Original Article

The Ameliorative Impacts of Berberine on Testicular Ischemic/Reperfusion Injury in Rats: An Experimental Study

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ABSTRACT

Ischemia/reperfusion is one of the emergency cases that frequently occurs in testis. This pathologic event is one of the reasons for infertility in men. Inflammation and oxidative stress induce ischemia/reperfusion injury in testis. Consequently, agents possessing antioxidant activity are applied in the treatment of testicular ischemia/reperfusion. In the present study, the effect of berberine administration in the treatment of testicular ischemia/reperfusion injury is investigated. In this experiment, 24 Wistar rats were randomly divided into four groups (n = 6): Sham (receiving normal saline 0.9%), control (ischemia/reperfusion), treatment I (ischemia/reperfusion receiving 50 mg/kg berberine), and treatment II (ischemia/reperfusion receiving 100 mg/kg berberine). All injections were performed through the intraperitoneal route. Histopathological findings demonstrated that in the Sham group, testis has normal structure and normal spermatogenesis occurs. In the control group, severe hyperemia, coagulative necrosis, and interstitial edema are observed and spermatogenesis has severe damage. In treatment I group moderate interstitial edema, hyperemia, and coagulative necrosis are observed. Besides, spermatogenesis has moderate damage. In treatment II group all damages are mild. This experiment reveals that berberine exerts its protective impact in a dose-dependent manner so that the highest protective impact is observed in the group treated with 100 mg/kg of berberine. With respect to the major role of testicular ischemia in infertility and the results of the present study, berberine can be used as a valuable plant extract in the treatment of testicular ischemia and preventing its harmful impacts.

Introduction

The spermatic cord twisting leads to the development of a surgical emergency known as testicular torsion.1 Hypoxia is the most challenging problem resulted from testicular torsion. During testicular torsion, the blood supply undergoes a remarkable reduction resulting in ischemia.2 It seems that torsion-associated ischemia is related to adverse effects on germ cells.3 In spite of the role of reperfusion...
in the survival of cells, a growing body of evidence demonstrates that reperfusion is responsible for poor prognosis by induction of ischemic/reperfusion (I/R) injury. It has been shown that the harmful effect of reperfusion is due to the enhanced generation of reactive oxygen species (ROS). Furthermore, an increase occurs in the concentrations of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) deteriorating this pathologic event. The elevation in ROS production leads to damages in lipids, proteins and DNA. Besides, ROS induce the intrinsic pathway of apoptosis by stimulation of mitochondrial dysfunction. Hence, using naturally occurring antioxidants is of importance in amelioration of I/R injury.

Berberine (Brb) is a natural alkaloid present in the root, rhizome and stem barks of Berberis vulgaris. This plant-derived natural product has a number of pharmacological activities such as antioxidant, anti-inflammatory, anti-diabetic, hepatoprotective, and cardioprotective. Besides, this compound has demonstrated great potential in treatment of neurological disorders (NDs) such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Brb inhibits the aggregation of amyloid-beta (Aβ) plaques and improves memory deficits. These studies highlight this fact that Brb is capable of being used as an efficient drug in the treatment of pathological conditions. At the present study, we investigate the protective effects of Brb on the adverse effects of testicular I/R injury.

Materials and Methods

Animal Housing and Treatment

Twenty four Wistar rats with the weight of 250-300 g were purchased from histology department of Urmia University. All of the necessary procedures were considered in the conduction of this experiment. The rats were housed in a standard condition with 12:12 h. There were four groups and rats randomly divided into these groups as following: A) Sham, B) I/R receiving normal saline, C) I/R receiving Brb (50 mg/kg), and D) I/R receiving Brb (100 mg/kg). Brb was administered through intraperitoneal route once a day for 14 days.

Induction of Testicular I/R

After the stimulation of anesthesia using xylazine (5 mg/kg) and ketamine (90 mg/kg), the testicular region was shaved and then scrubbed using povidone-iodine solution. In order to expose testis, a vertical paramedian incision was made. The surgery was made on the right testis after incising tunica vaginalis. Next, the right testis was rotated 720° in a clockwise direction to induce ischemia. The torsion position was preserved by fixing testis to the scrotum using 4-0 silk suture for 2 hours. After spending 2 hours, the right testis was de-rotated and re-perfused.

Histopathological Analysis

The testis samples were selected for histopathological analysis. The fixed samples using 10% neutral buffered formalin were dehydrated in graded ethanol. Then, they were cleared in xylol, loaded in paraffin wax and sectioned at about 5-6 μm. The hematoxylin and eosin (H&E) was applied to stain prepared samples.

Results

Photomicrograph of Sham rat testis showing seminiferous tubules lined with series of spermatogenic cells including spermatogonia, primary spermatocytes and round (early) spermatids. Sertoli cells are seen with attached sperms. Tubules are surrounded by basement membrane enclosing myoid cells. The interstitial spaces in-between the tubules contains interstitial cell of Leydig having vesicular nucleus with prominent nucleolus. H and E-stained sections of I/R exposed rats’ testicles revealed the irregular outline of the seminiferous tubules. Many degenerating and reduced germ cells were observed. The basement membrane was thickened and irregular. Interstitial cells of Leydig had scanty cytoplasm with deeply stained or normal vesicular nuclei (Figure 1).

In this study, we evaluated dose-dependent protective impacts of Brb against I/R injury in testis. In order to evaluate dose-dependent activity of Brb, we used 50 and 100 mg/kg of Brb. In group treated with 50 mg/kg of Brb, moderate interstitial edema, coagulative necrosis and hyperemia were observed, while these damages were mild in group treated with 100 mg/kg, showing dose-dependent effect of Brb that enhances with an increase in concentration of Brb (Figure 2).

Discussion

Notably, berberine has demonstrated great potential in protection of kidney against toxins and other harmful agents. In addition to kidney, other organs of body including liver, brain, and testis can be protected against toxic agents due to protective impact of Brb.
In the present study, we investigated the role of Brb in amelioration of testicular I/R injury. It seems that Brb exerts its protective impacts in a dose-dependent manner. The damages are moderate in 50 mg/kg administration, while these damages are mild in group treated with 100 mg/kg. Coagulative necrosis, interstitial edema and hyperemia are observed upon I/R, while administration of 50 and 100 mg/kg of Brb significantly diminishes these damages. This is a basic experiment evaluating protective impacts of Brb against I/R injury, and further studies are needed to approve these results. Furthermore, upcoming studies can focus on using nanoparticles for delivery of Brb to promote its bioavailability, resulting in its improved therapeutic effect against I/R injury.

**Conflict of Interest**

The authors declare no conflict of interest.
References


