

Experimental model of mesenteric ischemia/reperfusion by abdominal aorta clamping in Wistar rats

Modelo experimental de isquemia/reperfusão intestinal por clampeamento de aorta abdominal em ratos Wistar

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A B S T R A C T

Objective: To develop an experimental model of global normothermic ischemia able to demonstrate the transient ischemia and reperfusion periods required for development of ischemia/reperfusion injury in the small intestines of Wistar rats by clamping the abdominal aorta. **Methods:** Twenty adult male Wistar rats weighing 250-350g were randomly divided into five groups with four rats each and submitted to increasing times of ischemia (0 - 30 - 45 - 60 - 90 minutes). Within each group, except the control one, two rats underwent 60 minutes of reperfusion and two 90 minutes. After the procedures, histological analysis was conducted by measurement of areas of necrosis. **Results:** The degree of intestinal necrosis ranged from 15% to 54% ($p = 0.0004$). There was progressive increase in the degree of injury related to increase in ischemic time. However, greater degrees of injury were observed in the lowest times of reperfusion. The analysis of the coefficient of variation of necrosis among the ten groups of ischemia/reperfusion showed a statistically significant difference in 15 areas, 13 related to the control group. **Conclusion:** The model was able to show the periods required for the occurrence of ischemia/reperfusion injury by aortic clamping and can serve as a basis to facilitate the development of studies that aim at understanding this kind of injury.

Key words: Experimental development. Ischemia. Reperfusion. Constriction. Aorta, abdominal. Intestine, small.

INTRODUCTION

After a critical period of ischemia, restoration of blood flow in a specific organ triggers the process of ischemia/reperfusion (I/R)¹. This is because the ischemia creates an environment that, at the time of reperfusion, activates enzymes that participate in reduction of molecular oxygen, generating oxirradicals².

I/R injury is a phenomenon that attracts the interest of researchers, which through experimental models seek to understand its pathophysiology and possible therapeutic measures. It is noted, however, that the majority of I/R models are directed to regionalization³⁻⁵, using selective clamping of vascular pedicles of specific organs, rather than I/R models clamping source blood vessels for various organs. In this context, there are models of clamping of the aorta, which would simulate operations on the aorta and

its systemic repercussions, such as neurological, intestinal and renal impairment⁶⁻⁸.

The objective of this study was to develop an experimental model of normothermic global ischemia able to demonstrate the transient times of ischemia and reperfusion injury necessary for I/R development in the small intestines of rats, by clamping of the suprarenal abdominal aorta.

METHODS

We used 20 adult male Wistar rats weighing 250-350g, maintained under controlled conditions, with water *ad libitum* and fed with commercial chow. The experimental procedures were approved by the Ethics Committee of the institution.

Study conducted at the Experimental Research Center, Department of Experimental Surgery and Surgical Specialties, College of Medicine, Federal University of Bahia (UFBA) in partnership with the Gonçalves Moniz Research Center, Laboratory of Pathology and Biointervention (LPBI), Fiocruz. From the Faculty of Medicine, Federal University of Bahia and Fiocruz, Salvador, BA, Brazil.

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The animals were randomly divided into five groups (Table 1). All were anesthetized with ketamine (75 mg/kg, intramuscular) and xylazine (10 mg/kg, intramuscular). They were maintained in spontaneous ventilation, positioned on the operating table in supine position, immobilized in four points and subjected to abdominal trichotomy and antisepsis with povidone detergent. A xifopubic laparotomy, with exposure of the abdominal cavity, was performed. The left kidney was shifted to the right and the perirenal fat divided. After dissection, the abdominal aorta was clamped just above the emergence of the right renal artery with an atraumatic clamp (Figure 1), interrupting the mesenteric blood flow.

After completing the time of ischemia and reperfusion, we performed a section of the inferior vena cava and resection of abdominal organs. In Group I (control), after laparotomy the small intestine was put in formalin solution for 24 hours and kept in 70% alcohol till histologic evaluation. Six sections of small intestine were obtained from each rat; from them, two were randomly chosen for histopathological analysis after photos of the intestinal walls were taken. All images were obtained with a 280x1024 pixels resolution camera (CX41, Olympus, Tokyo, Japan) coupled to a microscope with 20x magnifying lens and analyzed with ImageJ software (National Institutes of Health, Bethesda, USA). Image analysis occurred through measuring the areas of necrosis in relation to the total area of the intestinal wall in the sections. Three investigators, blinded to the groups of rats, performed the measurements. The average areas measured by each of the researchers was considered for analysis of results.

Descriptive and inferential statistical analysis was performed using the nonparametric Kruskal-Wallis test and subsequent Student-Newman-Keuls test, when applicable, using BioStat 5.0 software. Was assumed the findings to be statistically significant when $p < 0.05$.

RESULTS

The degree of intestinal damage, in terms of necrosis, was evaluated in 20 rats subjected to different times of ischemia and reperfusion (Table 1). The average degree of intestinal necrosis varied from 15% to 54% (Figure 2).

There was no significant difference ($p=0.0004$) between the degree of necrosis in all groups, with a trend of gradual increase related to increased ischemic times (0 - 30 - 45 - 60 - 90 minutes). However, the degrees of necrosis were higher in the lower reperfusion times (60 minutes) compared with the groups of longer time (90 minutes). There were no statistically significant differences between the different times of reperfusion for the same duration of ischemia.

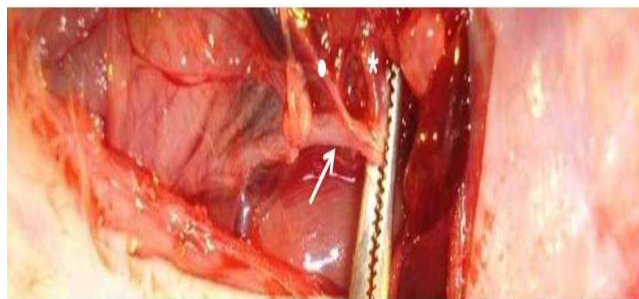


Figure 1 - Left renal artery (●), abdominal aorta (arrow) clamped just above the emergence of the right renal artery (*).

Table 1 - Study design.

Group	Ischemia (minutes)	Reperfusion (minutes)	n
I	0	0	4
II	30		4
a		60	2
b		90	2
III	45	4	
a		60	2
b		90	2
IV	60	4	
a		60	2
b		90	2
V	90	4	
a		60	2
b		90	2

The control group proved to have the lowest degree of necrosis in relation to the other groups. When the degree of necrosis between groups was analyzed individually, the coefficient of variation showed a statistically significant difference between the means in 15 of 45 areas, 13 directly involved in the control group (Table 2). This group showed a statistically significant difference when compared to other groups of IR in 76% of the time.

DISCUSSION

In rats, the emergence of the superior mesenteric artery is located below the emergencies of the renal arteries. Therefore, the clamping of the aorta above the renal arteries' emergencies implies termination of mesenteric flow.

The ischemic tissue injury is a common occurrence in surgical practice. The restoration of blood flow is crucial to prevent cell death, and is associated with aggravation of the ischemic damage by I/R injury¹. The aortic clamping in

Table 2 - Difference of averages between I/R groups

Groups	Coefficient of variation	p-value
Control x I45R60	29.3333	0.00
Control x I60R60	38.5	0.00
Control x I60R90	25.8333	0.01
Control x I90R60	30.5	0.00
Control x I90R90	23.5	0.02
Control x I30R60	22.3333	0.03
Control x I30R90	19.8333	0.05
Control x I45R60	32.6667	0.00
Control x I45R90	20.1667	0.05
Control x I60R60	41.8333	< 0.0001
Control x I60R90	29.1667	0.00
Control x I90R60	33.8333	0.00
Control x I90R90	26.8333	0.01
I30R90 x I60R60	22	0.03
I45R90 x I60R60	21.6667	0.03

(p) Nonparametric Kruskal-Wallis test, p=0.0004; subsequent Student-Newman-Keuls test, p<0.05=statistically significant

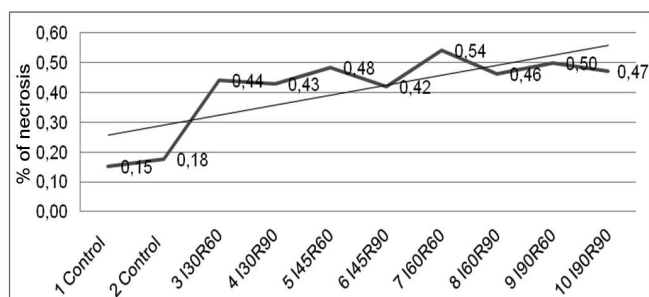


Figure 2 - Curve of ischemia / reperfusion.

the open repair of aortic aneurysms is associated with hemodynamic changes that lead to I/R injury⁹. This damage has been studied by various experimental models based on clamping of the mesenteric vascular pedicle^{3,4,10}.

As observed on previous studies, there is no standardization of time of ischemia and reperfusion for the occurrence of tissue injury, a fact already noted by Silveira *et al.*¹¹. Aiming to better evaluate this issue in the intestine, distinct periods of clamping were tested to analyze the degree of histological changes, as well as the specific duration of ischemia and reperfusion necessary for the occurrence of lesions in the organ in question.

The small intestine is an organ known to suffer a lot with I/R injuries. Schoenberg & Berger¹² determined that in one hour of ischemia the intestinal mucosa already displayed important damage; in two, this damage would be irreversible. Several studies have been developed, generally with the time varying from one to two hours of ischemia. According to Sola *et al.*¹³, after 90 minutes of ischemia by clamping the mesenteric artery and 30 minutes

of reperfusion, the intestine presented with a histologic lesion grade 5, according to the modified classification by Chiu *et al.*¹⁴. Santos *et al.*¹⁵ demonstrated that, with 30 min of ischemia followed by 60 min of reperfusion, the degree of histological injury obtained can vary between 3 and 4. Li *et al.*¹⁶ found that clamping the superior mesenteric artery for 60 min of ischemia and allowing 60 min of reperfusion causes severe mucosal damage. Brito *et al.*¹⁰ demonstrated that, for the same time of ischemia, a greater degree of ischemic injury is associated with longer reperfusion time. Miranda *et al.*¹⁷ used 45 min of ischemia in their studies, followed by seven days of reperfusion.

In this study, we chose to conduct aortic clamping. The reasons for this vessel were lack of studies with this type of clamping and easy handling. With respect to ischemia, these results are corroborated by literature data, showing that the longer the duration of ischemia the greater is the degree of histological injury¹⁸. In this study we show that after 30 min of aorta ischemia it is already possible to observe histopathologic changes in the intestinal wall of rats. No statistically significant difference was found between injuries of the times 60 and 90 min of reperfusion in either group.

In conclusion, the model was able to show the time needed to cause intestinal I/R injury by aortic clamping and might serve to facilitate the development of studies aimed at understanding this type of injury.

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R E S U M O

Objetivo: desenvolver um modelo experimental de isquemia global normotérmica transitória capaz de demonstrar os tempos de isquemia e reperfusão necessários para desenvolvimento de lesão de isquemia/reperfusão em intestinos delgados de ratos Wistar através clameamento de aorta abdominal suprarrenal. **Métodos:** Vinte ratos Wistar adultos machos, pesando entre 250 e 350g, foram distribuídos aleatoriamente em cinco grupos, com quatro ratos cada, e submetidos a tempos crescentes de isquemia (0 - 30 - 45 - 60 - 90 minutos). Dentro de cada grupo, à exceção do grupo controle, dois ratos foram submetidos à 60 minutos de reperfusão e dois à 90 minutos. Após os procedimentos, procedeu-se análise histológica através de medição de áreas de necrose. **Resultados:** O grau de necrose intestinal variou de 15 a 54% ($p=0,0004$). Houve tendência de aumento progressivo no grau de lesão relacionado ao aumento no tempo de isquemia, contudo, os maiores graus de lesão foram observados nos menores tempos de reperfusão. A análise do coeficiente de variação de necrose entre os dez grupos de isquemia/reperfusão demonstrou diferença estatisticamente significativa em 15 postos, sendo 13 relacionados ao grupo controle. **Conclusão:** O modelo foi capaz de demonstrar os tempos necessários para que ocorra lesão de isquemia/reperfusão intestinal através de clameamento aórtico e poderá servir como base para facilitar o desenvolvimento de estudos voltados para a compreensão deste tipo de lesão.

Descritores: Desenvolvimento experimental. Isquemia. Reperfusão. Constrição. Aorta abdominal. Intestino delgado.

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