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Original Article

The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats

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ABSTRACT

Objective: Ischemia/reperfusion (I/R) injuries result in damage to endothelial and parenchymal cells. Oxytocin (OXY) stimulates uterine contraction during parturition and myoepithelial cells during suckling. OXY has been used as a protective antioxidant. Kisspeptin plays a key role in the central control of reproductive functions and onset of puberty. Recent studies show that these reproductive hormones have protective potential as antioxidant. The aim of this study is to investigate the potential protective effects of Kisspeptin and OXY as antioxidants on I/R injured ovary and uterus of female rats.

Materials and methods: Rats were separated into five groups. Group 1, is control group; Group 2, rats were subjected to ischemia followed by reperfusion. Group 3, OXY administration 30 min prior to I/R applied rats; Group 4, Kisspeptin administration 30 min prior to I/R applied rats; Group 5, OXY and Kisspeptin administration 30 min prior to I/R. Ovary and uterus were removed for histopathological and biochemical observations. Malondialdehyde, glutathione levels, and superoxide dismutase activities were analyzed in order to observe antioxidant potential of OXY and Kisspeptin. Hematoxylin and Eosin staining was applied for histopathologic scoring.

Results: Stromal and granulosa cells in ovary, endometrial cells in uterus were damaged in I/R group. The cellular damage of ovary and uterus were reduced in OXY and Kisspeptin administered I/R group when compared to only Kisspeptin injected I/R group and I/R group. There is no significant difference between OXY and OXY + Kisspeptin injected I/R groups. MDA levels were decreased in Kisspeptin and/or Oxytocin applied I/R group compared to I/R group. SOD activity and GSH levels were increased in Kisspeptin and/or OXY applied I/R group compared to I/R group.

Conclusions: The present results suggest that exogenous application of oxytocin and kisspeptin can have antioxidant effects on the uterus and ovary.

Introduction

Ovarian torsion with a prevalence of 2.7% is a gynecological emergency. Ovarian torsion causes ischemia in the tissue and the blood flow can be restored by surgical intervention. The diagnosis and the treatment may be delayed because of the non-specific symptoms and clinical findings of this pathological condition. Detorsion, laparoscopy or laparotomy can be performed for twisted adnexa. Detorsion causes ischemia/reperfusion injury while providing circulation to ovary. Reactive oxygen species (ROS) released during reperfusion worsens acute ischemic injury [1].

Overproduction of ROS, such as superoxide anion, nitric oxide (NO), hydrogen peroxide, hydroxyl radical and free radicals causes some pathological states, although normal amounts of them are vital for normal physiology. The cells are protected against ROS damage by various ways such as scavenging enzyme systems including Catalase which converts hydrogen peroxide into hydrogen oxide (water) and superoxide dismutase (SOD). SOD catalyzes the

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partitioning of the superoxide (O^{2-}) radical into either ordinary molecular oxygen (O^2) or hydrogen peroxide (H^2O^2). Superoxide is produced as a by-product of oxygen metabolism. It needs to be regulated; otherwise it causes many types of cell damage. Lipid peroxidation which plays a role in decreased binding of hypothalamic hormones to their receptors causes a decline in antioxidant systems [2].

Oxytocin which is a nonapeptide produced in paraventricular and supraoptical nuclei of hypothalamus have central and peripheral effects. It plays a role in uterine contraction, milk ejection reflex, cardiovascular and hydroelectrolytic regulations and modulation of release of adenohypophyseal hormones as well as behaviors such as maternal, sexual and social. Many tissues such as heart, thymus and adipocytes contain OXY receptors. Recent studies show that OXY has a role in wound healing and modulation of immune and inflammatory processes by acting as acute phase reactants and interleukins. OXY may also activate the growth factors and anti-inflammatory mechanisms. Therefore, it helps the ischemic skin flaps to survive [1].

Kisspeptin (metastin) is a 145 amino acid protein which has a role in controlling reproductive functions and puberty with its G protein coupled membrane receptors (GPR 54). Studies reveal that kisspeptin induces the hypothalamic pituitary gonadal axis and adjusts an antioxidant enzyme expression against oxidative damage [3].

The goal of this study was to evaluate the protective effect of exogenous oxytocin and kisspeptin as antioxidants, together or alone, against ischemia/reperfusion injury in ovary and uterus.

Material-method

Animals

The study was performed on the 24 adult Wistar-Albino rats with body weight of 150–200 g in accordance with institutional guidelines. Animals were placed in a quiet and temperature (22 ± 2 °C) and humidity (60 ± 5%)-controlled room in which a 12/12 h light/dark cycle was maintained. All experiments, scheduled between 09:00 and 17:00 h, were performed in accordance with the guidelines for animal research and were approved by the Yeditepe University Ethical Committee of Animal Care as approved number 434, Istanbul, Turkey.

Experimental procedure

The rats were anesthetized with a combination of 50 mg/kg ketamine hydrochloride and 7 mg/kg xylazine hydrochloride, intraperitoneally. The depth of the anesthesia was kept at a level to preserve spontaneous respiration while providing necessary analgesia. No other venous cannula was inserted and all animals spontaneously breathed the room air during surgical procedures. All animals were anticoagulated with intravenous heparin 10 min before the ischemia to prevent thrombosis in the occluded artery.

The abdominal aorta, just above the bifurcation point, was exposed except control group. In the control group, the abdominal aorta was not occluded. Blood-flow occlusion was confirmed with the visual assessment of color changes of the sole of the foot, by palpation of bifurcation point of abdominal aorta pulse. I/R procedure were done according to our previous data (1). Oxytocin (0.5 μg/kg was dissolved in phosphate-buffered saline (PBS), Sigma–Aldrich Co., St Louis, MO, USA) was injected intraperitoneally (i.p.) 30 min before the ischemia in the I/R + OXY group (n:6), Kisspeptin (0.5 μg/kg was dissolved in phosphate-buffered saline (PBS), Sigma–Aldrich Co., St Louis, MO, USA) was injected intraperitoneally (i.p.) 30 min before the ischemia in the I/R + Kiss group (n:6) whereas the vehicle solution of an equal volume was injected 30 min before the ischemia in the I/R group (n:6). The ovary and uterus was rendered ischemic for 90 min and reperfusion was achieved by releasing the clamp and was confirmed by restoration of the pulsatile blood flow in the aorta, and disappearance of color change of the sole. Animals in I/R group (n:6) were subjected to 90 min of abdominal aorta occlusion followed by 90 min reperfusion. In oxytocin pretreated I/R group (I/R + OXY) (n:6), oxytocin were given i.p. 30 min before ischemia. In kisspeptin pretreated I/R group (I/R + Kiss) (n:6), kisspeptin were given i.p. 30 min before ischemia. In oxytocin and kisspeptin pretreated I/R group (I/R + OXY + Kiss) (n:6), oxytocin and kisspeptin were given i.p. 30 min before ischemia. At the end of the reperfusion period of 90 min, right ovary and uterus tissue samples were taken from all groups to assess histopathology and left ovary and uterus tissue samples were taken for freeze clamp biopsies for biochemical analysis from all groups. All animals survived until the end of the reperfusion period.

Histopathologic examination

Samples from the ovary and uterus were incubated in 10% formalin solution and routinely processed afterwards, embedded in paraffin blocks. 5 μm ovary and uterus tissue sections were stained with hematoxylin and eosin (H&E) for histopathological scoring. Every fifth section for each block was evaluated from all animals for both uterus and ovary by a blind observer. The stained sections were examined by Leica BM500 (German) photomicroscope for light microscopical examination. All sections were scored according to below scoring system.

Scoring of histopathological damage was performed for uterus: vasocongestion, infiltration of inflammatory cells, epithelial desquamation [4]. And for ovary: degeneration of follicles in the cortical area (cellular dispersion and degeneration of follicular cells), vascular congestion, haemorrhaging, oedema, infiltration by inflammatory cells [11]. Each criterion was evaluated as according to normal (0), mild (1), moderate (2), severe (3). Maximum score was 9 for uterus and 15 for ovary. All results were calculated as mean ± SD statistically.

Biochemical examination

Malondialdehyde (MDA) analysis

MDA determination is used for lipid peroxidation. The lipid peroxidation level in the ovary and uterus tissue was measured according to the concentration of thiobarbituric acid reactive substances [21]. Briefly, one volume of the test sample and two volumes of stock reagent (trichloroacetic acid and thiobarbituric acid) were mixed in a centrifuge tube. The solution was heated in boiling water for 20 min. After cooling, the precipitate was removed by centrifugation at 2500 rpm for 5 min, and then absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer. The thiobarbituric acid reactive substance level was expressed as nanomoles per gram tissue.

Total glutathione (GSH) analysis

GSH plays role as a co-substrate in the metabolism of xenobiotics and it is also a co-factor for several metabolic enzymes and is involved in intracellular transport, functions as an antioxidant and radioprotectant and facilitates protein folding and degradation. GSH (μmol/mL) was determined by a spectrophotometric method based on the use of Ellman's reagent [5]. The samples were precipitated by a solution containing metaphosphoric acid, disodium EDTA, and then centrifuged at 3000 rpm for 30 min. The reaction mixture contained 0.25 mL of supernatant, 1 mL of Na_2