Archives of Biochemistry and Biophysics 608 (2016) 1–7

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Protection from ischemia by preconditioning, postconditioning, and combined treatment in rabbit testicular ischemia reperfusion injury

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ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form
27 August 2016
Accepted 27 August 2016
Available online 29 August 2016

Keywords:
Testicular torsion
Ischemia reperfusion injury
Ischemic preconditioning
Ischemic postconditioning
Combined treatment
Cell apoptosis

ABSTRACT

This study aimed to investigate the protection of ischemic preconditioning (IPreC), ischemic postconditioning (IPostC) and combined treatment on ischemia reperfusion injury (IRI) of testis. A rabbit testicular ischemia reperfusion (IR) model was established with determining of rabbit serum testosterone, nitric oxide (NO), malondialdehyde (MDA), protein carbonyl (PC), superoxide dismutase (SOD), myeloperoxidase (MPO), glutathione peroxidase (GSH-Px), and tissues pathology. After IR, the NO, MDA, PC, SOD, MPO, and GSH-Px expression significantly increased in torsive testis, and significantly decreased after IPreC, IPostC, and combined treatment in torsive testis when compared to contralateral testis. In torsive testis, testicular tissues was severely damaged with spermatogenic cells disappearing, and were filled with light eosin edema liquid. Cell apoptosis index significantly increased, and the ratio of Bcl-2/Bax significantly decreased. After IPreC, IPostC, and combined treatment, testicular tissues were restored to normal, cell apoptosis index significantly decreased, and the ratio of Bcl-2/Bax significantly increased. It indicates that IPreC, IPostC, and combined treatment has an obvious protective effect on testicular IRI, by decreasing the oxidative stress index and cell apoptosis, provides a significant reference for the treatment of testicular torsion induced infertility, and exhibits a great value in clinical applications.

1. Introduction

Testicular torsion is a common adolescent male urological disease and is often induced by strong contraction of the cremaster when the scrotum is damaged by strenuous exercise or violence [1,2]. It is an acute blood circulation disturbance of testis that manifests as acute scrotal pain with the symptoms of nausea and vomiting [3]. If testicular torsion is not immediately treated, the testis would suffer an irreversible pathological injury, often accompanied by serious infertility [4,5] and spermatogenic barrier [1,2]. Recently, early restoration of testicular torsion by surgery has been a key treatment approach [6]. However, testicular torsion and restoration is a process of ischemia reperfusion injury (IRI), and subsequently, a secondary injury in the testicular tissues develops when ischemic tissues are restored by hemoperfusion, which may lead to an irreversible injury [7–10]. Therefore, how to effectively manipulate and/or reduce the secondary injury of IRI has become a challenge; some treatment approaches have been documented, including ischemic preconditioning (IPreC) and ischemic postconditioning (IPostC) [11].

IPreC or IPostC, which indicate that the organism was subjected to a series of short ischemic episodes and reperfusion just before ischemia, are significant defense phenomena in the organism, and could induce endogenous protective substances to tolerate or adapt to the secondary reperfusion injury [11]. IPreC and IPostC had a significant protective effect on the IRI of brain, liver, heart, muscle, intestine, kidney, retina, and testis, but especially testis [12–14]. Shimizu et al. documented that IPreC, consisting of 3 applications of 5 min of ischemia and 5 min reperfusion before 60 min ischemia, and then 120 min reperfusion, could alleviate the pathological change intesticular tissue and decrease the mRNA expression of malondialdehyde (MDA), 8-Hydroxy-2-deoxyguanosine (8-OHdG), myeloperoxidase (MPO), and heat shock protein 70 (HSP70) [11]. Gozen et al. reported that IPreC, which was characterized by 3 applications of 5-min ischemia, 5-min reperfusion before 180-min

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http://dx.doi.org/10.1016/j.abb.2016.08.020
0003-9861/

ischemia, and 60-min reperfusion, could decrease lipid peroxidation, MPO, total antioxidants, total oxidative status, and the oxidative stress index of testicular tissue, *etc* [15]. Similarly, with 3 applications of 10-min ischemia and 10-min reperfusion before 180-min ischemia, IPreC could reduce the expression of MDA and nitric oxide (NO) [16]. IPostC, 5 applications of 10-s reperfusion and 10-s ischemia, after 60-min ischemia, and followed by 120-min reperfusion, could decrease the mRNA expression of 8-OHdG and HSP70 and also alleviate DNA damage, which was induced by oxidative stress and cell apoptosis [11]. IPostC, consisting of 3 applications of 10-s reperfusion and 10-s ischemia, was the most effective approach, and first validated that it could reduce the expression of MDA and MPO [17]. These data indicated that, regardless of IPreC and IPostC, different intervals and different cycle numbers had a different protective effect on testicular IRI. Significantly, although combined treatment of IPreC and IPostC has been shown to provide significant protection from IRI of heart, lung, brain, and spinal cord, no study has been validated in its protection from testicular IRI thus far [18]. We hypothesized that a combined treatment of IPreC and IPostC may have a significant protective effect on testicular IRI by altering the oxidative stress index and cell apoptosis. As expected, after testicular torsion, the expression level of NO, MDA, PC, SOD, MPO, and GSH-Px could significantly increase in the torsive testis, and that of IPreC, IPostC, and the combined treatment could significantly decrease. Similarly, testicular torsion could severely damage the morphological structure of testicular tissues, and increase the ratio of Bcl-2/Bax mediated cell apoptosis, and IPreC, IPostC, and the combined treatment could alleviate the damage to testicular tissues and decrease the ratio Bcl-2/Bax mediated cell apoptosis. These results indicate that IPreC, IPostC, and combined treatment has an identical protection on testicular IRI by decreasing oxidative stress and cell apoptosis and exhibits a great value in clinical applications.

2. Materials and methods

2.1. Experimental animals

A total of 25 New Zealand white rabbits (male, body weight: 2.88 ± 0.26 kg, age: 28.7 ± 3.29 weeks) were purchased, and raised in the Laboratory Animal Center of the Academy of Military Medical Sciences with the temperature of 25 ± 2 °C and the humidity of 50%–85%, and randomly divided into five groups (n = 5) Group A was the Sham control, and Group B was the Ischemia reperfusion (IR) group, which was subjected to 60 min ischemia +3 days reperfusion. Group C was the IPreC group, which was subjected to 3 × (5-min ischemia + 5-min reperfusion) + 60-min ischemia + 3-day reperfusion. Group D was the IPostC group, which was subjected to 60-min ischemia +3 × (5-s ischemia + 5-s reperfusion) + 3-day reperfusion; Group E was the Combined treatment group, which was subjected to 3 × (5-min ischemia + 5-min reperfusion) + 60-min ischemia +3 × (5-s ischemia + 5-s reperfusion) + 3-day reperfusion. These studies were approved by the Experiment and the Chinese People's Liberation Army General Hospital Ethics Committee.

2.2. Establishment of rabbit testicular ischemia reperfusion model by testicular torsion and restoration

All of the rabbits were fasting the food and the water for 6-h before operation, and anesthetized with 3% pentobarbital sodium (1.0 mL/kg) intravenously in the ear, and fixed on the operation table to establish a rabbit testicular ischemia reperfusion model by testicular torsion and restoration as follows. After sterilization of the scrotum, the right testis was chosen as the torsion side, and a longitudinal incision was made along the root of scrotum, and blunt dissected to expose the spermatic cord. In order to minimize the suffering of the animals, a small operation incision was prepared, and the condition of animals was monitored every 30 min, and once paining of animals, the maintained anesthesia with 3% pentobarbital sodium was performed through intraperitoneal injection during operation. The spermatic cord, which is about 1 cm from the testis, was occluded using a non-invasive endoclip while monitoring via ultrasound. When two-dimensional images stably displayed, the images were regulated to the color flow display based on no signal of CDFI as a criterion. Subsequently, the spermatic cord was exposed for 75 min and 15 s, and did not clamp in group A followed by suturing layer by layer. For group B, the spermatic cord was exposed and clamped in ischemia for 60 min, and immediately reperfusion for 3 days. For group C, after exposing the spermatic cord, ischemic preconditioning was performed with 3 cycles of 5-min ischemia and 5-min reperfusion, and subsequently clamped for 60 min in ischemia, and immediately reperfusion for 3 days. In group D, after 60-min ischemia, the tissue was subsequently postconditioned with 3 cycles of 5-s reperfusion and 5-s ischemia, and then immediately reperfusion for 3 days. In group E, after exposing the spermatic cord, ischemic preconditioning was performed with 3 cycles of 5-min ischemia and 5-min reperfusion, and subsequently clamped for 60 min in ischemia, and also performed ischemic postconditioning with 3 cycles of 5-s reperfusion and 5-s ischemia, and then immediately reperfusion for 3 days. For 3 days reperfusion, when rabbits were revived, they were regularly raised in the Laboratory Animal Center of the Academy of Military Medical Sciences with the temperature of 25 ± 2 °C and the humidity of 50%–85%, and animal behavior was observed every day, and no any unintended deaths of animals.

After operation, the whole blood was collected to detect the change of serum testerone, and the testicular tissues, including right and left testis, were collected to assay the change of NO, MDA, PC, SOD, MPO, and GSH-Px, and also sliced followed HE staining and cell apoptosis staining.

2.3. Rabbit serum testosterone assay by enzyme linked immunosorbent assay (ELISA)

Whole blood (about 3 mL) was collected using a direct heart puncture at precordium after intravenous anesthesia in the ear, and the sample was incubated for 30 min at room temperature followed by centrifugation at 4000 rpm for 10 min to collect the serum. Subsequently, the content of serum testosterone was examined by a rabbit serum testosterone ELISA kit (ZKP-150001, Suzhou Zeke Biotech Co., Ltd, Suzhou, China) according to the manufacturer's instructions. Sucked up 20 μL of serum and 80 μL of sample diluents to 96-wells plate, and gently mixed. Closed the plate with a closure plate membrane, and incubated at 37 °C for 30 min. Washed the plate with 100 μL of washing buffer for 5 times (30 s/time), and then added 100 μL of HRP-Conjugate reagent except for blank wells, and incubated at 37 °C for 30 min. Washed the plate with 100 μL of washing buffer for 5 times (30 s/time), and added 100 μL of diaminobenzidine (DAB) substrate to develop at 37 °C for 15 min, and then added 50 μL of stop solution. The data were recorded at 450 nm using a microplate reader during 15 min, and analyzed by SPSS software (version 21.0, http://spss.en.softonic.com/; Chicago, IL, USA), and histogram analysis was performed using Origin 9.5 software (http://www.originlab.com/).

2.4. Rabbit testicular tissue NO, MDA, PC, SOD, MPO, and GSH-Px levels by ELISA

A total of 20 μg testicular tissues was weighed, rapidly frozen in liquid nitrogen, homogenized with a grinder, and centrifuged at