

# Evaluation of ischemic postconditioning effect on mesenteric ischemia treatment. Experimental study in rats

*Avaliação do pós-condicionamento isquêmico no tratamento da isquemia mesentérica. Estudo experimental em ratos*

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## Abstract

**Objective:** To assess the preconditioning and postconditioning effect on intestinal mucosal lesions in rats undergone mesenteric ischemia and reperfusion procedure.

**Methods:** Thirty Wistar rats were studied and divided into three groups: Group A, 10 rats undergone mesenteric ischemia (30 minutes) and reperfusion (60 minutes); Group B, 10 rats undergone mesenteric ischemia and reperfusion preceded by ischemic preconditioning for three cycles of ischemia and reperfusion for two minutes each; Group C, 10 rats undergone mesenteric ischemia and reperfusion and, preceding the beginning of reperfusion, ischemic postconditioning was performed for three cycles of reperfusion and ischemia for two minutes each. Then, a segment of small intestine was resected for histological analysis. We assessed the results by Chiu et al. score and the statistical analysis was performed.

**Results:** According to Chiu et al. score, the means of lesion degree were: In the group A, 3,5; Group B, 1,2; Group C, 1. The difference between group A with the groups B and C was considered statistically significant ( $P < 0,05$ ).

**Conclusion:** Ischemic pre- and postconditioning were capable of minimizing - in a similar intensity - the tissue injury on the intestinal mucosa of rats undergone mesenteric ischemia and reperfusion process.

**Descriptors:** Ischemia. Reperfusion injury. Ischemic preconditioning.

## Resumo

**Objetivo:** Avaliar o efeito do pré e pós-condicionamento isquêmico sobre a lesão tecidual na mucosa intestinal de ratos submetidos ao processo de isquemia e reperfusão mesentérica.

**Métodos:** Foram estudados 30 ratos Wistar, distribuídos em três grupos: grupo A, em que se realizou isquemia (30 minutos) e reperfusão (60 minutos) mesentérica; grupo B, isquemia e reperfusão mesentérica precedidos pelo pré-condicionamento isquêmico por três ciclos de isquemia e reperfusão com duração de dois minutos cada; grupo C, isquemia e reperfusão mesentérica e, precedendo o início da reperfusão, foi realizado o pós-condicionamento isquêmico por três ciclos de reperfusão e isquemia com duração de dois minutos cada. Ao final, ressecou-se um segmento do intestino delgado para análise histológica. Avaliaram-se os resultados pela classificação de Chiu et al. e procedeu-se ao tratamento estatístico.

**Resultados:** As médias dos graus de lesão tecidual segundo a classificação de Chiu et al. foram: no grupo A, 3,5; grupo B, 1,2; grupo C, 1. A diferença entre o resultado do grupo A com os resultados dos grupos B e C foi considerada estatisticamente significativa ( $P < 0,05$ ).

**Conclusão:** O pré e pós-condicionamento isquêmico foram capazes de minimizar, com intensidade semelhante, a lesão tecidual na mucosa intestinal de ratos submetidos ao processo de isquemia e reperfusão mesentérica.

**Descritores:** Isquemia. Traumatismo por reperfusão. Precondicionamento isquêmico.

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## INTRODUCTION

In 1986, two publications have brought a great advancement for the treatment of ischemia and reperfusion syndrome. The first of them was the study from Parks and Granger [1], showing that reperfusion is more damaging than ischemia alone, a fact unknown up to now, promoting a major change in current concepts and initiating several studies that came to elucidate the physiopathology of ischemia and reperfusion. The second publication of great importance was the study from Murry et al. [2], which brought the concept of ischemic preconditioning (IPC) as a way to minimize the lesions of ischemia and reperfusion. These authors described the short-term beneficial effect of coronary occlusion also followed by short periods of reperfusion, before initiating the stage of ischemia itself, by noting reduction in ischemic myocardial lesion in dogs.

However, there are situations in which ischemia is identified when the lesions are already installed, with no opportunity for employment of the preconditioning.

In 2003, Zhao et al. [3] presented the concept of ischemic postconditioning (IPC), that consisted in performing one or more short cycles of reperfusion followed by one or more short cycles of ischemia, immediately after the phase of ischemia and before establishment of permanent reperfusion. These authors showed that IPreC was as effective as the IPostC in the prevention of reperfusion lesions.

This study led to other studies that similarly have demonstrated the ability of IPostC to prevent the lesions of ischemia and reperfusion, as the IPreC [4].

If confirmed the effectiveness of IPostC in humans, this could be a breakthrough in the treatment of ischemia and reperfusion, since it is very common to perform the diagnosis of lesions of this process when the ischemia has already occurred, then there would be no space for use of IPreC. In experimental model, there is already evidence of a probable protective effect of IPostC on the intestinal mucosa of rats undergoing mesenteric ischemia and reperfusion [5].

Thus, considering the current evidences of the value of the IPostC in reducing tissue lesions resulting from ischemia and reperfusion process, it becomes of fundamental importance - and it is the purpose of this study - to evaluate comparatively the ability of the IPreC and IPostC to mitigate the intestinal tissue lesion in the mesenteric ischemia and reperfusion process.

## METHODS

This study was approved by the Ethics Committee of the Federal University of Mato Grosso do Sul.

## Animals studied

We studied thirty rats (*rattus norvegicus albinos*, Rodentia, Mammalia) of the Wistar strain, male, adults, with weight ranging from 270 to 350 grams, with an average of 305 grams, from the biotery of the Federal University of Mato Grosso do Sul.

## Groups formed

The animals were divided into the following groups:

- Group A - Ischemia and Reperfusion: ten rats undergone intestinal ischemia for 30 minutes by occlusion of the cranial mesenteric artery with vascular forceps, followed by 60-minutes reperfusion;
- Group B - Ischemic Preconditioning: Ten rats undergone ischemia procedure for 30 minutes by occlusion of the cranial mesenteric artery with vascular forceps and reperfusion for 60 minutes. The phase of ischemia was preceded by three cycles of ischemia (two minutes each) interleaved with three cycles of reperfusion (two minutes each);
- Group C - Ischemic Postconditioning: ten rats undergone ischemic procedure for 30 minutes by occlusion of the cranial mesenteric artery with vascular forceps and reperfusion for 60 minutes. Between the ischemia and reperfusion were performed three cycles of reperfusion (two minutes each) interleaved with three cycles of ischemia (two minutes each).

## Anesthesia

The animals were weighed on accuracy electronic scales and anesthetized with intraperitoneal injection of 2:1 solution of ketamine hydrochloride (Cetamin®), 50 mg/ml, and Xylazine hydrochloride (Xilazin®), 20mg/ml, respectively, in dose of (0.1 ml/ 100 g).

## Surgery procedure

The rats were maintained on spontaneous ventilation in room air throughout the procedure. They underwent median longitudinal laparotomy of approximately four centimeters, exteriorization of the small intestine, identification and dissection of the cranial mesenteric artery.

In group A, the cranial mesenteric artery was occluded by atraumatic vascular forceps, which remained for thirty minutes (ischemic phase). After placing the forceps, the small intestine was repositioned in the abdominal cavity and the surgical wound was closed with continuous suture of the skin using 4-0 monofilament nylon yarn (mononylon®). When the phase of ischemia has finished, the abdominal wall was opened again by the removal of the suture yarn and vascular forceps was removed, initiating the phase of reperfusion, for 60 minutes. In all three groups, the phase

of reperfusion was initiated, the abdomen was again closed by continuous suture of the skin using 4-0 monofilament nylon yarn until the end of the experiment.

In group B, before the stage of ischemia (30 minutes), the ischemic preconditioning was performed by three cycles of ischemia, lasting two minutes each (occlusion of the cranial mesenteric artery by atraumatic vascular forceps), intervealed by three cycles of reperfusion, also lasting two minutes each (atraumatic vascular forceps removed from the cranial mesenteric artery). After the stage of ischemia, reperfusion was performed for 60 minutes.

In group C, the phase of ischemia (30 minutes) and reperfusion (60 minutes) was performed. Preceding the reperfusion phase, the ischemic postconditioning was performed through three cycles of reperfusion (removal of atraumatic vascular forceps from the cranial mesenteric artery) lasting two minutes each, intervealed with three cycles of ischemia (occlusion of the cranial mesenteric artery by atraumatic vascular forceps), also lasting two minutes each.

After finishing of the reperfusion phase in the three groups, the abdominal wall was opened again by the removal of the suture yarn and a segment of approximately one cm of the ileum was resected, five centimeters proximal to the ileocecal transition, that was opened on its antimesenteric border, washed with saline solution and placed in a 10% formaldehyde solution for further histological analysis.

The animals were sacrificed by deepening the anesthetic plan.

### Histopathological study

The resected intestinal segments, after fixation in 10% formaldehyde solution, underwent histological processing and the slides were stained with hematoxylin-eosin and analyzed under optical microscopy by the pathologist, without his prior knowledge on the group that each rat belonged, and were classified according to the degree of tissue lesion according Chiu et al. [6]:

- Degree 0: mucosa without changes;
- Degree 1: well-formed villosity, without cell lysis or inflammatory process, but with formation of the Grunhagen's subepithelial space;
- Degree 2: presence of cell lysis, formation of the Grunhagen's subepithelial space and increased spacing between the villi;
- Degree 3: destruction of the free portion of the villi, presence of dilated capillaries and inflammatory cells;
- Degree 4: structural destruction of the villi, with only draft of one of them, formed by inflammatory cells and necrotic material, with hemorrhage and basal glandular ulceration;
- Degree 5: destruction of the entire tunica mucosa, no

longer being observed any glandular structure, but only amorphous material deposited on the submucose tissue.

### Statistical analysis

The results obtained underwent statistical treatment, by using the non-parametric Kruskal-Wallis test, with establishment of a significance level of  $P < 0.05$ . We used the Bioestat software 2.0.

### RESULTS

After the histological analysis of the degree of lesion of the intestinal mucosa according Chiu et al. [6], we found the results summarized in Table 1 and Figure 1.

Table 1. Result of histological analysis of the lesion degree of the intestinal mucosa of rats in groups A, B and C according to the classification of Chiu et. al. [6]

Rats	Mucosa lesion degree		
	Group A	Group B	Group C
1	3	0	0
2	3	1	1
3	3	1	3
4	3	2	1
5	4	3	2
6	4	3	1
7	4	1	0
8	4	0	0
9	4	0	1
10	3	1	1
Mean	3.5	1.2	1.0

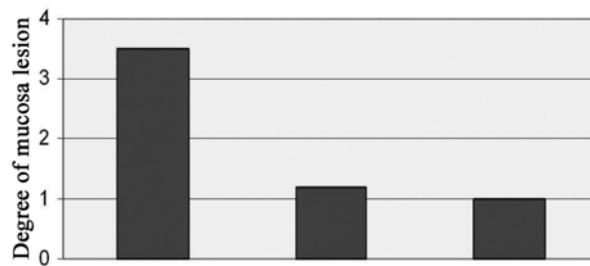


Fig. 1 – Comparative graph of the histological analysis of the lesion degree of the intestinal mucosa of rats in groups A, B and C according to the classification of Chiu et. al. [6]

In group A, the mean degree of tissue lesion was 3.5. Half of the animals in this group presented lesion degree 3 and the other half, degree 4.

In group B, the mean tissue lesion degree was 1.2. In this group, three animals showed no lesion (degree 0), four presented lesion degree 1, one degree 2, and two, degree 3.

In group C, the mean tissue lesion degree was 1. Three animals showed no lesion (degree 0), five presented lesion degree 1, one presented 2 and one degree 3.

By using the non-parametric Kruskal-Wallis test, it was observed that the groups B and C were similar statistically ( $P=0.7507$ ), however, there is a statistically significant difference between these groups and group A (between A and B  $P=0.0004$  and between A and C  $P=0.0002$ ).

Figures 2 to 6 show the histological aspect of the tissue lesion degree found.

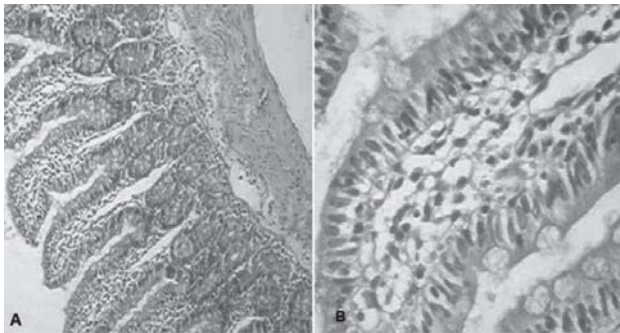


Fig. 2 – Photomicrography of the 0 degree according to Chiu. et. al. classification [6] (HE, A x 100, B x 400) – rat 1 from group B

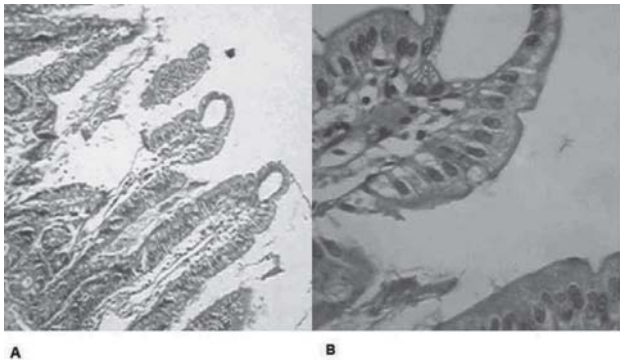


Fig. 3 – Photomicrography of the 1 degree according to Chiu. et. al. classification [6] (HE, A x 100, B x 400) – rat 2 from group C

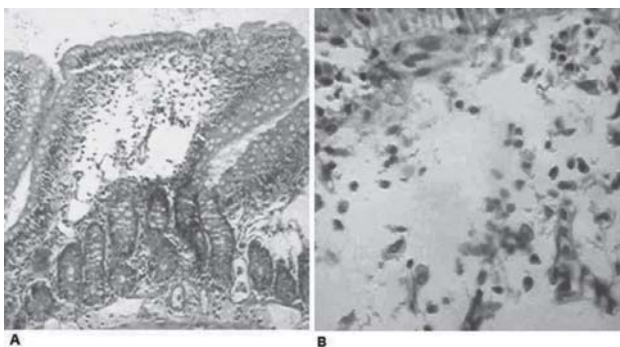


Fig. 4 – Photomicrography of the 2 degree according to Chiu. et. al. classification [6] (HE, A x 100, B x 400) – rat 4 from group B

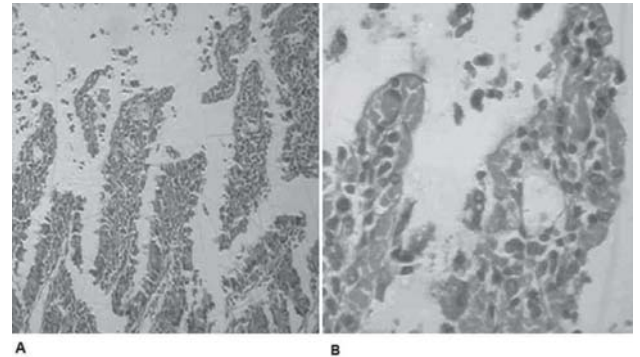


Fig. 5 – Photomicrography of the 3 degree according to Chiu. et. al. classification [6] (HE, A x 100, B x 400) – rat 4 from group A

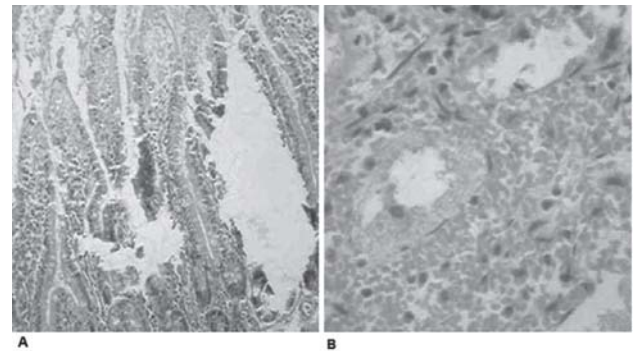


Fig. 6 – Photomicrography of the 4 degree according to Chiu. et. al. classification [6] (HE, A x 100, B x 400) – rat 8 from group A

## DISCUSSION

Since the publication of Murry et al. [2], ten years have passed for the assessment of the IPreC effects in the intestine. In 1996, Hotter et al. [7] published an article in which the authors used effectively the IPreC in the prevention of lesion caused by intestinal ischemia and reperfusion in rats. The authors compared a group that underwent only intestinal ischemia and reperfusion with another IPreC group, and showed lower intensity of the lesions in the second group.

In the present study the ability of the IPreC to minimize the harmful effects of the mesenteric ischemia and reperfusion was shown, since the means of tissue lesion degree were 3.5 and 1.2 in the control groups and IPreC, respectively, according to classification of Chiu et al. [6].

Also, Sola et al. [8] proved the benefit of the use of IPreC in mesenteric ischemia and reperfusion, in a study on which the authors found that in the group of rats undergone only ischemia and reperfusion, the mean degree of intestinal lesion according Chiu et al. [6] was 5, and in the group on



which the IPreC was performed, such degree was 3. The differences between the group ischemia-reperfusion and the group IPreC was statistically significant both in this study as in that from Sola et al. [8], although the values are quite different. The reason for this difference may be related to the time of ischemia and reperfusion and IPreC. In the study by Sola et al. [8], the animals underwent 90 minutes of ischemia and 30 minutes of reperfusion, in contrast with this study, on which is used 30 and 60 minutes respectively. The IPreC was also performed differently. Sola et al. [8] performed the IPreC with a single cycle of ischemia and reperfusion lasting ten minutes each, while in this study three cycles of ischemia and reperfusion were performed lasting two minutes each.

Moore-Olufemi et al. [9] also used the classification of Chiu et al. [6] in their study, on which means were 3 and 1.2 in groups ischemia and reperfusion and IPreC, respectively. Although very similar to the results of this study, the method used was different. Moore-Olufemi et al. [9] performed the ischemia and reperfusion lasting 30 minutes and six hours respectively. The IPreC was performed with three cycles of ischemia and reperfusion lasting four and ten minutes each, respectively.

Other studies also showed the ability of IPreC to reduce intestinal lesions of animals undergone mesenteric ischemia and reperfusion [10-16], in such a way to become evident that the IPreC is able to minimize the harmful effects of the mesenteric ischemia and reperfusion.

The IPostC has also presented similar results to IPreC in the prevention of lesions resulting from the process of ischemia and reperfusion. However, currently there is only one publication [5] that shows that this method may be similar to IPreC in respect to its effect in the aforementioned process in mesenteric circulation.

Zhao et al. [3] were the first to report the similarity of the outcomes of IPreC and IPostC in prevention of lesions from the process of ischemia and reperfusion. The study was performed in dogs, by performing occlusion of the left anterior descending artery for 60 minutes and reperfusion for three hours. The IPreC was performed by a cycle of ischemia lasting five minutes and reperfusion for ten minutes. The IPostC consisted of three cycles of ischemia and reperfusion lasting 30 seconds each. The area of infarction was similar in groups IPreC and IPostC and significantly lower than in the control group.

In the last 20 years, many forms of treatment for ischemia and reperfusion were tested without great success [17,18]. With the advent of IPreC, there was great progress in the treatment of Ischemia-Reperfusion Syndrome, however, specifically in relation to mesenteric ischemia and reperfusion, its use is limited due the fact that in most cases the ischemia is already installed at the time of diagnosis [19]. This is the main reason that values the IPostC because

when its efficacy is also confirmed in mesenteric ischemia and reperfusion, its clinical applicability could be of great importance. Nevertheless, up to now there is no studies assessing its action in mesenteric ischemia and reperfusion.

In this study we observed the mean of the tissue lesion degree 1.2 and 1 in groups IPreC and IPostC respectively, according to the classification of Chiu et al. [6], in clear contrast with the control group, in which the result was 3.5. This shows that in this study the IPreC and IPostC were similar in ability to prevent the lesion of ischemia and reperfusion in the intestine of rats.

Most of the studies that analyzed the effects of IPostC was performed in myocardial ischemia and reperfusion. Donato et al. [20] compared the IPreC and IPostC in isolated rabbit hearts, by performing ischemia and reperfusion of 30 minutes. The IPreC was performed by a cycle of ischemia and reperfusion of five minutes each, and the IPostC, two cycles of ischemia and reperfusion of 30 seconds each. The authors concluded that the IPostC reduces the size of infarction in the same magnitude as the IPreC without changing the post-ischemic ventricular dysfunction.

These results were also obtained by Darling et al. [21] in an experimental study that assessed infarction in rabbit hearts. The IPostC was able to minimize the area of infarction in comparison to the control group, by performing four cycles of ischemia and reperfusion lasting 30 seconds before initiation of the reperfusion phase.

It is believed that the IPostC may have protective effects by activation of the adenosine receptors, based on studies that showed that when administering agonists of such receptors during reperfusion, the infarction is significantly reduced [22,23].

Tang et al. [24] showed that the IPostC is as effective as the IPreC in prevention of lesions from the process of coronary ischemia and reperfusion in rats, since the time of ischemia does not exceed 45 minutes. After this period, according to these authors, the IPreC provides better results on the reduction of the infarcted area.

In addition to the assessment of the IPostC in animal hearts under ischemia and reperfusion, there is also a report of its use in spinal cord. Huang et al. [25] showed that the IPostC was as effective as the IPreC in the prevention of tissue lesions in the spinal cord of rabbits undergone ischemia and reperfusion.

The IPostC was also assessed in humans. Staat et al. [26] reported its beneficial effect by performing intermittent reperfusion during angioplasty in patients with acute myocardial infarction, noting reduction of myocardial lesion. Loukogeorgakis et al. [27] also performed an experimental study in humans on which caused the transient upper limb ischemia followed by reperfusion, observing the protective effect from IPostC.

The protection mechanism of the IPostC in the

mesenteric ischemia and reperfusion process is still not entirely clear, but there are evidences that the IPostC may be related to the significant decrease in levels of malondialdehyde and products related to lipid peroxidation. These observations suggest a reduction in the production of Reactive Oxygen Species (ROS) and less lesion mediated by oxidants with the IPostC [3].

An abundant production of ROS during the initial phase of reperfusion has been implicated as the main factor in the pathogenesis of tissue lesion. The peak production of ROS occurs between the first and seventh minutes after the initiation of reperfusion, although these substances are detectable in later periods [21,28,29]. The IPostC acts at this stage in such a way not yet fully clarified, possibly reducing the production of ROS by gradual release of oxygen to tissues [16,30]. For this reason, in this study the IpostC was performed soon after ischemia, by performing short cycles of ischemia and reperfusion, since there is no one definition of which is the better time to be used in these cycles, nor the number of cycles that would lead to better outcomes.

We should consider that this study has limitations regarding the assessment of tissue lesion. It was used the histological analysis, a method widely used for this purpose, however, thus becoming difficult to compare with other studies on which different methods for assessment of lesion by ischemia and reperfusion are used, such as the dosage of myeloperoxidase and plasma or tissue malondialdehyde.

Thus, it is believed that the results found in this study show that, similar to what is shown in the literature in respect to the equivalence of the IPostC and IPreC capacity to minimize the lesions caused by ischemia and reperfusion in heart and spinal cord in the intestine rats, the IPostC minimizes such lesions in the same proportion as the IPreC.

## CONCLUSION

The ischemic pre- and postconditioning were able to reduce tissue lesion in the intestinal mucosa of rats undergone process of mesenteric ischemia and reperfusion in a similar manner.

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