Life Sciences 92 (2013) 35–41

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Postconditioning with far-infrared irradiation increases heme oxygenase-1 expression and protects against ischemia/reperfusion injury in rat testis

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ARTICLE INFO

Article history:
Received 10 April 2012
Accepted 23 October 2012

Keywords:
Heme oxygenase-1
Far-infrared radiation
Ischemia/reperfusion
Rat testis
Postconditioning

ABSTRACT

Aims: Studies have shown that heme oxygenase-1 (HO-1) has a protective role in the mechanism underlying hypoxic preconditioning. We used a far-infrared radiation (FIR) heater to investigate the postconditioning protective role of HO-1 against ischemia/reperfusion (I/R) injury in rat testis.
Main methods: Forty rats were used. Testis ischemia was mimicked by total obstructive clamping of testis vessels for 1, 2, or 4 h, and concomitant postconditioning with 30 min FIR or heat light during initially 30 min reperfusion. HO-1 expression and apoptosis of testis tissues were examined by immunohistochemistry and in situ terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay, respectively. HO-1 protein level and caspase-3 activity were analyzed by Western blotting.
Key findings: There was less apoptotic activity in rat testis after FIR, as determined by TUNEL assay. Higher HO-1 protein expression was observed by immunohistochemistry and Western blotting ($p<0.01$) in testis cells after FIR postconditioning. In contrast, caspase-3 activity was significantly higher in heat light groups, as compared with FIR groups ($p<0.01$).
Significance: FIR postconditioning attenuated I/R injury in rat testis by inducing HO-1 expression, which might have a protective role in testis apoptosis after I/R injury.

Introduction

Testicular ischemia/reperfusion (I/R) injury due to testicular torsion is a serious urological condition (Nieschlag and Behre, 2000). Delayed treatment of testicular I/R injury can result in irreversible damage, infertility, and loss of the testis (Dokmeci, 2006). I/R injury can induce the overexpression of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the similar mechanism to other organs such as brain, heart and kidneys (Dokmeci, 2006). Thus, it is an important issue how to attenuate the severe effects of testicular I/R injury.

The initial definition of "conditioning" was a brief series of ischemic periods followed by reperfusion applied in the setting of major ischemia. Ischemic preconditioning, the conditioning stimulus was applied before the major (index) ischemic event, and in postconditioning it was applied after the event at the onset of reperfusion (Vinten-Johansen and Shi, 2011). All phases of conditioning have involved both mechanical interventions (direct or ischemic conditioning) and pharmacological conditioning. A review of the literature indicated that the protective effects of remote preconditioning (RP) were shown in heart and other organs, including kidney (Ates et al., 2002), intestine (Gho et al., 1996), and skeletal muscle (Addison et al., 2003). Postconditioning was another strategy that can modify RP-induced adverse incidents by moving the preconditioning "stimulus" to the beginning of reperfusion (Vinten-Johansen et al., 2005). Recently, Shimizu et al. have showed that ischemic preconditioning and postconditioning significantly decreased the testicular damage of I/R injury (Shimizu et al., 2009, 2011). So this technique may provide a new strategy to protect organs from I/R injury.

Heme oxygenase belongs to the heat-shock protein family. It was the rate-limiting enzyme for oxidizing heme to biliverdin and carbon monoxide (Choi and Alam, 1996). Biliverdin was further metabolized to bilirubin, which was a strong antioxidant (Stocker et al., 1987). Through guanylyl cyclase, carbon monoxide functions as an intracellular messenger and exerts vasodilatory effects similar to those of nitric oxide (Morita et al., 1995). Heme oxygenase-1 (HO-1) was found to be inducible under stress states, including hypoxia (Lee et al., 1997), exposure to free radicals (Keyse and Tyrrell, 1989), and heat shock (Ewing and Maines, 1991). Induction of HO-1 was associated with cellular adaptive protection (Poss and Tonegawa, 1997). HO-1 was shown to have a protective role in testis injury in models of hypoxic

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0024-3205/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2012.10.019

preconditioning (Tu et al., 2011) and heat shock stress (Ewing and Maines, 1995), especially in Leydig cells.

Far infrared radiation (FIR) therapy used the low energy of light emitted from an artificial radiator and had been used to treat many vascular disorders (Kihara et al., 2004; Inoue and Kabaya, 1989). Lin et al. observed that FIR therapy had a potent anti-inflammatory effect and induced HO-1 production (Lin et al., 2008). FIR irradiation of cultured human endothelial cells induced HO-1 protein, mRNA, and promoter activity that depended on activation of the antioxidant responsive element/NF-E2-related factor-2 complex (Lin et al., 2008).

In this study, we examined whether FIR postconditioning prevented progression of microtesticular tube apoptosis and reperfusion deficits after I/R injury in rat testis.

Materials and methods

General surgical procedures and FIR therapy

Male Wistar rats weighing 200–250 g were used. The rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Mean arterial blood pressure was recorded on a Gould polygraph (Quincy, MA). A heating pad was used to maintain body temperature at 36.5–37.5 °C. The anesthetized rats were randomized to the FIR group or the heat light control group. To avoid environmental interference, the experimental field was surrounded by a wooden barrier (Fig. 1). To ensure that abdominal skin temperature was increased gradually, the top radiator was placed 25 cm above the rats. The TY301 FIR radiator used in this experiment could be adjusted (manually or automatically) to maintain the surface temperature at 38–39 °C. I/R injury of the right testis was induced by total obstructive clamping of testicular vessels for 1, 2, or 4 h, then followed reperfusion by clamping release. During the initially 30-min reperfusion period, rats of the FIR group were exposed to FIR lighting at a distance of 25 cm. Similarly, heat light group were exposed to lamp light at the same distance. All testes were surgically harvested at 24 h after postconditioning and stored at −80 °C until analysis of testis HO-1 expression. All animal experiments and animal care were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by National Academy Press, Washington DC, 1996. All protocols used in this study were approved by the Laboratory Animal Care Committee of the National Taiwan University College of Medicine.

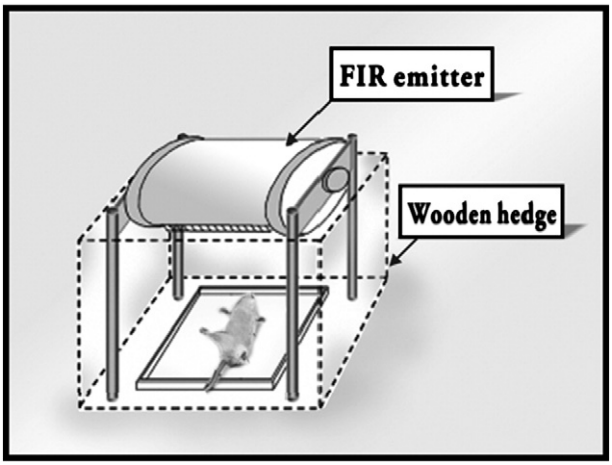

Fig. 1. Experimental setup for far-infrared radiation (FIR) postconditioning of rat testis. The device used was a WS TY101 FIR emitter. The light generated from the electrified ceramic plates ranged between 5 and 12 mm, with a peak at 8.2 mm (based on Yu et al., 2006, Fig. 1a).

Western blot analysis

The harvested tissues were homogenized in ice-cold lysis buffer (20 mM Tris–HCl, 137.5 mM NaCl, 1% Triton X-100 [pH 8.0]) containing protease inhibitors. For detection of HO-1 immunoreactive proteins, the resulting supernatants were collected and subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Using a Semiphor unit (Hoefer Scientific Instruments, San Francisco, CA), proteins were transferred to nitrocellulose membranes and blocked in Tris-buffered saline with 0.05% Tween 20 (TBS-T) buffer containing 5% skim milk. The membranes were then incubated with anti-HO-1 and anti-β-actin (Stressgen Biotechnology) primary antibodies (1:1000 dilution in TBS-T with 5% skim milk). All incubations were performed for 2 h at room temperature, and development occurred in an ECL chemiluminescence system. Computer-assisted densitometry was used to quantify protein signals.

Immunohistochemistry

In brief, sections were deparaffinized in xylene, rehydrated in graded alcohols, and then boiled in 0.01 M citrate buffer (pH 6.0) for 10 min. Hydrogen peroxide (0.3%) was added to block any endogenous peroxidase activity. To block nonspecific binding, sections were incubated with a goat serum blocking solution composed of 10% normal goat serum in phosphate buffer saline (pH 7.4) and 0.05% sodium azide. The sections were then incubated overnight with anti-HO-1 (at 1:100 dilution) antibody at 4 °C. PolyDetector secondary antibody was used to avoid contaminating endogenous biotin or streptavidin (Bio SB, Santa Barbara, CA). After washing, the antigen–antibody complex was applied and sections were stained with diaminobenzidine (Bio SB). Counterstaining was performed lightly with hematoxylin. As the negative control, pre-immune serum was used instead of the first antibody. All control slides yielded negative results. Evaluation of immunostaining was performed by one pathologist, who was unaware of the group assignment and tissue site.

TUNEL staining

The TUNEL assay detects DNA fragments, a sign of cell apoptosis. In situ apoptotic TUNEL assay was performed according to the instruction manual provided with the TUNEL staining kit (Calbiochem, USA). Briefly, 5-μm-thick sections of testis were prepared, deparaffinized, and stained with TUNEL-avidin–biotin-peroxidase complex. Six high-power fields were randomly selected in each section, and apoptotic cells were counted. Hematoxylin and eosin (HE) staining grade of testis injury was classified as previously described (Cosentino et al., 1986). In brief, tissue sections were stained with hematoxylin and eosin (HE staining). The injury grades by HE staining were examined under microscopic 100× magnification, and classified as grade 1: normal testis, grade 2: seminiferous tubules closely packed with focal "giant cells", grade 3: seminiferous tubules less distinct with very disordered arrangement and grade 4: loss of cellular detail.

Caspase-3 activity

Capspase-3 is widely used as an indicator of cell apoptosis, and the present caspase-3 activity assay was performed as previously described (Przyklenk et al., 1993). Briefly, cytosolic proteins were extracted by lysis buffer. Enzymatic reactions were performed in reaction buffer (50 mM Tris [pH 7.5], 5 mM $MgCl_2$, 1 mM EGTA, 0.1% CHAPS, 1 mM DTT) with 25 μg of proteins and the fluorogenic caspase-3 substrate Ac-DEVD-AMC (40 μM). Reactions were incubated at 37 °C for 2 h. Fluorescence was quantified with a spectrofluorometer (Photon Technology International, Lawrenceville, NJ) at an excitation wavelength of 405 nm.