Iranian Veterinary Surgery Association

Iranian Journal of Veterinary Surgery

Journal homepage: www.ivsajournals.com

Original Article

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

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ARTICLE INFO

Article History:

Received 21 December 2020
Revised 10 January 2021
Accepted 15 February 2021
Online 15 February 2021

Keywords:

Ischemia/reperfusion
Berberine
Testis
Rat

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

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www.ivsajournals.com© Iranian Journal of Veterinary Surgery, 2021
https://doi.org/10.30500/IVSA.2021.263095.1239

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.[4] It has been shown that the harmful effect of reperfusion is due to the enhanced generation of reactive oxygen species (ROS).[5] 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.[6,7] 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.[8,9] 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*.[10] This plant-derived natural product has a number of pharmacological activities such as antioxidant,[11] anti-inflammatory[12] anti-diabetic,[13] hepatoprotective,[14] and cardioprotective.[15] Besides, this compound has demonstrated great potential in treatment of neurological disorders (NDs) such as Alzheimer's disease (AD) and Parkinson's disease (PD).[16] Brb inhibits the aggregation of amyloid-beta (Aβ) plaques and improves memory deficits.[17,18] 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 [19]. 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.[20-22]

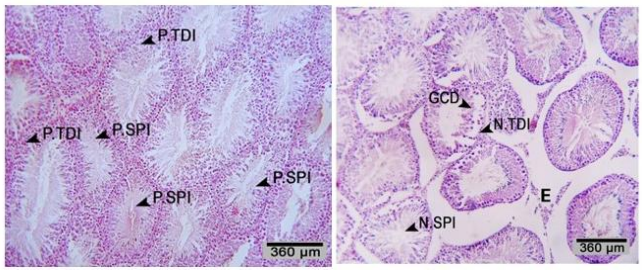

Figure 1: Cross-sectional of testis tissue of experimental groups. (H&E). Sham group receiving saline normal with no changes in testis structure (left figure). Positive tubular differentiation (P.TDI) and Positive spermiogenesis index (P.SPI). The presence of necrosis and hyperemia in rats undergoing I/R injury. Furthermore, number of cells have been reduced significantly in I/R group; germ cell-deficient (GCD). The structure of cells is abnormal (right figure). Negative tubular differentiation (N.TDI) and negative spermiogenesis index (P.SPI).

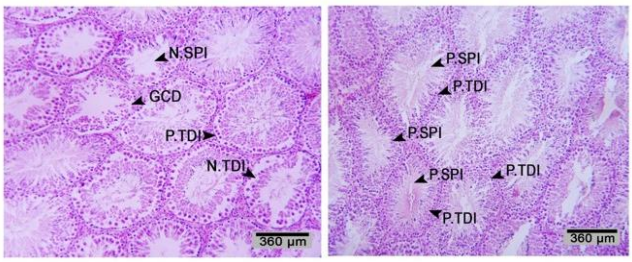

Figure 2: The histopathological profile of tests upon Brb administration (50 and 100 mg/kg). These figures demonstrate that Brb exerts its protective effect in a dose-dependent manner, so that group treated with 100 mg/kg of Brb demonstrates lower damages compared to 50 mg/kg. Left figure, 50 mg/kg Brb; right figure, 100 mg/kg Brb.

A same story occurs in the liver. Brb has high antioxidant capability and increasing evidence reveals that these antioxidant effects are mediated by its modulatory impact on Nrf2 signaling pathway.[23,24] In the liver of rats exposed to the CCl4, Brb improves hepatic injury by upregulation of Nrf2.[25] Besides, Brb administration is beneficial in treatment of patients with non-alcoholic fatty liver disease (NAFLD) since Brb ameliorates non-alcoholic hepatic steatosis.[26]

A newly published article by Razi and colleagues has investigated the protective effect of Brb in reproductive system. It is held that the therapeutic impact of Brb against varicocele-mediated injury in testis is a consequence of its anti-inflammatory and antioxidant effects. The antioxidant activity leads to an increase in the level of total antioxidant capacity and activity of superoxide dismutase, and a decrease in malondialdehyde and nitric oxide. The reduced levels of IL-6 and TNF-α emanate from the anti-inflammatory activity of Brb. These protective effects substantially elevate the sperm viability, motility and DNA integrity resulting in improvement in spermatogenesis.[27] In the present study, same protective effects were observed in testis. Brb exerted dose-dependent protective effects on testis, capable of reducing adverse impacts such as interstitial edema, coagulative necrosis and hyperemia. Moderate damages were observed in 50 mg/kg, while damages were mild in 100 mg/kg.

Increasing evidences are in agreement with capability of Brb in reducing I/R injury in different organs.[28] A recently published article has shown that Brb administration (25 and 50 mg/kg) is beneficial in reducing I/R injury via regulating inflammation severity. In this way, Brb inhibits NF-κB signaling and its nuclear translocation to prevent inflammation upon I/R injury.[29] In alleviation of renal I/R injury, Brb modulates anti- and pro-apoptotic factor. Brb administration is associated with Bcl-2 upregulation (anti-apoptotic protein) and Bax down-regulation (apoptotic protein) to prevent apoptotic cell death in renal cells. Besides, Brb diminishes serum creatinine, blood urea nitrogen and malondialdehyde levels in ameliorating renal I/R injury.[30] It has been reported that Brb administration is of importance in promoting proliferation of cells in attenuating I/R injury. For this purpose, Brb stimulates Pi3K/Akt pathway that subsequently leads to induction of endogenous neuroprotective mechanisms,[31] These studies advocate the fact that using Brb as a protective compound is an ideal strategy in alleviating I/R injury in rats.

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.

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