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Use of thiamine pyrophosphate to prevent infertility developing in rats undergoing unilateral ovariectomy and with ischemia reperfusion induced in the contralateral ovary

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ABSTRACT

Objective: To investigate whether thiamine pyrophosphate can prevent infertility developing in rats undergoing unilateral ovariectomy and with ischemia reperfusion induced in the contralateral ovary. Biochemical examinations of the ovaries were also performed.
Study design: Rats were divided into two main groups of three subgroups each. An ischemia reperfusion model was established in the first main group, while surgical unilateral ovariectomy was performed in the second. Thiamine pyrophosphate and melatonin were administered to the subgroups. No additional procedure was performed in the control groups. The rats were then left in laboratory environments and their fertility levels were determined. Malondialdehyde, total glutathione and DNA damage products were measured in those rats from which ovarian tissue was collected.
Results: The results showed that thiamine pyrophosphate prevented ischemia/reperfusion injury-related infertility, but melatonin did not provide adequate prevention. However, reproduction in healthy animals receiving melatonin began earlier compared to those receiving thiamine pyrophosphate. Melatonin suppressed oxidative stress caused by ischemia/reperfusion in ovarian tissue significantly better than did thiamine pyrophosphate.
Conclusions: We think that different mechanisms, in addition to antioxidant activity, are involved in the prevention of reperfusion-associated infertility after ischemia.

1. Introduction

Ovarian ischemia is a pathological condition that generally results from ovarian torsion and can lead to blockage of the ovarian artery that feeds the ovaries [1]. Delay in diagnosis and treatment of ischemic ovaries can result in ovariectomy [2]. In female children who have undergone unilateral ovariectomy, torsion in the contralateral ovary can lead to future infertility [3]. In order to protect the ischemic ovaries, reperfusion is established with detorsion [4]. In ischemia, cellular phosphorylation decreases when the blood flow reaching the tissue stops: the consumption of high-energy phosphate such as adenosine, ATP and phosphocreatine increases while production decreases [5]. This leads to the release of energy stores in the cell and to inhibition of the Na^+, K^+-ATPase pump. Inhibition of the ATPase pump stops Na^+ and Ca^{2+} ions leaving the cell and increases intracellular Na^+ and Ca^{2+} ion concentrations [6]. A rise in intracellular Ca^{2+} concentrations leads to pathological events in the cells [7].

As described above, since ATP use continues in the ischemic period although production ceases, AMP, adenosine, inosine and hypoxanthine form from ATP. The most significant characteristic of the ischemic period is that xanthine dehydrogenase (XDH) is converted into xanthine oxidase (XO). In aerobic metabolism, hypoxanthine is metabolized with XDH. Since nicotinamide adenine dinucleotide is used in the metabolism of hypoxanthine with XDH, toxic oxygen radicals do not form [8,9]. Since XO is dominant in ischemic tissue, hypoxanthine is metabolized with XO in ischemic tissue. Molecular oxygen (O_2) is used in the metabolism of hypoxanthine with XO, and toxic oxygen radicals

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are produced as a side-product. Due to insufficiency of O_2 in ischemic tissue, however, the hypoxanthine that accumulates in the absence of reperfusion cannot be converted into xanthine, and toxic oxygen radicals cannot be produced as a side-product [9]. With the provision of oxygen during reperfusion, however, the XO that forms during ischemia leads to the formation of excessive free oxygen radicals as the accumulated hypoxanthine is converted to xanthine, and to a decrease in antioxidant defence mechanisms [10,11]. Free oxygen radicals can cause severe damage to cell membrane lipids, proteins and DNA [12]. Reactive nitrogen products have been described in addition to reactive oxygen products that lead to necrosis or apoptosis during reoxygenation, although the specific molecular mechanisms in which these are involved are as yet unclear [10].

The thiamine pyrophosphate (TPP) we planned to test in reperfusion injury after ischemia is an active metabolite of thiamine, which is known to have antioxidant properties [13]. TPP is a coenzyme of the enzyme carboxylase that plays a role in oxidative decarboxylation in mitochondria [14]. It is also a co-factor in the enzyme transketolase, which plays an important role in the maintenance of cellular redox status, and the pyruvate and 2-oxoglutarate dehydrogenase complex essential for the mitochondrial synthesis of ATP [15,16]. Thiamine and TPP have been reported to possess antioxidant properties [17], but thiamine has been shown not to prevent tissue damage established with ischemia/reperfusion in rats, while TPP has been shown histopathologically to prevent such damage [18]. This shows that antioxidant therapy by itself is insufficient in ischemia/reperfusion injury, and suggests that TPP may be effective in preventing ovarian ischemia/reperfusion-related-infertility.

In order to evaluate this theory, TPP needed to be tested in rats subjected to unilateral ovariectomy and ischemia/reperfusion in the contralateral ovary, and compared with melatonin with its known antioxidant activity. The melatonin we compared with TPP in our study has been shown to inhibit hydroxyl ($HO^{\bullet}$), hydroperoxide (H_2O_2), singlet oxygen ($_1O_2$) and superoxide ($O_2^{\bullet -}$) radicals and non-radical species such as hypochloric acid (HOCl), nitric oxide (NO) and peroxynitrite (ONOO–), and to increase the egen expressions of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase [19,20].

As described above, infertility is seen in cases with a single ovary [3]. The fact that ovarian torsion leads to infertility in subjects with a single ovary, despite treatment, indicates that different mechanisms apart from oxidative stress may be involved in the pathogenesis of ischemia/reperfusion injury. The purpose of this study was therefore to investigate whether or not TPP is effective in the prevention of infertility in rats exposed to unilateral ovariectomy with ischemia/reperfusion injury established in the contralateral ovary, and to evaluate this by comparing TPP with melatonin with its known antioxidant activity.

2. Materials and methods

2.1. Animals

Ninety-six female albino Wistar rats weighing between 205 and 215 g and obtained from the Ataturk University Medical Experimental Practice and Research Center were used. Before the experiment, the animals were housed and fed in groups at normal room temperature (22 °C). All procedures were performed in accordance with ethical guidelines set out by the local ethical committee that were fully compatible with the "NIH Guide for the Care and Use of Laboratory Animals".

2.2. Chemical substances

Of the chemical substances used for the experiments, thiopental sodium was provided by IE Ulagay, Turkey. TPP was obtained from Biopharma, Russia, and melatonin was purchased from Przedsiebiorstwo Farmaceutczne, Poland.

2.3. General procedure

The 5–6-week-old female baby rats were kept for 12 weeks in cages in normal laboratory conditions. They were then randomly divided into six groups; a control group in which ischemia/reperfusion would be established (IRC, $n = 16$), an ischemia/reperfusion + TPP treated group (IRTP, $n = 16$), an ischemia/reperfusion + melatonin treated group (IRM, $n = 16$), a healthy group given a sham operation (SG, $n = 16$), a sham operation + TPP treated group (STP, n-16) and a sham operation + melatonin treated group (SM, $n = 16$).

2.4. Unilateral ovariectomy procedure

The surgical procedures were performed under sterile conditions in an appropriate laboratory environment on rats under anesthesia with 25 mg/kg thiopental sodium administered intraperitoneally (i.p.). Unilateral ovariectomy was performed on all rat groups (IRC, IRTP, IRM, SG, STP and SM) in the experiment. For ovariectomy, a 0.5–1.0-cm incision was made to the lower abdomen over the linea alba (2nd and 5th lumbar vertebrae region). First the left uterus was accessed. This was lifted slightly and the left ovary extracted. The uterus and the cranial part of the blood vessels were ligated using 4-0 absorbable thread. Subsequently, the entire left ovary and a small part of the uterus near the ovary were severed with sterile scissors and removed. The operation was concluded by suturing the incised area (cutaneous muscle). No procedure was performed for 4 weeks on rats subjected to ovariectomy [21].

2.5. Ischemia/reperfusion procedure

Surgical procedures on rats subjected to unilateral ovariectomy were performed under sterile conditions, in an appropriate laboratory environment. Anesthesia was performed with 25 mg/kg i.p. thiopental sodium. Once anesthesia had been established, the rats were kept until the appropriate time for the surgical procedure. This was regarded as when animals were immobile in the supine position. During this period, the ovaries were accessed by a vertical incision 2–2.5 cm in length in the lower abdomen. Three-hour ischemia was subsequently established by the application of an artery clip to the lower part of the right ovary (the region where the ovary joins the uterus) in the rats in the IRC, IRTP and IRM groups. No ischemia was established in the ovaries of the SG, STP or SM groups. At the end of this period, the artery clip was removed and reperfusion established. Following reperfusion, TPP (50 mg/2 ml) at 25 mg/kg was injected in the IRTP and STP groups and melatonin (1 mg/ml) at 2.5 mg/kg, was injected in the IRM and SM groups, i.p., dissolved in 1 ml distilled water once a day over 7 days. The IRC group was given distilled water as solvent by the same route.

At the end of that period, six rats from each group were sacrificed using high-dose anesthesia. The ovaries were extracted and biochemical examinations performed. The remaining rats were kept for 3 months in an appropriate laboratory environment together with male rats in order for them to reproduce. Those rats that did not become pregnant and give birth during that period were regarded as infertile.