

Protective effect of remote ischemic per-conditioning in the ischemia and reperfusion-induced renal injury in rats

Efeito protetor do per-condicionamento isquêmico remoto nas lesões da síndrome de isquemia e reperfusão renal em ratos

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A B S T R A C T

Objective: To evaluate the protective effect of remote ischemic per-conditioning in ischemia and reperfusion-induced renal injury. **Methods:** Fifteen rats (*Rattus norvegicus*) were randomized into three groups (n = 5): Group Normality (GN), Control – Ischemia and Reperfusion (GIR) and Group remote ischemic per-conditioning (GPER). With the exception of the GN group, all others underwent renal ischemia for 30 minutes. In group GPER we performed the ischemic remote per-conditioning, consisting of three cycles of ischemia and reperfusion applied every five minutes during the ischemic period, to the left hindlimb of the rats by means of a tourniquet. To quantify the lesions we measured serum levels of creatinine and urea, as well as analyzed renal histopathology. **Results:** The GPER group presented with better levels of urea ($83.74 \pm 14.58\%$) and creatinine ($0.72 \pm 26.14\%$) when compared to GIR group, approaching the GN group. Histopathologically, the lower levels of medullary congestion and hydropic degeneration were found in group GPER. **Conclusion:** The remote ischemic per-conditioning had a significant protective effect on renal ischemia and reperfusion.

Key words: Reperfusion injury. Warm ischemia. Reperfusion. Kidney. Rats.

INTRODUCTION

Ischemia and reperfusion lesions culminate in several deleterious effects for the most different kinds of organs. Paradoxically, reperfusion is largely responsible for the main lesions in the cells of the ischemic organ. Numerous practical situations in urology converge to the problem of renal ischemia and reperfusion, notably, renal transplantation being the most common occurrence of this type of injury in everyday Urology. Acute renal failure, characterized by abrupt elevation of serum urea and creatinine, has in the ischemia and reperfusion lesion its the main etiological factor¹.

As an alternative to reduce the deleterious effects of kidney ischemia and reperfusion syndrome, various substances have been tried, such as chlorpromazine, verapamil, allopurinol, octreotide, copaiba oil, vitamins C, D and E and cyclosporine A. However, the effects of the majority of them have been disappointing^{1,2}.

In addition, other techniques have already been reported, such as ischemic preconditioning (PreC) and postconditioning (PostC)^{3,4}, which consist of a few

interspersed cycles of ischemia and reperfusion before or after the period of main renal ischemia⁵. These procedures have also been applied with excellence in other organs such as intestines, myocardium and liver⁶. Such cycles of ischemia / reperfusion (I/R) can also be applied in an organ or tissue distant from that undergoing the index ischemia, which became known as remote ischemic preconditioning (rPreC) and remote ischemic postconditioning (rPostC), both also successful in minimizing reperfusion injury in various organs^{7,8}.

Schmidt et al. reported the per-ischemic remote technique (rPerC)⁹, which consists in applying remote ischemia through a tourniquet applied to the hind limb of pigs during the main duration of ischemia, which was actually effective in preventing reperfusion injury in ischemia myocardial. This protective effect was corroborated by further studies involving myocardial ischemia, and the procedure was expanded to cerebral ischemia^{10,11}.

The mechanisms involved in the protective effect against ischemic remote conditioning against I/R lesions are complex and are not yet fully understood. They appear to be linked to the activation of the RISK (reperfusion Injury

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Salvage Kinase), through phosphatidylinositol 3-kinase (PI3K)-Akt and the mitogen-activated protein kinase p44/p42, besides ERK 1/2 (Extracellular Signal-Regulated Kinase). It was also demonstrated the involvement of ATP-dependent potassium channels, of adenosine and the SAFE (Survivor Activating Factor Enhancement) pathway^{5,9,10,12}.

Although these Remote ischemic conditioning techniques possess interweaving mechanisms, the rPreC has limited clinical applicability, since it is usually impossible to predict when an organ will suffer from an ischemic event. In this sense, studies about rPerC are more interesting and promising⁹. However, there are no reports of the effect of rPerC in renal ischemia and reperfusion syndrome.

Thus, the present study aims to evaluate the effect of remote ischemic per-conditioning on lesions from kidney ischemia and reperfusion syndrome induced in rats.

METHODS

We used 15 young, male, Wistar rats (*Rattus norvegicus*), weighing 250-300g. The animals were kept in a vivarium of the Laboratory of Experimental Surgery, University of Pará (LCE-UEPA) with temperature, light, humidity and noise control, as well as water and food provided ad libitum. The research rigidly followed the rules of the Council for International Organization of Medical Sciences (CIOMS) ethical code for Animal Experimentations. Moreover, the project was approved by the Ethics Committee on Animal Use of the University of Pará (CEUA-UEPA) (54/11).

The animals were randomized into the following groups (n = 5): Normality Group (GN); Control - Ischemia and Reperfusion Group (GIR); and Ischemic Per-conditioning Group (GPRE).

In all the groups the adopted anesthesia protocol utilized the combination of ketamine and xylazine, administered intraperitoneally at doses of 60mg/kg and 6mg/kg, respectively. The rats of the GN group were used to determine the normal range of the samples. The rest of the groups underwent a median superior laparotomy of 3cm length, followed by a right nephrectomy. Subsequently, we dissected the left renal artery and applied ischemia to the kidney by occlusion of the renal artery with a microsurgical clamp for 30 minutes.

For the animals in GIR group, no additional procedure was adopted. In the GPRE group during the time of index renal ischemia we carried out three interspersed cycles of ischemia and reperfusion, every five minutes, with an elastic band tourniquet applied at the base of left hind limb of the animal, so that the remote ischemic cycles happened concurrently with renal ischemia (Figure 1). On completion of these procedures, there was free reperfusion for 24 hours.

The abdominal wall of the animals was sutured in two planes. After full recovery from anesthesia, the rats were comfortably placed in individual cages and received water and food ad libitum. After 24 hours, they were again subjected to the anesthetic protocol above, then had blood collected from a puncture of the inferior vena cava, as well as the left kidney resected for histopathological analysis. Subsequently, the animals were euthanized by lethal anesthetic doses.

Blood samples were immediately submitted to dose levels of urea and creatinine. The collected left kidney was fixed in 10% formaldehyde, subjected to impregnation with paraffin and stained with hematoxylin and eosin. Multiple sections were analyzed according to the presence of tubular necrosis, hydropic degeneration, medullary congestion and tubular dilation and atrophy, following the protocol developed by Shih et al.¹³, which considers degree '0' as normal kidney; degree '0.5' for small focal areas; degree '1' for commitment of less than 10% of the renal cortex; degree '2' for involvement of 10-25% of the renal cortex; grade '3' for involvement of 25-75% of the renal cortex; and degree '4' for commitment of more than 75% of the renal cortex.

The results were evaluated in different groups, the Kolmogorov-Smirnov (KS) test of Normality to confirm the Gaussian distribution of the data. After that, we carried out the analysis of variance (ANOVA) followed by Tukey test correction. Values were considered significant at $p < 0.05$.

RESULTS

Only the GIR group showed serum urea statistically different from GPRE and GN groups ($p < 0.01$) (Figure 2). The GPRE group ($83.74 \pm 14.58\%$) had lower serum urea when compared to the GIR group ($216.3 \pm 1.17\%$) ($p < 0.01$). However, the comparison of the group subjected to remote



Figure 1 - Remote ischemic per-conditioning applied at the base of the left hind limb by means of an elastic band tourniquet, using a strap.

ischemic per-conditioning with the GN group ($72.16 \pm 9.9\%$) did not show statistically significant results ($p > 0.05$).

As for serum creatinine, (Figure 3) we could observe a statistically significant difference between groups GPRE ($0.72 \pm 26.14\%$) and GN ($0.30 \pm 9.72\%$). The GIR group ($2.06 \pm 12.69\%$) had the highest levels when compared to the other groups. Therefore, all groups were statistically different ($P < 0.01$).

Regarding renal histopathology (Table 1), all animals subjected to procedures of renal ischemia showed medullary congestion and hydropic degeneration (Figure 4) in varying degrees. In GIR group, with the mostly altered histopathology, we observed extensive areas of hydropic degeneration, as well as mild tubular necrosis (Figure 5) and considerable medullary congestion, thus presenting with

a statistically higher degree of injury when compared to the other groups ($p < 0.05$). The group normality, being the standard for renal tissue integrity, was not statistically different when compared to the GPRE.

DISCUSSION

The remote ischemic per-conditioning is the latest technique described as a way to mitigate ischemia-reperfusion⁹. In the literature, there are descriptions of the per-conditioning in myocardial and cerebral ischemia^{10,11}. The present study aimed to evaluate its possible protective effects on renal ischemia. This, in turn, is the genesis of acute renal failure, characterized by elevated levels of urea and creatinine².

The results of analysis of serum levels of urea and creatinine of rats from GIR group (Figures 2 and 3), subjected only to ischemia and reperfusion, returned significantly higher values, proving the efficiency of the model of ischemia used. On the other hand, the serum levels for the group GN showed lower values, since they were not subjected to any procedure.

Serum urea followed expressively creatinine levels in different groups. Except for the comparison between groups GN and GPRE, where values were not statistically different, urea and creatinine levels differed between groups. It is known that creatinine is produced from muscle phosphocreatine, therefore depending on muscle cell metabolism, being little affected by diet. Urea, on its turn, has blood levels changed according to the type of food. Therefore, serum creatinine is more reliable than serum urea², thus characterizing the difference between the normal range and the GPRE group.

The values of urea and creatinine in rats of the GPRE group indicated a protective effect of this technique on induced ischemia and reperfusion renal injury, their levels being very close to the GN group rats. Therefore, such as the ischemic pre and post-conditioning that were initially described in myocardial ischemia and then were introduced to the study of renal ischemia and reperfusion, the per-

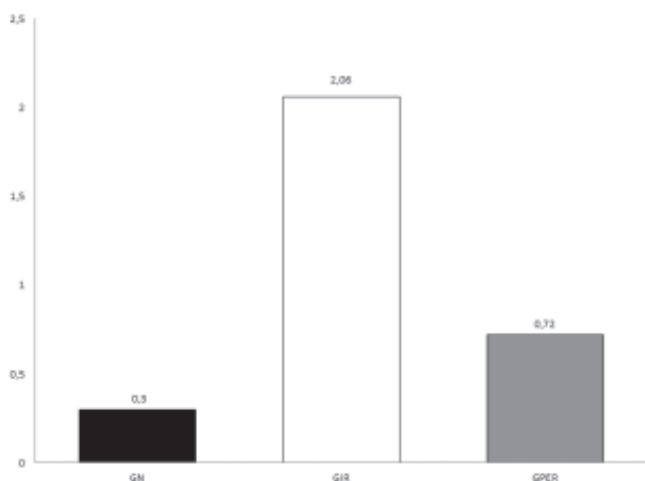


Figure 2 - Mean serum urea (mg / dl) of the experiment groups. $p < 0.01$. * $p < 0.05$ (ANOVA). GN, normality group. GIR, ischemia / reperfusion group. GPER, per-conditioning group.

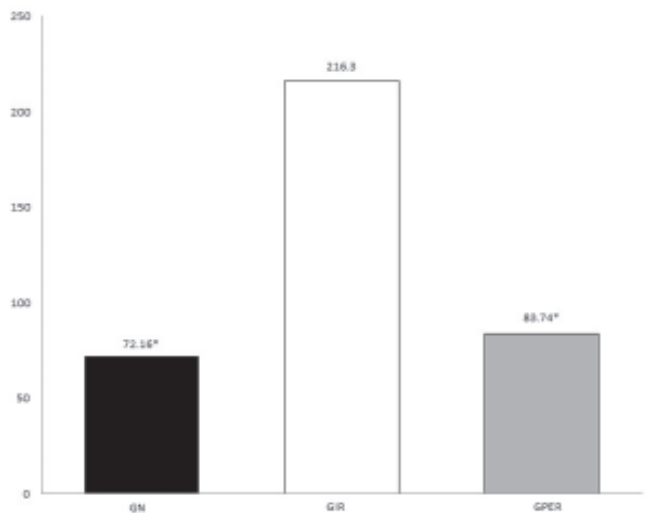


Figure 3 - Mean serum creatinine (mg / dl) of the experiment groups. $p < 0.01$ (ANOVA). GN, normality group. GIR, ischemia / reperfusion group. GPER, per-conditioning group.

Table 1 - Scores of histopathological analysis (Scale Shih et al.)¹³ of every animal, according to the groups of the experiment.

	GN	GIR	GPER
Rat I	0	1.0	0.5
Rat II	0	2.0	0.5
Rat III	0	1.0	0
Rat IV	0	2.0	0
Rat V	0	1.0	0.5
Mean	0	1.4	0.3

$p < 0.05$ (ANOVA). GN, normality group. GIR, ischemia / reperfusion group. GPER, per-conditioning group.

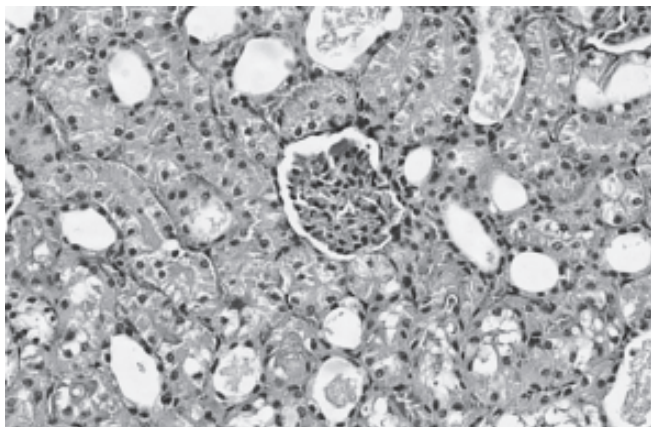


Figure 4 - Section of Kidney from Rat II of the GPER group, where mild tubular congestion and hydropic degeneration are seen, achieving 0.5 grade in the Shih et al. scale.¹³

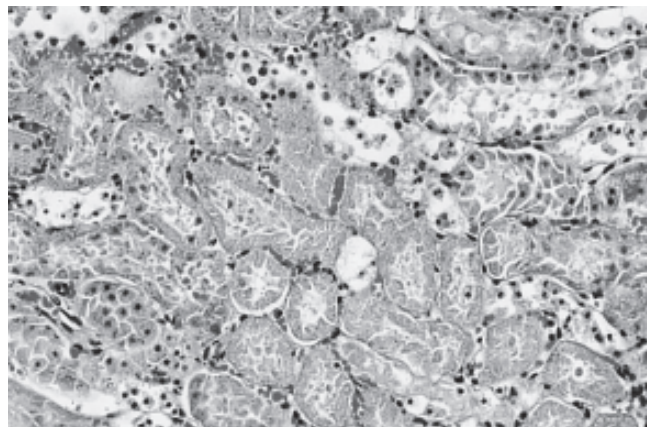


Figure 5 - Section of kidney from Rat II in the Control Group – Ischemia and Reperfusion, where hydropic degeneration, medullary congestion and tubular necrosis are seen. Grade 2 Shih et al. scale.¹³

conditioning follows the same path⁹, having now proven its effectiveness in protecting this type of kidney injury.

Renal histopathology has also been widely used to quantify the damage caused by renal ischemia and reperfusion syndrome¹³. Nevertheless, this study is innovative with regard to the histopathological evaluation of the effects of ischemic per-conditioning on renal ischemia and reperfusion.

For this analysis, we adopted the scale of Shih et al.¹³, which quantifies the extent of ischemia-reperfusion injury in the renal tissue. In this study, a more severe tissue injury was found to be grade 2 in GIR group (Table 3). This can possibly be explained by the reduced

time of 30 minutes of ischemia adopted, as well as the time of free reperfusion, 24 hours, since the inflammatory lesions requires at least 48 hours of observation for installation¹⁴, whereas, when the GN group was compared to the GPER group, it was not possible to identify significant differences, demonstrating the protective effect of this technique.

In conclusion, the remote ischemic per-conditioning showed a significant protective effect, according to serum and histopathological examination, when compared to the control group of ischemia and reperfusion. In addition, the per-conditioning substantially approached normal patterns.

R E S U M O

Objetivo: Avaliar o efeito protetor do per-condicionamento isquêmico remoto nas lesões de isquemia e reperfusão renal induzida.

Métodos: Quinze ratos (*Rattus Novergicus*) foram randomizados em três grupos (n=5): Grupo Normalidade (GN), Grupo Controle - Isquemia e Reperfusão (GIR) e Grupo Per-condicionamento isquêmico remoto (GPER). Com exceção do grupo GN, todos os demais foram submetidos à isquemia renal de 30 minutos. No grupo GPER, foi realizado o per-condicionamento isquêmico remoto, constituído de três ciclos de isquemia e reperfusão de cinco minutos cada aplicado, durante o período de isquemia, no membro posterior esquerdo dos ratos, por meio de torniquete. Para quantificar as lesões, foram dosados os níveis séricos de ureia e creatinina, bem como, analisada a histopatologia renal. **Resultados:** O grupo GPER apresentou-se com melhores níveis de ureia ($83,74 \pm 14,58\%$) e creatinina ($0,72 \pm 26,14\%$) quando comparado ao grupo GIR, se aproximando do grupo GN. Na histopatologia, os menores níveis de degeneração hidrópica e congestão medular foram encontrados no grupo GPER. **Conclusão:** O per-condicionamento isquêmico remoto apresentou importante efeito protetor na lesão de isquemia e reperfusão renal.

Descritores: Traumatismo por reperfusão. Isquemia quente. Reperfusão. Rim. Ratos.

REFERENCES

1. Brito MVH, Moreira RJ, Tavares MLC, Carballo MCS, Carneiro TX, Santos AAS. Efeito do óleo de copaíba nos níveis séricos de ureia e creatinina em ratos submetidos à síndrome de isquemia e reperfusão renal. *Acta cir bras.* 2005;20(3):243-6.
2. Percário S. Prevenção do estresse oxidativo na síndrome de isquemia e reperfusão renal em ratos com suplementação nutricional com antioxidantes. *Rev Nutr.* 2010;23(2):259-67.
3. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation.* 1986;74(5):1124-36.
4. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol.* 2003;285(2):H579-88.
5. Wever KE, Warlé MC, Wagener F, van der Hoorn JW, Masereeuw R, van de Vliet JA, et al. Remote ischaemic preconditioning by brief

- hind limb ischaemia protects against renal ischaemia-reperfusion injury: the role of adenosine. *Nephrol Dial Transplant*. 2011; 26(10):3108-17.
6. Zhang WX, Yin W, Zhang L, Wang LH, Bao L, Tuo HF, et al. Preconditioning and postconditioning reduce hepatic ischemia-reperfusion injury in rats. *Hepatobiliary Pancreat Dis Int*. 2009;8(6):586-90.
 7. Przyklenk K, Darling CE, Dickson EW, Whittaker P. Cardioprotection 'outside the box'—the evolving paradigm of remote preconditioning. *Basic Res Cardiol*. 2003;98(3):149-57.
 8. Kerendi F, Kin H, Halkos ME, Jiang R, Zatta AJ, Zhao ZQ, et al. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Res Cardiol*. 2005;100(5):404-12.
 9. Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, et al. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2007;292(4):H1883-90.
 10. Xin P, Zhu W, Li J, Ma S, Wang L, Liu M, et al. Combined local ischemic postconditioning and remote preconditioning recapitulate cardioprotective effects of local ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2010;298(6):H1819-31.
 11. Hahn CD, Manliot C, Schmidt MR, Nielsen TT, Redington AN. Remote ischemic per-conditioning: a novel therapy for acute stroke? *Stroke*. 2011;42(10):2960-2.
 12. Tamarelle S, Mateus V, Ghaboura N, Jeanneteau J, Croué A, Henrion D, et al. RISK and SAFE signaling pathway interactions in remote limb ischemic perconditioning in combination with local ischemic postconditioning. *Basic Res Cardiol*. 2011;106(6):1329-39.
 13. Shih W, Hines WH, Neilson EG. Effects of cyclosporine A on the development of immune-mediated interstitial nephritis. *Kidney Int*. 1988;33(6):1113-8.
 14. Kin H, Zhao ZQ, Sun HY, Wang NP, Corvera JS, Halkos ME, et al. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc Res*. 2004;62(1):74-85.
 15. Brito MVH, Nigro AJT, Montero EFS, Nascimento JLM, Silva PRF, Siqueira RBP. Viabilidade celular da mucosa do intestino delgado de ratos, após correção de choque hipovolêmico com solução NaCl 7,5%. *Acta cir bras*. 2003;18(4):326-31

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