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Original Article

Prevention of infertility induced by ovarian ischemia reperfusion injury by benidipine in rats: Biochemical, gene expression, histopathological and immunohistochemical evaluation

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ABSTRACT

Introduction. – Benidipine has been reported to prevent the ischemia/reperfusion (I/R) damage in heart tissue and to suppress oxidant and proinflammatory cytokine production, increased by I/R. However, There was no information about the effects of benidipine on I/R injury in the ovary and the damage of I/R-induced infertility.
Objectives. – The aim of the study was to investigate the effects of benidipine on bilateral ovarian I/R injury and whether or not effective in the treatment of I/R-induced infertility in rats.
Method. – Forty-eight females, albino Wistar rats were randomly divided into 4 groups: IRC group (ovarian I/R group, $n = 12$), IRB-2 group (ovarian I/R + 2 mg/kg benidipine group, $n = 12$), IRB-4 group (ovarian I/R + 4 mg/kg benidipine group, $n = 12$) and HG group (healthy group with sham operation, $n = 12$). In IRB-2 and IRB-4 groups, two hours ischemia and two hours reperfusion was performed following orally benidipine administration. After this I/R procedure, 6 rats from each group performed bilateral overectomy. Ovarian levels of malondialdehyde (MDA) and total glutathione (tGSH), ovarian gene expressions of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) and also apoptosis were evaluated. The other 6 rats from each group were put in together with six male rats in separated cages for 2 months in order to reproduce. During this period, rats which did not become pregnant were accepted as infertile.
Results. – MDA levels, expressions of TNF-α and IL-1β in IRC group were significantly higher than in the SGA group and tGSH was decreased. In total, 4 mg/kg benidipine has better prevented ovaries from the increase of oxidants and proinflammatory cytokines, the decrease of antioxidants than 2 mg/kg benedipine. In the histopathological examination hemorrhage, congestion, follicle degeneration, neutrophil infiltration and necrosis were seen in ovarian tissue of IRC group. Only dilated and congested blood vessels were found in the IRB-2 group. No histopathological finding was encountered in the IRB-4 group. I/R caused infertility in rats. In total, 4 mg/kg benidipine prevented from infertility better than the dose of 2 mg/kg benedipine.
Conclusion. – In total, 4 mg/kg benidipine reduced I/R injury and I/R-related infertility more significantly compared to 2 mg/kg benedipine in rat ovaries.

Introduction

Ovarian ischemia reperfusion (I/R) damage is a pathological condition which develops subsequent to a detorsion procedure applied with the intention to treat a torsioned ovary secondary to various causes. However, detorsion remains beneficial and recommended; as far as, detorsion procedure itself causes a more severe damage compared to the damage caused by the torsion in that tissue [1]. Although reperfusion procedure has been reported to be a method of treatment aimed to provide the normal functions of the ovary and to prevent possible infertility [2], the I/R procedure has been demonstrated to result in infertility in rats [3]. Also, IR-related

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infertility has been reported to result from oxidative stress [4]. Based on these data, it may be thought that antioxidative therapy after detorsion procedure applied to the torsioned ovary may be useful to prevent ovarian dysfunction related infertility. Although there are a large number of researches about the pathogenesis and treatment of the I/R damage in literature, its mechanism remains still unclear. However, it has been documented in many studies that I/R procedure increases proinflammatory cytokines in the tissues [5,6]. Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) emerge in the early period of inflammation and cause oxidative burst of neutrophils and release of free radicals [7,8]. It has been proposed that TNF-α is one of the significant factors in the development of fertility [9]. Besides, proinflammatory cytokines have been demonstrated to be increased, while anti-inflammatory cytokines have been demonstrated to be decreased in rats with experimentally induced infertility [10]. Increase in TNF-α has been suggested to be associated with increased intracellular calcium [11]. Increased intracellular calcium ion concentration in ischemic tissues has been demonstrated in a large number of studies [12]. Increased intracellular calcium ion concentration causes initiation of pathological events in the cells such as apoptosis [13]. These findings suggest that use of antioxidative and anti-inflammatory drugs and those that inhibit calcium channels and TNF-α and IL-1β prior to and after reperfusion might be beneficial in preventing the I/R damage and its complications. Benidipine, which is to be experimented in this study against the I/R damage and resulting infertility is an L-type calcium channel blocking antihypertensive drug [14,15]. Benidipine has been reported to prevent increase in the oxidative parameters in the heart tissue and decrease the antioxidatives [16] and suppress the oxidative stress increased by I/R [14]. In addition, benidipine has been reported to suppress production of proinflammatory cytokines [17]. This, in turn points out that benidipine can be beneficial in the treatment of ovarian I/R damage and resulting infertility. No information was encountered on the use of benidipine in infertility associated with ovarian I/R damage in the literature. Therefore, the aim of this study was to evaluate whether benidipine was effective or not in the treatment of ovarian I/R damage and this damage related infertility in rats.

Material and method

Animals

Experimental animals were obtained from the Medical Experimental Application and Research Center of the University of Ataturk. A total of 48 female albino Wistar rats weighing between 235–245 grams were randomly selected to be used in the experiment. The animals were kept in normal room temperature (220 °C) and fed until the experiment. Animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the local animal ethics committee of Ataturk University, Erzurum, Turkey (Ethics Committee Number: 111/3, Dated: 26.05.2016).

Chemical materials

Thiopental sodium (IE Ulugay, İstanbul, Turkey), meperidine (Liba Lab., İstanbul, Turkey) and benedipine (Deva, İstanbul, Turkey) were used in the study.

Experimental groups

Rats were randomly divided into 4 groups: IRC group (ovarian I/R group, $n = 12$), IRB-2 group (ovarian I/R + 2 mg/kg benidipine group, $n = 12$), IRB-4 group (ovarian I/R + 4 mg/kg benidipine group, $n = 12$) and HG group (healthy group with sham operation, $n = 12$).

Surgery and pharmacological procedures

Surgical procedures were performed in sterile conditions and in appropriate laboratory environment under intraperitoneal (i.p.) thiopental sodium anesthesia in a dose of 25 mg/kg. Also, for analgesia, 20 mg/kg meperidine was given intraperitonally. Immobile duration on supine position has been accepted as appropriate time for anesthesia that is needed for surgery in rats [18]. This interval is defined as 1,5 and/or 2 minutes in the present study. Prior to anesthesia induction, 2 mg/kg benidipine was administered orally through a catheter to the rats in IRB-2 group and similarly 4 mg/kg benidipine was administered to the rats in IRB-4 group Distilled water was administered in an equal amount through the same route as a solvent in rats in the IRC and HG groups. Ovaries of all rats were visualized through a vertical incision that was 2–2.5 cm in length in the lower abdomen, following the anesthesia induction. Subsequently, ischemia for two hours was created by application of a vascular clip to the inferior parts of the right and left ovaries (the part that connects the ovary to the uterus) in rats in the IRC, IRB-2 and IRB-4 groups. After two hours of ischemia period, the vascular clip was removed and reperfusion was provided for two hours. Subsequent to reperfusion, six rats from each group were sacrificed using high dose anesthesia and their ovaries were removed. Ovarian levels of malondialdehyde (MDA) and total glutathione (tGSH), ovarian gene expressions of IL-1β and TNF-α and also apoptosis were evaluated. Six mature male albino Wistar rats (12–13 weeks) were encountered to each group for the remainder rats to be able to be reproduced. As known, sperms are produced as from the 45th day after birth; however, optimum sperm production does not occur before the 75th day [19]. Therefore, for reproduction 12–13 weeks male rats were chosen. The groups in which female and male groups were together were kept for 2 months in an appropriate laboratory environment. During this period, the rats which became pregnant were taken and kept alone in separated cages. The rats which did not reproduce in these two months period were accepted as infertile.

Biochemical analysis of ovarian tissue

Malondialdehyde (MDA) analysis

The concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thio barbituric acid test [20].

Total glutathione (tGSH) analysis

The amount of GSH in the total homogenate was measured according to the method of Sedlak and Lindsay with some modifications [21]. The sample was weighed and homogenized in 2 mL of 50 mmol/L Tris–HCl buffer containing 20 mmol/L EDTA and 0.2 mmol/L sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid, and the precipitate was removed after centrifugation at 10,000 g for 40 min at 4 °C and the supernatant was used to determine GSH level. A total of 1500 μL of measurement buffer (200 mmol/L Tris–HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 μL supernatant, 100 μL DTNB (10 mmol/L) and 7900 μL methanol were added to a tube and vortexed and incubated for 30 min in 37 °C. 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) was used as an chromogen and it formed a yellow-colored complex with sulfhydry groups. The absorbance was measured at 412 nm using a spectrophotometer (Beckman DU 500, USA). The standard curve was obtained by using reduced glutathione.