

Postconditioning Does Not Improve Renal Function or Attenuate Tubular Damage in Ischemia/Reperfusion-Induced Acute Kidney Injury in Mice

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Abstract: Postconditioning (PostC), a series of brief ischemia/reperfusion (I/R) cycles at reperfusion onset, is a recently described approach to attenuate I/R injury in the heart and brain. Here, we examined its effect on acute kidney injury (AKI) induced by ischemia/reperfusion (I/R) injury in a mouse model. C57/black mice were subjected to right nephrectomy and 26-min left renal artery occlusion, and then divided into three groups: Group I, mice were only reperfused for 48 hr; Group II, mice received PostC that was initiated by three cycles of 30-s ischemia with a 30-s interval immediately after initial ischemia before reperfusion; Group III, mice were reperfused for 10 min after initial ischemia and then received the same regime of PostC prior to reperfusion. At 48 hr after reperfusion, renal function was assessed by measurement of serum creatinine and blood urea nitrogen (BUN), and tubular damage was evaluated by histology. Our results showed that I/R injury led to increased serum creatinine and BUN levels and tubular damage. However, PostC did not improve renal function, or attenuate pathological damage to tubules. These results suggest that PostC does not provide a protective effect on renal injury due to ischemia in C57/black mice with these two protocols.

Keywords: Postconditioning, ischemia/reperfusion, acute kidney injury, kidney, renal function.

INTRODUCTION

Ischemic acute kidney injury (AKI) is a common clinical problem. Although several decades of research have greatly improved our understanding of the mechanisms underlying AKI, effective drugs for treatment of AKI are still not available. Therefore, it is necessary to actively explore other approaches for this disease. Preconditioning, the application of brief, transient periods of nonlethal ischemia before a subsequent lethal episode of ischemia, has been reported to delay the development of renal damage [1-3]. However, this approach is not readily used in clinical practices since renal ischemia in most of cases is only identified when it has already occurred.

In 2003, Zhao *et al.*, introduced the concept of ischemic postconditioning (PostC), which consists of one or more short cycles of reperfusion followed by one or more short cycles of ischemia, immediately after an ischemic phase and before the permanent reperfusion occurs [4]. They showed that the PostC was as efficient as the preconditioning in reducing heart infarct size in a canine model [4]. In the subsequent studies, different observations have been reported regarding the effect of PostC in hearts. While most of studies showed the beneficial effect of this approach on cardiac injury, some of the studies indicated that PostC does not provide protection against heart tissue damage [5-8]. The reasons for those varying effects of PostC remains unclear, but may be related to the duration of ischemia, the algorithm of PostC, animal species, and the gender of animals [5].

The purpose of this study was to assess the effect of PostC on I/R-induced AKI. During the course of the study, other research groups published their results and suggested that PostC was effective in reducing renal damage in rat and murine models of I/R-induced AKI [1, 9, 10]. In contrast to these studies, we revealed that the PostC did not improve the renal function or attenuate the tubular damage in C57/black mice subjected to I/R injury.

MATERIALS AND METHODS

Experimental Procedures: For this study, a mouse model of right nephrectomy unilateral renal ischemia/reperfusion was used. Male C57/black mice (approx. 25 g) were anesthetized with pentobarbital and placed on a sterile disposable towel over a warming pad. A midline incision was made, and the renal artery and vein were isolated from surrounding tissue. The right kidney was removed and the left renal arteries and veins were then occluded with a non-traumatic vascular clamp (85grams pressure, Roboz Surg Instruments) for 26 min. This ischemia time was applied as our recent studies showed that serum creatinine and BUN levels were significantly elevated at 24 hr and remained at these levels at 48 hr after ischemic injury [11]. At the end of ischemia, the vascular clamp was removed and subjected to the following treatments: 1) reperfusion for 48 hr (I/R group); 2) three cycles of 30 seconds ischemia with a 30 second interval immediately after initial ischemia before 48 hr reperfusion (PostC group); 3) 10 min reflow after initial ischemia and then the same regime of PostC before reperfusion (delayed PostC group). In addition, a sham operation was performed for a group of mice in which the renal pedicles were isolated, but no clamp was applied (Fig. 1). There are 6 mice in each group. At the end of experiments, the kid-

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ney was harvested and prepared for histological analysis as described below.

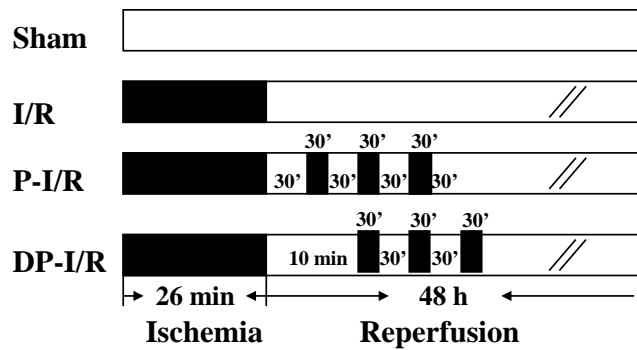


Fig. (1). Experimental algorithm used to determine the effect of PostC on renal function after ischemia and reperfusion. I/R, ischemia/reperfusion; P-I/R, ischemia/reperfusion with PostC; DP-I/R, ischemia/reperfusion with delayed PostC.

Histological Examination: Renal tissues were fixed in 4.5% buffered formalin, dehydrated, and embedded in paraffin. Paraffin sections were deparaffinized and rehydrated before staining for hematoxylin and eosin and examined for tubular injury resulting from I/R injury using light microscopy. To assess tubulointerstitial injuries, three representative sections of each kidney ($n = 6$ for each condition) and 10-12 fields/section were examined and scored using semiquantitative indices. Extent of tubular cast formation, tubular dilatation, and tubular degeneration (loss of brush border, detachment of tubular epithelial cells) were scored according to following criteria by two blinded observers: 0, normal; 1 < 10%; 2, 11 to 25%; 3, 26 to 50%; 4, 51-75%, 5 > 75% of the pertinent area. After scoring, the scores were summed to show the overall tubular damage in the kidney.

Measurement of Creatinine and Blood Urea Nitrogen (BUN): Blood was taken before I/R and at 24 and 48 hr after reperfusion. Serum creatinine and BUN were determined by an automated chemistry analyzer (VITROS 250, Ortho-Clinical Diagnostics, Rochester, NY, USA) at the MUSC Clinical Laboratory.

Statistical Analysis: All values were expressed as means \pm standard deviation, and one-way analysis of variance (ANOVA) was used with Newman-keuls test for post hoc analysis. In all comparisons, a p value less than 0.05 was considered significant.

RESULTS

Effect of PostC on Renal Function: Our recent studies showed that mice subjected to 26 min of renal I/R had significantly increased serum creatinine and BUN levels at 24 hr and remained at these levels at 48 hr after ischemic injury [11]. To examine the effect of PostC on the renal function in ischemia/reperfusion-induced AKI, we measured serum creatinine and BUN levels at 48 hr after PostC or delayed PostC in this model (see experimental procedures and Fig. (1)). The regimes of PostC were selected based on its protective effects on the heart demonstrated in the dog and rabbit [4, 12, 13]. Delayed PostC was used as a negative control since it has been reported that the protective effect of PostC in the heart was usually observed when it is started immediately after initial ischemia [5, 14]. Surprisingly, administra-

tion of PostC did not significantly reduce the levels of serum creatinine and BUN in mice subjected to I/R injury (Fig. 2). These data suggest that PostC is unable to offer a renoprotective effect in this model.

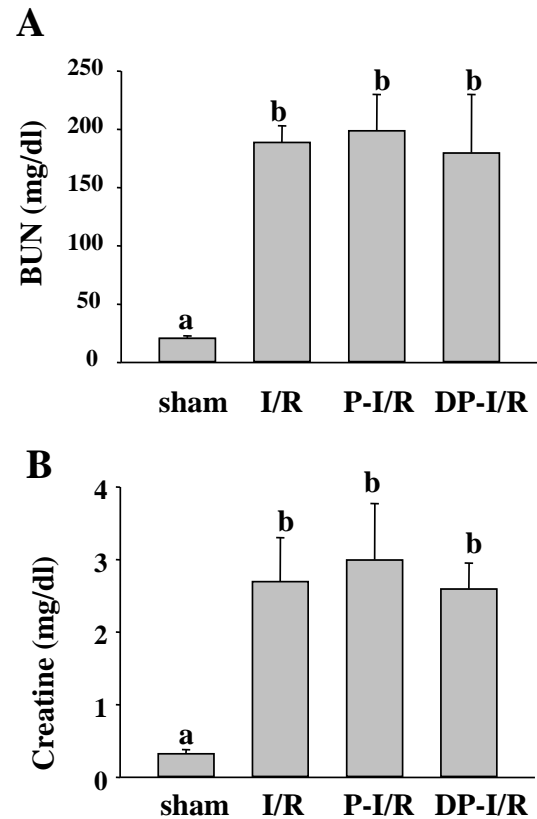


Fig. (2). Effect of PostC on serum creatinine and BUN after ischaemia and reperfusion injury. Mice were subjected to right nephrectomy and 26-min of left kidney occlusion, and then allowed to recovery for 48 hr after treatment with/without PostC as described in the experimental procedures. Data are shown as means \pm SD. Bars with different superscript letters are significantly different from each other ($P < 0.05$).

Effect of PostC on Kidney Morphology After I/R Injury: We also examined the effect of PostC on the kidney morphology. In line with many other observations, ischemic kidney displayed a classic morphology of renal damage, which includes tubular cast formation, tubular dilatation and necrosis, and tubular degeneration (loss of brush border, detachment of tubular epithelial cells). Induction of PostC did not alter these pathological changes (Fig. 3A). Furthermore, scoring of kidney sections for histopathologic damage to the tubules showed an equal degree of tubular damage in all three groups of mice injured by I/R with or without PostC treatment. Therefore, we suggest that PostC did not reduce tissue damage in this model.

DISCUSSION

It has been reported that PostC provides a renoprotective effect on I/R-induced renal injury in rat and mice [1, 9]. In contrast to these studies, we failed to demonstrate a protective effect of PostC on ischemic AKI in a mouse model. Consistent with our studies, the ineffectiveness of PostC has also been reported in myocardial I/R injuries. For example, Schwartz *et al.*, showed that 30-s cycles of repetitive ische-

mia during reperfusion, does not exert a protective effect on pig hearts subjected to lethal ischemia [6]. Dow *et al.*, demonstrated that PostC does not reduce myocardial infarct size in an *in vivo* regional ischemia rodent model [7]. Furthermore, Hale *et al.*, reported that PostC failed to reduce the extent of the anatomic no-reflow and necrotic damage in hearts of rabbits after acute ischemia [15].

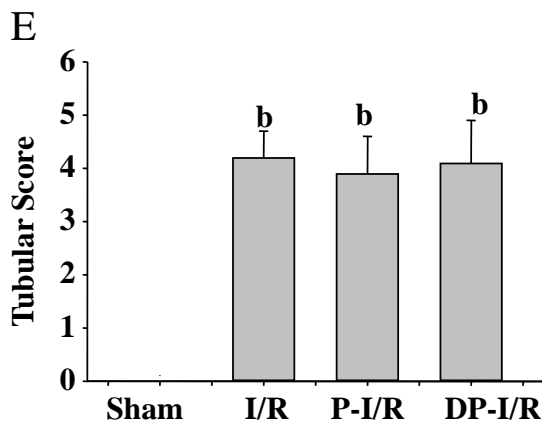
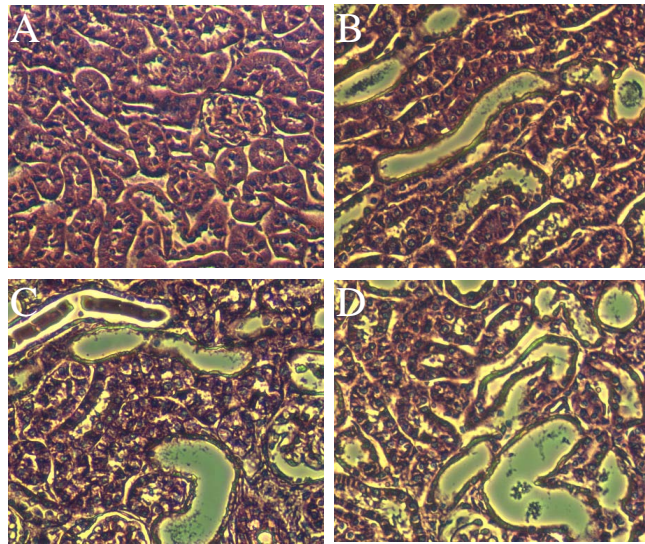


Fig. (3). Effect of PostC on the renal morphology after ischemia and reperfusion. Mice were subjected to right nephrectomy and 26-min of left kidney occlusion, and then allowed to recovery for 48 hr after treatment with/without PostC as described in the experimental procedures. (A) Sham; (B) I/R plus saline; (C) I/R plus PostC; (D) I/R plus delayed PostC; (E) tabulated data from scoring of renal tissue damage. Data are shown as means \pm SD. Bars with different superscript letters are significantly different from each other ($P < 0.05$). Magnification = X 200.

The reasons for the discrepancies in the effectiveness of PostC on renal injury between the present study and previous studies remain unknown. In the heart, it has been reported that different PostC algorithms plays an important role in determining the effect of PostC. For example, Chairi and co-workers tested the effect of PostC on I/R-induced cardiac injury using two protocols of PostC in rabbits. While three cycles of 20-s PostC resulted in a significant cardioprotective effect, three cycles of 10-s PostC ischemia failed to reduce infarct size [16]. Schwarz and Lagranha reported that while

PostC obtained with 3 cycles 30-s ischemia/reperfusion had no beneficial effect in open chest pigs, PostC with intermittent cycles of 1 min ischemia/reperfusion was effective [6]. However, we used the same regime that was used by Szwarc *et al.*, [9] in that three cycle of 30-s of ischemia and reperfusion, was effective in attenuating renal damage in Swiss mice. Therefore, we suggest that PostC algorithms may not be the sole factor that determines the protective effect of this approach on the kidney.

Recently, Mainintveld *et al.*, have reported that the duration of the preceding period of index ischemia is another key factor in regulating the cardiac effects of PostC [17]. In this study, authors observed that cardioprotection occurred with three cycles of 30-s of ischemia and reperfusion following the 45 min and 60 min coronary artery occlusion (CAO), while protection was lost with the longer occlusion duration of 90 and 120 min. Paradoxically, they also observed that with 15-min CAO as well as 30-min CAO, three cycles of 30-s of ischemia and reperfusion aggravated irreversible damage [17]. Therefore, PostC can be beneficial, have no effect or be detrimental, depending on the duration of the index ischemia. In the present study, we used 26 min of ischemia whereas Szwarc *et al.*, [6] used 30-min of ischemia in their study. Although it remains unknown whether our shorter duration of the index ischemia relative to their longer period of ischemia accounts for the inability of PostC to offer a protective effect on renal damage, it will be intriguing to further assess the relationship between the duration of ischemia and the protective effect of PostC in AKI.

In addition, animal gender may also affect the effect of PostC on AKI. Although comparative studies on the effectiveness of PostC have not been conducted in renal ischemia/reperfusion injury in male and female kidneys in the same mouse strain, the effect of PostC on male and female hearts has been examined. In a specifically designed study, Crisostomo *et al.*, showed that while PostC protective effects against ischemia injury was observed in isolated male rat hearts after 25 min ischemia, the protective effect was absent in female rat hearts exposed to the same time of ischemia [18]. In another study, Penna *et al.*, reported that PostC is less protective against infarct in female than in male rat hearts after 30-min of ischemia [5]. In our current study, we did not observe the renal protective effect of PostC in male mice whereas Szwarc *et al.*, reported that PostC offered a renal protective effect using the same protocol in the female mice with ischemia/reperfusion injury [9]. It is too early to offer an explanation for the differential effect of PostC observed in heart and kidney in terms of gender although different organs may have different responses. PostC warrants further studies to elucidate differences in response of male and female kidneys.

In conclusion, the present study showed that PostC protocols with 30-s cycles of reperfusion and reocclusion failed to improve renal function and reduce tubular damage in a mouse model of I/R-induced AKI.

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