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Original research

Classical and remote post-conditioning effects on ischemia/reperfusion-induced acute oxidant kidney injury

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HIGHLIGHTS

- Protective effect of ischemic conditioning on the IR-induced damage is already shown.
- This study compares the effects of two models of ischemic postconditioning on renal injury.
- Both methods have protective effects on renal function and histology possibly by a reduction in IR-induced oxidative stress.

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ABSTRACT

The present study aimed to analyze and compare the effects of classical and remote ischemic post-conditioning (POC) on rat renal ischemia/reperfusion (IR)-induced acute kidney injury. After right nephrectomy, male rats were randomly assigned into four groups ($n = 8$). In the IR group, 45 min of left renal artery occlusion was induced followed by 24 h of reperfusion. In the classical POC group, after induction of 45 min ischemia, 4 cycles of 10 s of intermittent ischemia and reperfusion were applied to the kidney before complete restoring of renal blood. In the remote POC group, 4 cycles of 5 min ischemia and reperfusion of left femoral artery were applied after 45 min renal ischemia and right at the time of renal reperfusion. There was a reduction in renal function (increase in blood urea and creatinine) in the IR group. Application of both forms of POC prevented the IR-induced reduction in renal function and histology. There were also significant improvements in kidney oxidative stress status in both POC groups demonstrated by a reduction in malondialdehyde (MDA) formation and preservation of antioxidant levels comparing to the IR group. We concluded that both methods of POC have protective effects on renal function and histology possibly by a reduction in IR-induced oxidative stress.

1. Introduction

In recent years, the protective effects of ischemic pre-conditioning (IPC), pre-application of brief ischemia-reperfusion (IR) episodes on the reduction of subsequent ischemia/reperfusion-induced damages have been suggested [1]. In a study conducted in 2004, we demonstrated that IPC preserved vitamin E levels, but it could not markedly improve renal function in the early phase (1–2 h) of reperfusion. We concluded that IPC may be a useful method for antioxidant preservation in organ transplantation [2]. However, IPC is of little practical use as the onset of an IR is usually unpredictable.

More recently, post conditioning (classical POC, post-application of brief IR episodes in the same organ) has been discussed as a more practical approach. In the heart, POC affords persistent infarct size reduction and improves long-term functional recovery in patients with acute myocardial infarction [3]. Similar to preconditioning, POC triggers signaling pathways and activates effectors implicated in other cardioprotective maneuvers. However, the detailed mechanisms underlying these actions are unknown. It was suggested that POC reduces the cardiac reperfusion-induced injury, blunts oxidant mediated damages and attenuates the local inflammatory response to reperfusion [4].

POC can also be applied at a distant organ, termed remote POC, by intermittent ischemia and reperfusion of a distal organ. In male rats, limb remote ischemic POC is shown to protect against focal ischemia after a brain stroke is generated [5]. It was then suggested that this intervention may confer a stronger protection than classical POC. In the heart, it is shown that not only the classical form but also remote application of short time IR provides resistance against lethal IR [6]. Remote conditioning by brief renal ischemia

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and reperfusion reduces acute myocardial ischemia and reperfusion-induced myocardial apoptosis in rabbits [7].

The beneficial outcome of the application of various forms of conditioning, including remote pre- and post-conditioning, suggests involvement of a universal protection in these processes. Remote ischemic conditioning can offer widespread systemic protection to other organs which are susceptible to acute ischemia-reperfusion injury. However, eventhough preconditioning is attractive, it is not easily applicable in clinical surgery.

Reactive oxygen species are produced during re-introduction of oxygen to a previously ischemic tissue. This will result in a reduction in the amount of anti-oxidant factors as well as increasing the pro-oxidant substances. Previous clinical studies demonstrated that various pro- and antioxidative factors not only influence post-operative kidney function in both allogeneic and autologus model of renal ischemia injury, but also were of clinical value for prediction of post-operative kidney function in human [8]. In a study by Dołęgowska et al., they found that, during renal transplantation, significant changes in xanthine metabolizing enzymes occur that are associated with early post-transplant graft function and have potential value to discern between early and delayed graft function [8]. Thus, the objective of the present investigation was to analyze and compare the effects of classical and remote POC on the rat renal ischemia/reperfusion-induced acute oxidant kidney injury.

2. Materials and methods

The animal protocols followed in this study were approved by the Animal Ethics Committee of Tehran University of Medical Sciences. After right nephrectomy, male Sprague–Dawley rats weighing 250–300 g, were randomly assigned into four groups ($n = 8$) as follows:

1 Sham operated for IR
2 IR
3 IR-classical POC
4 IR-remote POC

Rats were anaesthetized by intra-peritoneal injection of sodium pentobarbital (60 mg/kg Sigma–Aldrich, Steinheim, Germany) and then placed on a warming pad. Body temperature was maintained at 37 ± 1C. Tail vein was cannulated (using Venflon 22GA, 0.98IN, ID 0.8 mm, Helsingborg, Sweden) and 0.9% normal saline was infused to maintain euvolemia. Systolic blood pressure was measured by the tail-cuff connected to a pressure transducer (MLT 0380, ADInstruments, Castle Hill, Australia).

In the IR group, with the use of non-traumatic micro-vascular clip (Biemerclip, Aesculap, Germany) 45 min of left renal artery occlusion was induced followed by 24 h of reperfusion. In the sham group, all of the above surgical procedures were applied except that IR was not induced. In the classical POC group, after induction of 45 min ischemia, 4 cycles of 10 s of intermittent ischemia and reperfusion were applied to the kidney before complete restoration of blood to the kidney. In the remote POC group, 4 cycles of 5 min intermittent ischemia and reperfusion of left femoral artery were applied after ischemia, at the same time of blood restoration to the kidney. At the end of the experiments, serum and renal tissue samples were collected for renal functional and histological evaluation and assessment of oxidative stress status.

Blood samples were centrifuged at 4000 g for 10 min at 4 °C, and serum was collected for chemical analysis. Kidney tissues were fixed in formalin (10% phosphate-buffered, pH = 7.4) for histological evaluations. A part of the renal tissues were washed in cold phosphate-buffered saline and the rest were snap-frozen in liquid nitrogen. The samples were stored at −70 °C until further study.

Biochemical assay: Blood urea nitrogen (BUN) and creatinine (Cr) were used as renal functional indices. The plasma samples were taken at the end of 24 h reperfusion and were measured by commercially available kits.

2.1. Measurement of renal oxidative stress markers

The tissue MDA level was determined by the method of Esterbauer and Cheeseman [9] based on its reaction with thiobarbituric acid at 90–100 ° C and measurement of the absorbance at 532 nm. MDA reacts with thiobarbituric acid (TBA) and produces a pink pigment which has a maximum absorption at 532 nm. The value of each sample was obtained from the standard curve and was expressed as μmol/g tissue. Superoxide dismutase (SOD) activity was measured according to the Paoletti et al. method [10]. In this assay, superoxide anion is generated from molecular oxygen in the presence of Ethylenediaminetetraacetic acid (EDTA), manganese (II) chloride, and mercaptoethanol. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidation is linked to the availability of superoxide anions in the medium. Catalase activity was measured in renal homogenate fractions by spectrophotometric analysis (at 240 nm) of the rate of hydrogen peroxide decomposition according to the method of Aebi [11].

2.2. Histological analysis

After formalin fixation (10% phosphate-buffered) and dehydration, paraffin-embedded renal sections (4 μm) were stained by hematoxylin and eosin. Tubules were evaluated for the presence of necrosis, cellular degeneration & vacuolization, tubular obstruction and formation of luminal debris & casts.

2.3. Statistical analysis

The results are given as mean ± SEM. Statistical analysis was performed by analysis of variance using a post-hoc Tukey test. The null hypothesis was rejected at the 0.05 level of significance. SPSS 11.0 software (Chicago, IL, USA) was used for data analysis.

3. Results

3.1. Changes in renal function in different groups

Serum levels of creatinine and BUN were significantly increased in the IR group compared with the sham group ($p < 0.05$, Fig 1). These indices were significantly lower in the classical POC group compared with the IR group ($p < 0.05$). There was no significant difference between the classical POC group and the remote POC group in these parameters.

3.2. Changes in renal oxidative stress indices in different groups

Renal MDA levels were increased and renal catalase and SOD activities were decreased in the IR group compared to the sham group ($p < 0.05$, Fig. 2). Induction of classical POC prevented the IR-induced reductions in SOD and catalase activities. No significant differences were seen between classical POC group and remote POC group in these parameters. MDA levels were lower in both POC groups comparing to IR, although not significant.

3.3. Changes in renal histology in different groups

In the kidneys of the sham group, despite the unilateral nephrectomy, there were no detectable changes observed using light microscopy. In the IR group, extensive changes could be seen in