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Effects of melatonin and metformin co-administration on testicular ischemia/reperfusion injury in rats

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Summary

Introduction
Torsion of the spermatic cord is a common urologic emergency among infants and adolescents. It requires early diagnosis and surgical intervention to prevent subfertility and infertility.

Objective
The aim of this study was to investigate the effects of melatonin (MEL) and metformin (MET) co-administration on experimental testicular ischemia/reperfusion (I/R) injury in rats.

Material and methods
Fifty male Wistar rats were randomly divided into five experimental groups ($n = 10$), as follows. Group 1 was sham operated. In group 2, 1-hour ischemia was induced by the left testicular artery and vein clipping followed by 7 days of reperfusion. In groups 3 and 4, MEL (3 mg/kg) or MET (100 mg/kg) was administered orally for 7 days via oral gavage after ischemia, and in group 5 both agents were co-administered. At the end of trial, the left testis was removed for histological analysis and oxidative stress measurement. Histological findings in seminiferous tubule were evaluated according to Johnsen's scoring system.

Results
I/R reduced superoxide dismutase (SOD) activities and testicular Johnsen's scores accompanied by an elevation in malondialdehyde (MDA) and myeloperoxidase (MPO) levels ($p < 0.05$). MEL and MET, and their combination restored SOD activity, tissue scores, MDA and MPO levels ($p < 0.05$). There was no significant difference among individual or combined treatment of these parameters ($p > 0.05$).

Discussion
In the present experiment, using a rat model it has been demonstrated that testicular I/R caused a significant increase in testicular injuries. This was in accordance with previous studies that have demonstrated the effect of I/R in testicular tissue. Treatment of MEL and MET had a benefit effect, but, there was no significant difference among individual or combined treatment.

Conclusions
The results of the present study suggest that MEL and MET may be useful for protecting the testes from the I/R injury. However, the combined use of these agents does not further increase the protection from this damage.

Table Histological scores in the experimental groups.

Group	Histological score
Sham	9.95 ± 0.16
Ischemia/reperfusion	2.99 ± 0.12*
Melatonin	8.50 ± 0.52
Metformin	8.30 ± 0.52
Melatonin + metformin	8.67 ± 0.53

* $p < 0.05$ compared with the other groups.

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Introduction

Testicular torsion is a common urologic syndrome mainly caused by torsion of the spermatic cord that affects newborns, children, and adolescents. The primary pathophysiologic event in testicular torsion is ischemia followed by reperfusion; thus, testicular torsion—detorsion is an ischemia/reperfusion (I/R) injury to the testis. This syndrome often leads to male infertility. In the course of testicular I/R, overgeneration of reactive oxygen species (ROS) is a major initiating component of the testicular injury [1]. ROS, such as superoxide anions, hydrogen peroxide, hydroxyl radicals, and peroxynitrite anion, can oxidize cell membrane lipids, proteins, and DNA, leading to cellular dysfunction or death [2]. The neutrophil is one of important sources of ROS generation [3].

Enzymatic antioxidant defense systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) protect tissues from ROS and oxidative damage [1,4]. In recent years, many anti-inflammatory, antioxidants, and free-radical scavengers have been used for the treatment of testicular I/R-induced male infertility. Furthermore, treatment of antioxidants and ROS scavengers such as SOD, catalase, curcumin, allopurinol, *N*-acetylcysteine, resveratrol, and pentoxifylline have been proved by some research to prevent I/R injury in testes [5—8].

Melatonin (MEL, *N*-acetyl-5-methoxytryptamine) is a hormone produced by the pineal gland in a circadian rhythm and it is the most powerful endogenous antioxidant known [9]. Antioxidant effects of MEL can occur by either a direct or an indirect mechanism. MEL itself exerts direct antioxidant effects via scavenging the hydroxyl radical, peroxyl radical, singlet oxygen, peroxynitrite anion, and superoxide anion [9]. Additionally, it acts as an indirect antioxidant by stimulating several antioxidative enzymes, including glucose-6-phosphate dehydrogenase, GPx, and SOD [10].

Metformin (MET) is a molecule of the biguanide family, and has the ability to decrease ROS [11,12]. At the cytoplasmic level, MET is able to lower the activity of mitochondrial complex I, which results in less ROS. Indeed, Zhou et al. [13] have described the activation of the AMP-activated protein kinase (AMPK) by MET. AMPK is a key regulator of cellular energy balance, and activated AMPK switches cells from an anabolic to a catabolic state. MET also has a beneficial effect on the cardiovascular system. The cardioprotective effect of MET has previously been investigated by Charlon et al. [14], who reported that MET treatment in rats for 5 days reduced infarct size by approximately 22%. Wang et al. [15] showed that low doses of MET may attenuate renal I/R injury by increasing the energy supply to the ischemic tissue and reducing the expression of inflammatory cytokines.

In the present study, we aimed to evaluate the effects of MEL and MET co-administration on testicular damage in a rat testicular I/R injury model by assessing histological and biochemical parameters.

Methods

Animals

Fifty healthy adult male Wistar rats, (weighing 250—300 g) were purchased from the Pasteur Institute. They were maintained under constant room temperature of 22 ± 1 °C, relative humidity of $40 \pm 2\%$, on a 12-hour light/dark cycle with commercial food and filtered tap water ad libitum. This study was conducted according to the guidelines of the animal care review board of the Islamic Azad University, Faculty of Veterinary Medicine, adhering to the guide for care and use of laboratory animals; the study was approved by the ethics committee (no. 41-02-11/09).

Experimental groups

The subjects were randomly divided into five experimental groups, each with 10 rats: group 1 (sham-operated group) were subjected to all operative procedures, except vessels occlusion. group 2 (I/R group) were subjected to I/R. The animals in groups 1 and 2 received physiologic saline orally for 7 days via oral gavage. Group 3 (I/R + MEL group) were subjected to I/R. A solution of 3 mg/kg MEL [16] in 0.9% saline solution was administered orally for 7 days via oral gavage after ischemia. Group 4 (I/R + MET group) were subjected to I/R then were received oral administration of 100 mg/kg MET [17] in 0.9% saline solution for 7 days via oral gavage after ischemia. In group 5, a combination of MEL and MET (as above) was given in the same fashion.

Experimental protocol

All surgical procedures were performed under anesthesia by intraperitoneal (i.p.) injection of 60 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. After clipping, disinfecting with antiseptic povidone—iodine solution, and draping, an abdominal incision was made; then the testicular artery and vein of the left testis were occluded with a mini vascular clamp for 1 h; after this process, the clamp was removed and the organ was allowed to reperfusion for 7 days. Sham operations were performed in a similar fashion, except the vessels were not clamped. The rats were euthanized by overdose of pentobarbital injection (300 mg/kg, i.p.) at the end of the reperfusion period. The left testis was harvested, cleared of adhering connective tissue. First, the testicle was divided into two by a sagittal section and one half was fixed in Bouin's solution. The second half of the testicle tissue was stored at −80 °C for the biochemical analysis.

Preparation of testicular tissue homogenates

The sample of testicular tissues was washed three times in cold normal saline solution (0.9%). Then, the tissues were homogenized in ice-cold Tris—HCl buffer solution within a homogenizer for 2 min at $11{,}200 \times g$. The homogenate was centrifuged at $3500 \times g$ (4 °C) for 60 min, and supernatant was obtained. The levels of myeloperoxidase (MPO) were determined in the supernatant, and malondialdehyde (MDA) levels were studied in the homogenate. For a further extraction procedure, the supernatant was extracted in ethanol/chloroform mixture (5/3 v/v). After a second centrifugation at $3500 \times g$ for 20 min, the clear upper layer was taken and used for SOD activity determination [18].

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