenized on ice with use of an Ultra-Turrax homogenizer (IKA, Werke, Germany) for 15 min. Homogenates were filtered and centrifuged using a refrigerator centrifuge at $4\text{ }^{\circ}\text{C}$. Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature.

1.4.2. Superoxide dismutase (SOD) analysis

Superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye [21]. Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction and expressed as millimoles per minute per milligram of tissue.

1.4.3. Nitric oxide synthase (tNOS) activity

Nitric oxide synthase activity of rat ovaries was measured spectrophotometrically using the oxidation of oxyhemoglobin to methemoglobin by NO as described by others [22]. The absorption difference between 401 and 421 nm was continuously monitored with a dual wave length recording spectrophotometer at $37\text{ }^{\circ}\text{C}$. For the total NOS (tNOS) assay, the incubation medium contained 1.6 mmol/L oxyhemoglobin, 200 mmol/L CaCl_2 , 1 mmol/L MgCl_2 , 100 mmol/L L-arginine, 100 mmol/L of the reduced form of nicotinamide-adenine dinucleotide phosphate, 40 mmol/L potassium phosphate (pH 7.2), 1 mmol/L NG-nitro-L-arginine, and 10% (vol/vol) tissue extract with 50 mmol/L L-valine to inhibit arginase [23].

1.4.4. Malondialdehyde (MDA) analysis

Concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test [24]. The rat ovaries were rinsed with cold saline. The corpus mucosa was scraped, weighed and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added to a solution containing 2-thiobarbiturate (1.5 ml of 8 g/l), acetic acid (1.5 ml of 200 g/l), sodium lauryl sulfate (0.2 ml of 80 g/l), and distilled water (0.3 ml). The mixture was incubated at $98\text{ }^{\circ}\text{C}$ for 1 h. n-butanol:pyridine 5 ml (ratio:15:1) was then added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The standard curve was obtained using 1,1,3,3-tetramethoxypropane.

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