Effects of hypertonic saline solution associated to remote ischemic perconditioning in kidney ischemia/reperfusion injury in rats[1]

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Abstract

Purpose: To evaluate the effects of hypertonic saline solution associated to remote ischemic perconditioning in renal ischemia/reperfusion injury in rats.
Methods: Twenty five male rats (Wistar) underwent right nephrectomy and were distributed into five groups: Sham group (S); Ischemia/Reperfusion group (I/R) with 30 minutes of renal ischemia; Remote ischemic perconditioning group (Per) with three cycles of 10 minutes of I/R performed during kidney ischemia; Hypertonic saline solution group (HSS) treated with hypertonic saline solution (4ml/kg); remote ischemic perconditioning + Hypertonic saline solution group (Per+HSS) with both treatments. After reperfusion, blood samples were collected for BUN and creatinine serum levels analyzes. TBARS were evaluated in plasma and renal tissue to assess oxidative stress. Kidney histopathological examination were performed.
Results: Per+HSS group showed a lower degree of renal dysfunction in relation to I/R group, whereas the technique of remote ischemic perconditioning isolated or associated with saline solution significantly reduced oxidative stress and histological damage.
Conclusion: Remote ischemic perconditioning associated or not to saline solution promoted reduction of acute renal injury induced by ischemia/reperfusion.
Key words: Ischemia. Reperfusion. Saline Solution, Hypertonic. Kidney. Rats.

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■ Introduction

Ischemia/reperfusion (I/R) injury culminate in several deleterious effects for different organs. In contrast, reperfusion is the most responsible for main lesions in cells of the ischemic organ[1].

Renal ischemia/reperfusion often results from shock or surgical procedures, such as renal transplantation, resection of tumors and traumas, which act as a major cause of morbidity and mortality in clinical settings[2,3].

Several substances have been tested as alternatives to reduce deleterious effects of ischemia/reperfusion syndrome, such as chlorpromazine, verapamil, allopurinol, octreotide, copaiba oil, vitamins (C, D and E) and cyclosporin A. However, the effect of most them was disappointing[1-4].

According to literature, crystalloid solutions, among them the hypertonic saline solution (NaCl 7.5%), showed a lower rolling of neutrophils to kidney, consequently, promoting a lower inflammatory renal injury[5]. However, studies to understand the current repercussions of intensity and establish the different degrees of ischemia and reperfusion are necessary.

In addition, Schmidt *et al.*[6] reported the remote ischemic perconditioning, which consists of application of remote ischemic conditioning applied to hind limb of the swine during the time of major ischemia, which has also been shown to be effective in preventing reperfusion injury in myocardial ischemia. This protective effect was corroborated by subsequent studies involving myocardial ischemia, and the procedure was expanded to cerebral and renal ischemia[7-9]. Thus, the aim of this study was to evaluate the effects of hypertonic saline solution associated to remote ischemic perconditioning in renal ischemia/reperfusion injury in rats.

■ Methods

All experiments were performed in accordance with Brazilian law for scientific use of animals, and this project was formally approved by the Committee of Ethics in Animal Experimentation, Universidade Estadual do Pará (Protocol 02/2014).

Twenty-five adult male Wistar rats aged 10-12 weeks (250-350g), were obtained from the Evandro Chagas Institute. The rats were maintained with free access to regular food and water, at 22±1°C under a 12-h light/dark cycle.

The animals were randomly distributed into five experimental groups, each with five rats: sham group were submitted to all operative procedures, except vessels occlusion. I/R group were undergoing 30-minutes of ischemia and 72 hours of reperfusion. Remote ischemic perconditioning group (Per) were submitted to three cycles of 10-minutes of I/R with a tourniquet on left hind limb[9]. Hypertonic saline solution (HSS) group received i.v. administration of 4 ml/kg[10] hypertonic saline solution 7.5% after ischemic period. Remote ischemic perconditioning + HSS group (Per+HSS) received both treatments (Figure 1).

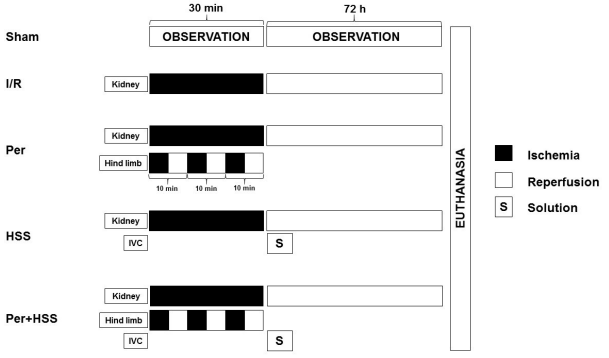

Figure 1 - Procedures between groups. IVC – inferior vena cava.

The rats were fasted overnight before the experiments, but were given free access

to water. They were weighed and anesthetized using an intraperitoneal injection of ketamine hydrochloride 10% and xylazine hydrochloride 2% (70mg/kg and 10mg/kg, respectively). During the operations, additional doses were administered if necessary.

Experimental protocol

All operations were performed under sterile conditions. An abdominal incision was made; then the left renal artery and vein were occluded with a microvascular clamp for 30-minutes; after this process, the clamp was removed and the organ was allowed to reperfusion for 72 hours. Sham operations were performed in a similar fashion, except the vessels were not clamped.

Serum analysis

After two hours of reperfusion, blood samples were collected by inferior vena cava puncture to measure blood urea nitrogen (BUN) and creatinine (Cr)[11] and evaluating oxidative stress by measurement of thiobarbituric acid reactive substances (TBARS) in peripheral blood and left kidney tissue fragment[12].

Histopathology

The left kidney was collected 72h after reperfusion. It was fixed in 10% buffered formalin, embedded in paraffin, sectioned at a thickness of 4 mm according to a standard procedure[13]. The sections were dewaxed and gradually hydrated before being stained with hematoxylin-eosin. The presence of renal tubulointerstitial injury was defined by parameters: tubular necrosis, tubular dilation and/or atrophy, inflammatory cells infiltration and cellular edema. Kidney histopathology scores range from 0 to 4, with the highest levels represent major damage[14]. The rats were euthanized by overdose of ketamine hydrochloride and xylazine hydrochloride injection (triple dose of anesthetic) at the end of the reperfusion period.

Preparation of kidney tissue homogenates

The samples were washed three times in cold normal saline solution (0.9%). Then, the tissues were homogenized in ice-cold Tris-HCl buffer solution within a homogenizer for 2min at 11.200×g. The homogenate was centrifuged at 3500×g (4°C) for 60min, and supernatant was obtained. TBARS levels were studied in the homogenate and for a further extraction procedure, the supernatant was extracted in ethanol/chloroform mixture (5/3 v/v). After a second centrifugation at 3500×g for 20min[15].

Thiobarbituric acid analysis

TBARS levels in kidney tissues and plasma were analyzed by a method based on the reaction with thiobarbituric acid at 95°C[16]. In the thiobarbituric acid test reaction, MDA or MDA-like substances and thiobarbituric acid react together to produce a pink pigment with an absorption maximum of 532 nm.

Statistical analysis

BioEstat 5.4 was used and to confirm normal distribution samples by Kolmogorov-Smirnov test. Data are expressed as means ± SE. ANOVA with *post hoc* Tukey test was applied to renal function and oxidative stress, whereas the histopathological parameters by Kruskal-Wallis with *post hoc* Newman-Keuls. Values of $p<0.05$ were considered statistically significant.

■ Results

Compared to I/R group, all groups showed a reduction in TBARS levels, both in blood sample and renal tissue fragment. However, there was a statistically significant reduction with Per ($p=0.0320$) and Per+HSS

(p=0.023) in accordance to I/R group. In addition, all groups reduced BUN and creatinine levels when compared to I/R; besides, perconditioning (p=0.027) and HSS (p=0.019) group were statistically significant in relation to I/R (Table 1).

Table 1 - Serum levels of renal function and oxidative stress (TBARS) according to groups.

	Renal function (mg/dl)		TBARS (mg/ml)	
Groups	BUN	Creatinine	Plasma	Renal tissue
Sham	90.6 ± 56.8	0.89 ± 0.37	0.15 ± 0.04	0.18 ± 0.01
I/R	207.2 ± 69.7[a]	1.94 ± 0.84[a]	0.58 ± 0.08[a]	0.83 ± 0.04[a]
Per	83.3 ± 2.8[b]	0.70 ± 0.06[b]	0.07 ± 0.20[b]	0.17 ± 0.03[b]
HSS	59.8 ± 1[b]	0.61 ± 0.15[b]	0.12 ± 0.03	0.31 ± 0.02
Per+HSS	72.7 ± 7.5	0.81 ± 0.08	0.10 ± 0.02[b]	0.21 ± 0.02[b]

Data are expressed as means ± SD. Teste ANOVA (Tukey). [a]p<0.05 *versus* grupo Sham. [b]p<0.05 *versus* grupo I/R.

Furthermore, there was statistically significant difference between Perconditioning and Per+HSS group in all microscopic parameters: tubular necrosis (Figure 2), tubular dilation and/or atrophy (Figure 3), inflammatory cells infiltration (Figure 4) and cellular edema (Figure 5), compared to I/R group.

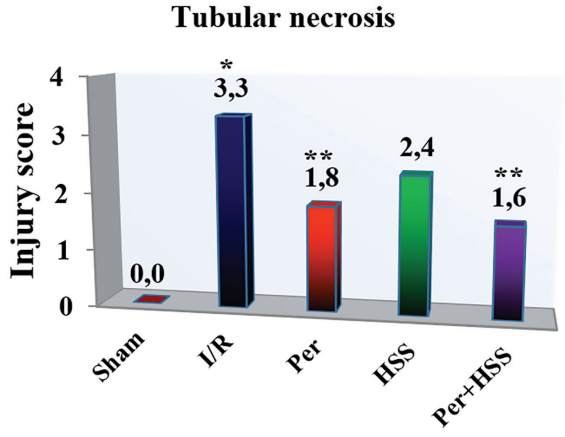

Figure 2 - Tubular necrosis between groups. *p<0.05 *versus* Sham group; **p<0.05 *versus* I/R group. Kruskal-Wallis (Newman-Keuls) test. Source: Protocol research.

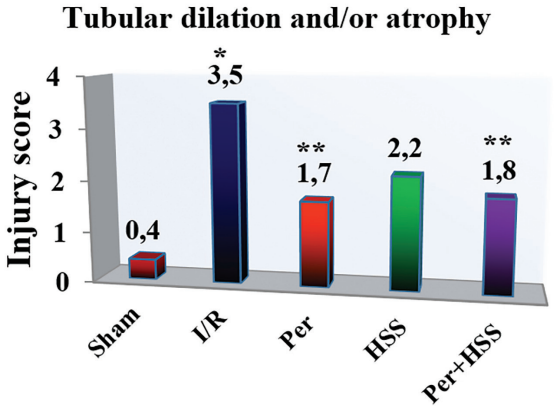

Figure 3 - Evaluation of tubular dilation and/or atrophy between groups. *p<0.05 *versus* Sham group; **p<0.05 *versus* I/R group. Kruskal-Wallis (Newman-Keuls) test. Source: Protocol research.

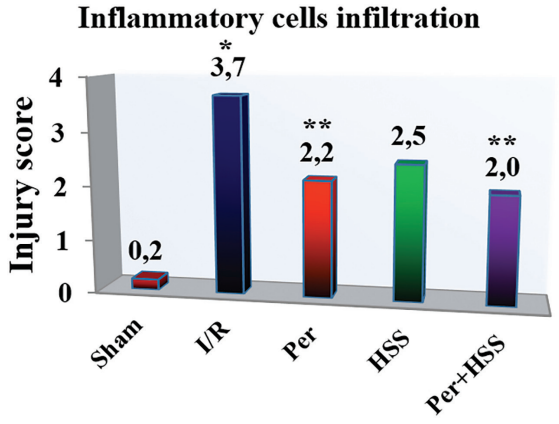

Figure 4 - Presence of inflammatory cells infiltration between groups. *p<0.05 *versus* Sham group; **p<0.05 *versus* I/R group. Kruskal-Wallis (Newman-Keuls) test. Source: Protocol research.

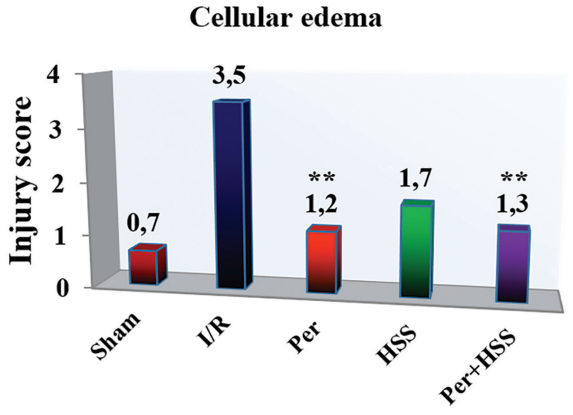

Figure 5 - Cellular edema between groups. *p<0.05 *versus* Sham group; **p<0.05 *versus* I/R group. Kruskal-Wallis (Newman-Keuls) test. Source: Protocol research.

■ Discussion

The present study showed that remote ischemic perconditioning isolated was as effective as Per+HSS in reducing kidney reperfusion injury in I/R model in rats.

The protective effect of perconditioning in renal ischemia was first reported by Kadkhodaei *et al.*[17], who promoted four cycles of 5 min of ischemia and 5 min of reperfusion, clamping left femoral artery before initiating renal reperfusion phenomenon.

Yamaki *et al.*[9] described an alternative model of rat conditioning for renal I/R performed by a tourniquet with an elastic band at the base of left hind limb. The authors performed three cycles of 5 min of ischemia and 5 min of reperfusion, which significantly reduced serum creatinine levels and histological damage.

I/R group showed significantly higher serum values and evidence of renal histological lesions than Sham, demonstrating that experimental model of ischemia was effective promoting oxidative and histological behavior as expected. In addition, Cr and BUN levels in Per and HSS group were significantly lower than in the I/R, which proves the protective effect of these treatments.

HSS group decreased parameters of renal biochemistry. Many studies have described that the use of hypertonic saline solutions promotes intravascular space expansion, which may be associated with an increase in blood volume and consequent elevation of glomerular filtration pressure, stimulating the urinary excretion of Cr and BUN.

A parasympathetic neural response was identified as one of the effector mechanisms of remote ischemic perconditioning, reducing nonperfusion lesion by vasospasm mechanism and contributing to the reestablishment of blood flow in microcirculation of the organ submitted to I/R injury, which may be associated with improvement of glomerular filtration capacity[18,19].

In this context, application of ischemic perconditioning to a remote organ can induce the release of humoral factors - such as adenosine[20], bradykinin[21], and opioids[22], which under local innervation, would trigger activation of neural pathways that promotes kidney protection[19].

TBARS levels were significantly higher in I/R group when compared to Per and Per+HSS,

suggesting that they were capable to maintain an antioxidant defense higher than the I/R. This maintenance of antioxidant defense, promote destruction of oxygen free radicals, reducing lipid peroxidation mediated by these reactive species originated in renal parenchyma[23].

The results indicated that HSS associated or not with remote perconditioning was able to reduce intensity of renal inflammation, protecting from the injury of I/R. Hirano *et al.*[24] showed that HSS reduced rolling of neutrophils in renal cortex. The use of this solution also showed promising results when used in mesenteric I/R models[10,25].

Although the mechanisms of action of saline solutions and remote ischemic perconditioning were not fully explained, it was evidenced that in an acute ischemic injury, perconditioning isolated or associated to saline solutions obtained similar results regarding the analysed parameters, proving the effectiveness of both treatments.

■ Conclusions

The protective effect of remote ischemic perconditioning, when isolated or associated to hypertonic saline solution, was demonstrated in reduction of kidney ischemia/reperfusion injury. However, there was no difference between Per and Per+HSS groups.

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