

# Kidney ischemia and reperfusion syndrome: effect of lidocaine and local postconditioning

## *Síndrome de isquemia e reperfusão renal: efeito da lidocaína e do pós-condicionamento local*

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### ABSTRACT

**Objective:** to evaluate the effects of blocking the regulation of vascular tone on the ischemia and reperfusion syndrome in rats through the use of lidocaine in the postconditioning technique. **Methods:** we randomized 35 rats into seven groups of five animals: Group 1- Control; Group 2- Ischemia and Reperfusion; Group 3- Ischemia, Reperfusion and Saline; Group 4- Ischemic Postconditioning; Group 5- Ischemic Postconditioning and Saline; Group 6- Lidocaine; Group 7- Ischemic Postconditioning and Lidocaine. Except for the control group, all the others were submitted to renal ischemia for 30 minutes. In postconditioning groups, we performed ischemia and reperfusion cycles of five minutes each, applied right after the main ischemia. In saline and lidocaine groups, we instilled the substances at a rate of two drops per minute. To compare the groups, we measured serum levels of urea and creatinine and also held renal histopathology. **Results:** The postconditioning and postconditioning + lidocaine groups showed a decrease in urea and creatinine values. The lidocaine group showed only a reduction in creatinine values. In histopathology, only the groups submitted to ischemic postconditioning had decreased degree of tubular necrosis. **Conclusion:** Lidocaine did not block the effects of postconditioning on renal ischemia reperfusion syndrome, and conferred better glomerular protection when applied in conjunction with ischemic postconditioning.

**Keywords:** Reperfusion Injury. Warm Ischemia. Reperfusion. Ischemic Postconditioning. Lidocaine. Rats.

### INTRODUCTION

The ischemia and reperfusion syndrome contributes to 60-70% of the morbidity and mortality related to the Acute Kidney Injury (AKI) present in various clinical situations, such as renal transplantation and renal artery embolism<sup>1-5</sup>. Although the immediate arterial blood reperfusion is the best approach to eliminate the ischemic process<sup>6</sup>, cell reoxygenation is associated with an increase in lipid peroxidation, in cellular damage and in deterioration of the function<sup>7,8</sup>.

The recent description of "tissue conditioning" techniques, consisting of alternate cycles of brief ischemia and reperfusion, is a promising approach for controlling damage by prolonged ischemia and reperfusion injury<sup>6</sup>.

In 2003, Zhao *et al.*<sup>9</sup> developed the concept of ischemic postconditioning (POS), which consists of small ischemia and reperfusion cycles before free reperfusion in

a previously ischemic tissue. This technique can be easily applied in unexpected ischemias, especially when compared with preconditioning, with studies showing beneficial effects in humans<sup>10,11</sup>.

The mechanisms involved in the protective effect of POS against ischemia and reperfusion injury are poorly understood. It is known that this procedure works in modulation of mitochondrial potassium channels, the mitochondrial permeability transition pore and via p13-kinase-pAkt signaling<sup>12</sup>.

The exact POS intracellular mechanisms have not been fully determined. A mechanism suggested by some studies would be the vascular tone modulation<sup>13,14</sup>, wherein the postconditioning would increase the number and duration of contraction of the arterioles, preventing a large influx of oxygen and therefore the generation of reactive oxygen species. However, in the literature review, we identified no study directly assessing this route.

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Lidocaine is a local anesthetic, often used to perform minor surgical procedures. It acts by blocking sodium channels, preventing the nerve impulse and the painful feeling<sup>15,16</sup>. However, when administered systemically, it has a vasodilatory effect, which could be used to block the vascular tone modulation<sup>17</sup>.

Thus, this study aims to assess the effects of blocking the regulation of vascular tone in ischemia and reperfusion syndrome in rats, through the use of lidocaine in the ischemic postconditioning technique.

## METHODS

We used thirty-five male Wistar rats (*Rattus norvegicus*) aged 15 to 20 weeks, weighing 250-300 g study. We kept the animals in a vivarium of the Experimental Surgery Laboratory of the Pará State University, in a controlled environment, with water and food offered *ad libitum*. This research followed the Brazilian law of animal experimentation (11,794/08). The research project was approved by the Ethics in Research Committee of the Pará State University (43/12)

We randomly divided the animals into seven groups of five animals each: 1) Control group (CON), in which renal ischemia was not induced; 2) Group ischemia and reperfusion (IR), which underwent renal ischemia for 30 minutes followed by reperfusion without any conditioning technique; 3) Group ischemia and saline reperfusion (IRS), which underwent renal ischemia for 30 minutes followed by saline infusion in the renal artery at a rate of two drops per minute (Figure 1); 4) Ischemic postconditioning group (POS), subjected to 30 minutes of renal ischemia followed by 30 minutes of local postconditioning (three cycles of five minutes

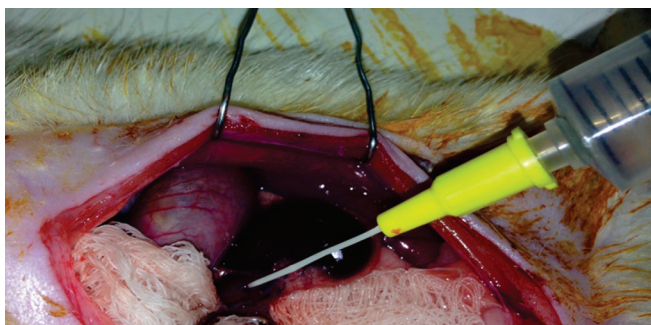


Figure 1. Technique of instillation in the left renal artery.

of perfusion interspersed by five minutes of ischemia)<sup>13</sup>; 5) Ischemic postconditioning and saline group (POSS), submitted 30 minutes of renal ischemia followed by 30 minutes of the local postconditioning (3 cycles of 5 minutes of renal perfusion interspersed with five min of renal ischemia)<sup>11</sup> and instillation of saline in the renal artery at a rate of two drops per minute; 6) Lidocaine group (LIDO), subjected to 30 minutes of renal ischemia followed by 30 minutes of lidocaine instillation into the renal artery at a rate of two drops per minute; 7) Postconditioning and lidocaine group (POLI), submitted 30 minutes of renal ischemia followed by 30 minutes of the local postconditioning and instillation of lidocaine in the renal artery.

We performed all procedures under anesthesia with ketamine 70mg/kg and xylazine 10mg/kg intraperitoneally. In all groups, we performed right nephrectomy and dissected the left renal artery with the aid of a microsurgical microscope. We induced renal ischemia by applying a microsurgical clamp on the left renal artery for 30 minutes. We maintained the temperature of the animals at 37°C using a thermal blanket throughout the procedure. We performed postoperative rehydration through saline injection (10ml/kg) subcutaneously.

During the observation period, the animals received analgesia with dipyrone 30mg/kg every eight hours and water and food *ad libitum*. The animals were individual in cages postoperatively. We evaluated the amount of food and water consumed before and after surgery.

Twenty-four hours after surgery, animals were again anesthetized. We obtained blood samples from the vena cava and immediately sent them to biochemical analysis. Serum urea and creatinine were measured using the colorimetric assay in a Selectra-E device using specific Labtest® kits. We removed the left kidney and fixed it in 10% buffered formalin. The pieces were stained with hematoxylin and eosin. We analyzed the presence of tubular necrosis, medullary congestion and retraction of the glomerular tuft, graduating it as 0 – absent; 1 – mild; 2 – moderate; and 3 – intense. We euthanized the animals by anesthesia overdose.

**Table 1. Serum levels of urea and creatinine.**

Group	Urea (mg/dl)	Creatinine (mg/dl)
CON	39.40 ± 3.43	0.50 ± 0.10
IR	216.30 ± 2.52	2.06 ± 0.26
IRS	208.40 ± 4.66	2.11 ± 0.19
POS	141.40 ± 43.77	0.93 ± 0.18
POSS	140.20 ± 30.98	1.02 ± 0.26
LIDO	187.80 ± 53.73	1.27 ± 0.41
POLI	80.50 ± 15.08	0.76 ± 0.08

*p*<0.05 CON vs other groups; *p*>0.05 IR and IRS vs POS, POSS and LIDO; *p*>0.05 vs IRS and POS vs POSS.

We expressed the results as mean ± standard deviation. We used the BioEstat® 5.4 software (Belém, PA, Brazil) for statistical analysis. We used the ANOVA test followed by Tukey's post-test to compare the levels of urea and creatinine between groups, and the Kruskal-Wallis test to compare the histopathological scores. We adopted a value less than 0.05 or 5% to reject the null hypothesis.

## **RESULTS**

During the entire study period, no animal died or needed resuscitation maneuvers. There was no change in the feeding patterns of animals during pre and postoperative periods (18.63 g/day vs 17.44 g/day, *p*<0.05). We noticed that the IR group had higher urea levels than CON, POS and POLI groups (*p*<0.01), showing no significant difference when compared with the LIDO group (*p*=0.62). The POLI group achieved better serum levels than the POS group (*p*=0.045), and was the only group that showed urea levels similar to the CON group (*p*=0.32).

With respect to creatinine, the IR group showed the highest levels when compared with all other groups (*p*<0.001); the POLI group achieved better serum levels than the POS group (*p*=0.043) and was the only group that showed creatinine levels statistically similar to the CON group (*p*=0.57). The LIDO group showed higher creatinine levels than POS and POLI groups (*p*<0.01). Table 1 shows the serum levels of urea and creatinine per group.

Table 2 shows the results of the histopathological analysis. We identified a lesser degree of glomerular

injury in all treated groups (*p*<0.05), but only in the postconditioning groups there was a reduction in the level tubular injury (*p*<0.05).

## **DISCUSSION**

The exact mechanism of ischemic postconditioning is poorly understood. It is known that its effects are mediated through potassium channels<sup>16,18</sup>, modulated by neural and humoral mechanisms that critically depend on the time at which the conditioning is applied. When performed immediately after the main ischemia, the neuronal pathway is dominant, through a parasympathetic action<sup>19</sup>. However, when applied shortly after the main ischemia, the humoral mechanism is predominant, through the RISK and SAFE pathways<sup>8</sup>.

One of the parasympathetic actions is the regulation of vascular tone that, when activated, causes an increase in blood flow by vasodilatation<sup>20</sup>. The tone of the renal artery is regulated primarily by the sympathetic pathway, which promotes dilation when inhibited<sup>21</sup>. Our hypothesis with lidocaine instillation in the renal artery is that it should generate a parasympathetic effect through of the sympathetic-type blocking of the vascular tone, resulting in a sustained vasodilation with blocking of the postconditioning neurogenic pathway.

The blood markers of renal function were decreased in the group subjected to the postconditioning, as identified in several other studies. However, lidocaine-treated animals also showed a beneficial effect,

**Table 2. Average histopathological scores.**

Parameter	CON	IR	IRS	POS	POSS	LIDO	POLI
Tubular necrosis	0.00	2.60	2.40	1.40	1.20	2.20	1.20
Medullary congestion	0.00	2.80	2.60	1.20	1.40	2.20	1.00
Glomerular retraction	0.00	2.40	2.60	1.40	1.00	1.40	1.20

$p < 0.05$  CON vs other groups;  $p > 0.05$  IR vs IRS and POS vs POSS. Tubular necrosis and Medullary Congestion:  $p < 0.05$  POS, POSS and POLI vs CON, IR, IRS and LIDO; Glomerular retraction:  $p < 0.05$  POS, POSS, POLI and LIDO vs CON, IR and IRS.

particularly in relation to creatinine, displaying an additional glomerular protection<sup>22</sup>. We did not observed this difference regarding urea levels, demonstrating a higher tubular compromise since, under normal conditions, the loop of Henle is responsible for 60% of urea urinary secretion<sup>23</sup>.

It is important to note that the groups treated with saline had no effect similar to the lidocaine group, indicating that the reduction of markers was due to the pharmacological properties of lidocaine and not to a possible volume overload of the instilled lidocaine.

One possible mechanism involved in the lidocaine protective effect of is the blockade of NaV 1.9 channels, which are closely related to the pathophysiology of ischemia and reperfusion syndrome<sup>16</sup>. When this channel is blocked, there is a decrease in the intracellular influx of sodium and less reperfusion injury. However, Lee *et al.*<sup>24</sup> demonstrated that the continuous infusion of lidocaine in rats' subcutaneous tissue had deleterious effects on the ischemia and reperfusion syndrome, suggesting that there is still need for greater understanding of this drug effects and the influence of the route of administration.

Histologically, no significant differences were evident when comparing the histological grade of glomerular and tubular injury, with results similar to those identified in the analysis of serum urea and creatinine and confirming the initial conclusions. We observed no further lesion by the use of lidocaine, which some studies proved to be toxic to the renal epithelium<sup>24,25</sup>.

Further studies are required before the clinical use of lidocaine, especially using other analysis parameters such as oxidative stress and cell viability, not tested in this initial work<sup>26</sup>. Furthermore, there is controversy in the literature on whether the ischemic postconditioning is influenced by the number of cycles or by the conditioning time<sup>27</sup>. Five-minute cycles may not have been the appropriate model to determine the maximum protective effect on ischemia and reperfusion. Thus, if we had applied an ideal cycle, lidocaine solution could not have presented the described effects.

In conclusion, lidocaine did not block the postconditioning effects on the ischemia and reperfusion syndrome, but determined a better result in glomerular protection when applied in conjunction with this technique.

## R E S U M O

**Objetivo:** avaliar os efeitos do bloqueio da regulação do tônus vascular por meio do uso da lidocaína na técnica de pós-condicionamento isquêmico na síndrome de isquemia e reperfusão renal em ratos. **Métodos:** trinta e cinco ratos foram randomizados em sete grupos de cinco animais: Grupo 1- Controle; Grupo 2- Isquemia e Reperfusão; Grupo 3- Isquemia, Reperfusão e Solução Salina; Grupo 4- Pós-condicionamento Isquêmico; Grupo 5- Pós-condicionamento Isquêmico e Solução Salina; Grupo 6- Lidocaína; Grupo 7- Pós-condicionamento Isquêmico e lidocaína. Com exceção do grupo controle, todos os demais foram submetidos à isquemia renal de 30 minutos. Nos grupos de pós-condicionamento, foi realizado o ciclo de isquemia e reperfusão de cinco minutos cada, aplicado logo após a isquemia principal. Nos grupos salina e lidocaína foram instiladas as substâncias numa taxa de duas gotas por minuto. Para comparar os grupos, foram dosados os níveis séricos de ureia e creatinina e análise histopatológica renal. **Resultados:** os grupos pós-condicionamento e pós-condicionamento + lidocaína apresentaram uma redução nos valores de ureia e creatinina. O grupo lidocaína apresentou apenas uma redução nos valores de creatinina. Na análise histopatológica, apenas os grupos submetidos ao pós-condicionamento isquêmico apresentaram redução do grau de necrose tubular. **Conclusão:** a lidocaína não bloqueou os efeitos do pós-condicionamento na síndrome de isquemia e reperfusão renal, mas conferiu melhor na proteção glomerular quando aplicada em conjunto com o pós-condicionamento isquêmico.

**Descritores:** Traumatismo Por Reperfusão. Isquemia Quente. Reperfusão. Pós-Condicionamento Isquêmico. Lidocaína. Ratos.

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