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Preconditioning with Hyperbaric Oxygen Induces Tolerance Against Renal Ischemia-Reperfusion Injury *Via* Increased Expression of Heme Oxygenase-1

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Objective. **Renal ischemia/reperfusion (I/R) injury occurs in both native and transplanted kidneys. Hyperbaric oxygen (HBO) has been shown to prevent I/R injury in different tissues. The aim of this study was to evaluate the effect of HBO on renal I/R injury in rats.**

Materials and methods. **Male Sprague-Dawley rats were randomly assigned to three groups. The sham group ($n = 8$) received right nephrectomy. The I/R ($n = 8$) and HBO + I/R groups ($n = 8$) received 45 min left renal ischemia followed by 24 h of reperfusion after right nephrectomy. The HBO + I/R group ($n = 8$) received 100% oxygen at 2.5 atmosphere absolute (ATA), for 1 h at every 12 h interval for 2 d. Reperfusion was performed 24 h later after the last HBO exposure.**

Results. **In HBO + I/R group, blood urea nitrogen (BUN) and creatinine levels decreased significantly compared with the sham and I/R groups ($P < 0.01$). Activities of superoxide dismutase (SOD) were increased in renal tissue in the HBO + I/R groups. The content of malondialdehyde (MDA) were decreased in the HBO + I/R groups. Kidney samples from HBO + I/R group rats revealed markedly reduced histological damage under histopathological examination. The animals treated with HBO showed significantly elevated heme oxygenase-1 (HO-1) protein and mRNA levels expression compared with I/R group ($P < 0.05$).**

Conclusions. **Hyperbaric oxygen preconditioning (HBO-PC) can protect renal I/R injury against oxidative stress, and the up-regulation of HO-1 expression plays an essential role in HBO induced preconditioning effect.**

Key Words: **ischemia/reperfusion (I/R) injury; hyperbaric oxygen preconditioning (HBO); heme oxygenase-1 (HO-1); reactive oxygen species (ROS); malondialdehyde (MDA); superoxide dismutase (SOD).**

INTRODUCTION

Ischemia reperfusion injury is commonly seen in clinical practice, usually in renal surgeries, kidney transplantation, acute renal arterial occlusion and hypovolemic shock, which can lead to acute renal failure or delayed functional recovery of the transplanted kidney [1–3]. Therefore, to effectively prevent all the acute or chronic kidney diseases resulting from kidney I/R injury remains to be solved urgently. The pathogenesis of ischemia-reperfusion injury involves calcium overload, generation of oxygen free radicals, activation of the apoptosis gene, and the disorder of mitochondria function. Oxygen free radicals are considered to be one of the important factors involved in the pathophysiology of ischemia-reperfusion. Thus, reducing the production of oxygen free radicals in the kidney after reperfusion may alleviate the I/R injury.

Hyperbaric oxygen preconditioning induces tolerance against brain ischemia reperfusion injury by up-regulation of antioxidant enzymes [4], and induces ischemia tolerance in other organs including the spinal cord [5], myocardium [6], and liver [7]. The HO-1 system, the rate-limiting step in the conversion of heme,

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is one of the most critical cytoprotective mechanisms activated during cellular stress [8]. HO-1 protein overexpression protects the kidney from free radical-mediated injury [9]. To induce the overexpression of HO-1 chemically or physically is an effective method for preventing or treating I/R injury. Huang *et al.* [10]. found that HBO pretreatment significantly induced the expression of HO-1 in lung injury. However, the effect of HBO pretreatment on HO-1 expression in the kidneys of rats experiencing renal I/R injury remains unstudied. The aim of the current study was to determine whether HBO preconditioning could induce tolerance against on renal I/R injury, and, if so, to identify the mechanism for the tolerance induction.

MATERIALS AND METHODS

Animals

A total of 24 male Sprague-Dawley rats weighing 225–250 g were used in the present study, which was approved by the Institutional Animal Care and Use Committee. All experiments were performed in accordance with the National Institute of Health guidelines (NIH pub. no. 86-23, revised 1985). Before the experiments, the animals were fed a standard rat chow, drank water *ad libitum*, and were housed in metabolic cages under controlled temperature in 12-h light/dark cycles for at least 1 wk. The rats were randomly assigned to one of three groups. The sham group ($n = 8$) received right nephrectomy. Animals in the I/R ($n = 8$) group received 45 min left renal ischemia followed by 24 h of reperfusion after right nephrectomy. In the HBO + I/R ($n = 8$) group, 1 h at every 12 h interval for pretreatment with hyperbaric oxygen was performed for 2 d. I/R models were performed 24 h later after the last HBO.

Surgical Procedure

All procedures were performed aseptically. The animals were anesthetized with a combination of ketamine hydrochloride (85 mg/kg) and xylazine hydrochloride (15 mg/kg), and placed on a heating pad. Laparotomy was performed through a midline incision. The right renal artery and right ureter were ligated and right nephrectomy was performed. The left renal pedicle was exposed and occluded with a non-traumatic vascular clamp for 45 min (except for the sham group) and left kidneys were reperfused for 24 h in the I/R and HBO + I/R groups. Twenty-four hours after the initiation of renal ischemia, rats were sacrificed under anesthesia, blood was drawn, and the left kidneys were harvested and frozen in liquid nitrogen.

HBO Treatment Procedure

A special animal hyperbaric chamber was used for HBO-PC. The HBO-PC procedure was conducted by consecutive four times of 1 h HBO exposure (2.5 ATA, 100% O_2) at an interval of 12 h. The sham group was not exposed to HBO in order to determine the basal levels of antioxidative enzymes. Before pressurization, 100% medical oxygen was flushed through the chamber for 10 min to displace ambient air. Oxygen pressure was then increased slowly and reached 2.5 ATA in 5 min. The chamber was ventilated during HBO therapy to avoid carbon dioxide accumulation. After 60 min at 2.5 ATA, the chamber was decompressed to normal atmospheric pressure in 5 min. Accumulation of CO_2 was prevented by using a small container with calcium carbonate crystals. Chamber temperature was maintained in the range 22–25°C. No seizures were observed in any animals during all HBO-PC procedures. I/R group rats were placed in the same rodent chamber for 1 h at 12 h interval for 2 d in room air without increased pressure. All HBO-PC administrations were started at the same time in the morning (08:00 AM) to prevent biological rhythm changes.

Analysis of Renal Function

Twenty-four hours after renal ischemia, blood was used to assess renal function by measuring serum blood urea nitrogen (BUN) and creatinine (Cr). The samples were analyzed on a COBAS Mira chemical analyzer (Roche, Basel, Switzerland) with commercial kits from Sigma (St. Louis, MO).

Histopathologic Evaluation

Four-micron-thick sections were cut and stained with hematoxylin and eosin (HE). Samples were blindly analyzed by a pathologist who determined the extent of kidney injury based on a technique outlined by Erdogan *et al.* [11]. Briefly, 24 areas corresponding to the kidney proximal tubules were graded for the degree of renal damage based on each of the following parameters: tubular cell necrosis, cytoplasmic vacuole formation, hemorrhage, and tubular dilatation. Specifically, one whole deep coronal section was examined under the microscope and graded according to the extent of damage, based on the percentage of involvement of the kidney. Higher scores represent more severe damage, with maximum score being 4. [0, normal kidney; 1, minimal damage (0%–5% injury); 2, mild damage (5%–25% injury); 3, moderate damage (25%–75% injury); and 4, severe damage (75%–100% injury)]. The mean score for each parameter was determined and subjected to statistical analysis.

Immunohistochemistry

Kidney tissue sections (4-μm thick) were subjected to immunohistochemical analyses. Sections were dewaxed in xylene, rehydrated through graded ethanol solutions, rinsed in PBS for 5 min, and immersed in 3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. The slides were then rinsed in PBS for 5 min, blocked with 5% BSA at room temperature for 15 min, then incubated with primary monoclonal antibody against HO-1 (1:100 dilution; Boster Biotechnology Ltd. Co., Wuhan, China) at 4°C overnight. Rabbit IgG Isotype was used as the negative control. The slides were then incubated with biotinylated mouse anti-rabbit IgG secondary antibody (Maixin Biotechnology Ltd. Co., Fuzhou, China). Finally, incubated with H_2O_2-DAB for 1 min. Sections were counterstained with hematoxylin. The estimates were performed by a blinded observer on coded sections (3 to 4 sections per kidney and 10 to 12 fields per section). The observer performed light microscopy and scored semiquantitatively the quantity of HO-1 staining in the whole section (0 = none, 1 = weak, 2 = moderate, 3 = strong).

Measurement of Antioxidant Enzyme Activity and Malondialdehyde Content

Kidney MDA and SOD content were performed according to the technical manual of the detection kits (Nanjing Jiancheng Biochemistry Co., Nanjing, China). Kidney tissue was homogenized in phosphate buffer (pH 7.4). After centrifugation at 12,000 *g* for 20 min, the MDA and SOD content in the supernatant were measured using the corresponding kits. MDA content was measured with thiobarbituric acid (TBA) reaction. The method was used to obtain a spectrophotometric measurement of the color produced during the reaction of TBA with MDA at 532 nm; estimated MDA level was expressed as nmol/mg-protein. SOD activity was measured using nitroblue tetrazolium (NBT) reduction assay following the reduction of nitrite by a xanthine-xanthine oxidase system, which is a superoxide anion generator. One unit of SOD is defined as the amount that shows 50% inhibition.