Ischemic preconditioning attenuates remote pulmonary inflammatory infiltration of diabetic rats with an intestinal and hepatic ischemia-reperfusion injury[1]

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ABSTRACT

PURPOSE: To assess ischemic preconditioning (IPC) effects in pulmonary lesion in intestinal and hepatic ischemia-reperfusion (IR) injury models using diabetic rats.

METHODS: Diabetes (DM) was induced in 28 male Wistar rats by alloxan (42 mg/kg, IV). After 28 days, severe DM rats were submitted to intestinal or hepatic IR injury with or without IPC. Intestinal IR (30 min of mesenteric artery occlusion and 30 min of reperfusion; n=6) and IPC groups (10 min ischemia, 10 min reperfusion, followed by intestinal IR; n=6), and Hepatic IR (30 min of hepatic pedicle occlusion and 30 min of reperfusion; n=5) and IPC groups (10 min ischemia, 10 min reperfusion, followed by hepatic IR; n=5), were compared to DM rats group (n=6). Plasmatic lactate, glycemia were measured before and after IR injury. Histomorphology of lung was performed counting inflammatory cells. Data was expressed in mean± SE. $P<0.05$.

RESULTS: Glycemia and lactate were similar among groups. IPC did not interfere in these parameters. On histological evaluation, IR increased inflammatory cells infiltration in pulmonary parenchyma compared to control in both IR injury models. IPC attenuated inflammatory infiltration in lungs.

CONCLUSION: Ischemic preconditioning protects against remote ischemia-reperfusion injury in lung on intestinal or hepatic ischemia-reperfusion model with acute diabetes.

Key words: Ischemia. Reperfusion. Ischemic Preconditioning. Lung. Diabetes Mellitus. Rats.

Introduction

Life expectancy has increased in parallel with the occurrence of chronic degenerative diseases such as Diabetes Mellitus. This disease is characterized by permanent elevated blood glucose levels, with subsequent macrovascular and microvascular injuries as endothelial dysfunction, atherosclerosis and chronic inflammatory disease[1-3].

According to the World Health Organization, projections estimate over 300 million people with diabetes in 2025 (WHO 2001). About 50% of these patients will need some surgical procedure during their lives[4], with increased risk for surgical complications, such as the phenomena of ischemia and reperfusion. The absence or reduction of arterial or venous blood flow to a particular area interrupting the supply of oxygen and nutrients characterize ischemia, which can lead to tissue death[5]. Reperfusion is needed to avoid irreversible damage, but can produce oxygen free radicals by the hypoxanthine-xanthine oxidase system, alter the distribution of ions, edema and cellular acidosis, among others culminating in circulation loss and increasing the injury[6-8].

During ischemia, free radicals can attract and activate neutrophils, secreting proteolytic enzymes, releasing more free radicals, *thrombi* in the microcirculation, platelet aggregation and tissue and cellular edema, culminating in the phenomenon of non-reperfusion[9] whose progression causes irreversibility[10].

Ischemia reperfusion injury has an impact not only local, but a systemic response, and often leads to respiratory syndromes and even a multiple organs failure[11]. In order to protect the ischemic areas caused by reperfusion injuries, various methods are used, including ischemic preconditioning. This constitutes a small induction period of ischemia followed by reperfusion before a long period of ischemia[12].

The protective mechanisms of ischemic preconditioning has been recently studied, showing beneficial effects locally and systemically, decreasing mucosal damage, cell apoptosis and the effects of reperfusion[13], however, the influence in the presence of diabetes mellitus has not been studied.

The objective of this study was to evaluate the protective effect of remote ischemic preconditioning in the lungs of diabetic animals submitted to intestinal or hepatic reperfusion injury.

Methods

The experimental protocol was submitted and approved by UNICID Ethics Committee on Animal Research. Adult, male Wistar rats (n=28) were used weighing between 270 and 320g, from the Center for experimental models development in medicine and biology-Federal University of Sao Paulo.

Diabetes Mellitus was chemically induced by intravenous administration of alloxan (monohydrate dioxiuracil 5.6) at a single dose of 42 mg/kg body weight. Only animals with clinical and laboratory signs of obvious severe diabetes were used, characterized by weight loss, increased water intake, food intake and urine output and values of fasting plasma glucose greater than 200 mg/dL. No treatment of the diabetic state was made in the diabetic animals.

Following four weeks after induction of diabetes, the animals were randomly subjected to surgical protocols to study the remote effect of ischemic preconditioning on lung by intestinal or hepatic reperfusion injury:

a) Intestinal IR - diabetic animals subjected to intestinal ischemia reperfusion injury (n=6)

b) Intestinal IPC - diabetic animals subjected to Intestinal ischemia reperfusion injury + ischemic preconditioning (n=6)

c) Hepatic IR - diabetic animals subjected to hepatic ischemia reperfusion injury (n=5)

d) Hepatic IPC - diabetic animals subjected to ischemic preconditioning and hepatic ischemia reperfusion injury (n=5)

e) Control - diabetic animals, sham surgery (n=6)

After weighing, animals were anesthetized with a combination of xylazine (5mg/kg) and ketamine (25mg/kg) intramuscularly. Midline laparotomy was performed and dissection was made in the surgical microscope (16-fold increase) and microsurgical instruments. The ischemic preconditioning was performed 10 minutes before a 30 minutes ischemia, and consists of 10 minutes of ischemia followed by 10 minutes of reperfusion.

Intestinal ischemia reperfusion injury

The superior mesenteric artery was clamped for 30 minutes for all animals, except in the sham surgery. After 30 minutes of reperfusion, animals were sacrificed to collect material.

Hepatic ischemia reperfusion injury

The hepatic pedicle was clamped for 30 minutes for all animals, except in the sham surgery. After 30 minutes of reperfusion, animals were sacrificed to collect material.

The blood was collected and analyzed for glucose and glycated hemoglobin to confirm diabetic condition and, for lactate to assess damage overall. Fragments of the lungs of all groups were

fixed in 10% formalin and embedded in paraffin. Tissue sections of five micrometers were stained with hematoxilin and eosin (HE) for histomorphometry. The lungs were investigated by counting cells in 30 fields (HE-40X) in the pulmonary parenchyma, and the difference was indicative of the inflammatory cell infiltration (cells/mm^2), using an appropriate image captured using a light microscope (Axiolab Standart 20, Carl Zeiss, Jena, Germany) coupled to a video camera (AxionCam, Carl Zeiss, Jena, Germany).

Statistical analysis

Data are presented as mean ± SD or median (interquartile range). Analysis of variance (ANOVA) or analysis of variance on ranks (Kruskal-Wallis) were used to analyze the biochemical and morphometric parameters among groups, and complemented by post-hoc test, when appropriate. Paired *t*-test was used for comparison of glycemia and lactate between pre- and post-surgery and, Student t-test or Mann-Whitney rank sum test used for comparison between IR and IPC subgroups. Statistical differences were considered when p≤0.05.

Results

Tables 1 and 2 summarize mean of body weight, glycemia and lactate of intestinal and hepatic injury groups, respectively. There were no differences in parameters among groups, except for preoperative values of lactate in the IR group compared to Control group.

TABLE 1 - Body weight, glycemia, lactate animals subjected to intestinal ischemia and reperfusion (IR), with or without ischemic preconditioning (IPC), animals of sham surgery (control).

	Control	IR	IPC	P
Body weight (g)	223±40	192±34	199±35	ns
Preoperative glycemia (mg/dL)	335±118	379±150	365±127	ns
Postoperative glycemia (mg/dL)	440±120	359±158	359±167	ns
Preoperative lactate (mmol/L)	4.4±1.4	3.0±0.7	3.6±0.6	ns
Postoperative lactate (mmol/L)		3.7±0.7	3.6±1.8	ns

ns: p>0.05

TABLE 2 - Body weight, glycemia, lactate animals subjected to hepatic ischemia and reperfusion (IR), with or without ischemic preconditioning (IPC), animals of sham surgery (control).

	Control	IR	IPC	P
Body weight (g)	223±20	192±20	223±48	ns
Preoperative glycemia (mg/dL)	335±118	486±147	410±78	ns
Postoperative glycemia (mg/dL)	440±120	521±37	441±51	ns
Preoperative lactate (mmol/L)	4.4±1.4	2.8±0.6*	3.7±0.5	0.05
Postoperative lactate (mmol/L)		4.0±0.9	3.2±0.3	ns

ns: p>0.05; *, p≤0.05 vs control group.

Remote lung injury

Histological assessment of pulmonary parenchyma indicated increased inflammatory cell infiltration in response to intestinal or hepatic ischemia reperfusion injury, when compared to controls. Remote ischemic preconditioning applied before intestinal ischemia reperfusion injury showed important attenuation of inflammatory infiltration in pulmonary parenchyma compared to IR group, but more intensive than control group (Figure 1A). Remote ischemic preconditioning applied before hepatic ischemia reperfusion injury showed important attenuation of inflammatory infiltration in pulmonary parenchyma compared to IR group and, similar to Control group (Figure 1B).

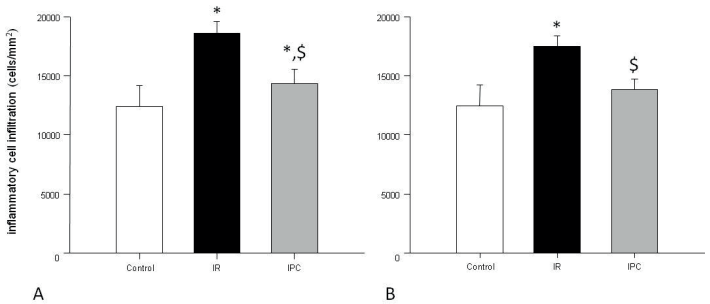

FIGURE 1 – Inflammatory cell infiltration within lung parenchyma in diabetic rats subjected to **A**) intestinal ischemia and reperfusion (IR) and/or ischemic preconditioning (IPC) compared to diabetic control animals and **B**) hepatic ischemia and reperfusion (IR) and/or ischemic preconditioning (IPC) compared to diabetic control animals. *, p<0.05 vs. Control; $, p<0.05 vs. IR.

Discussion

The relevant finding of this study is that even in diabetic rats remote ischemic preconditioning is a strategy for preservation of pulmonary parenchyma. The pulmonary inflammatory infiltrate was reduced by ischemic preconditioning in both situations: intestinal and hepatic ischemia reperfusion injuries.

The interruption of blood flow to a particular organ followed by reperfusion causes local and remote damage, mainly due to the release of reactive oxygen species, nitrogen and inflammatory mediators[14]. Similarly, ischemic preconditioning acts on the primary organ and as well as remote organs, by humoral or neural pathways promoting systemic effects[15-18].

Tamion *et al*.[19] observed that intestinal preconditioning prevents local inflammatory response in hemorrhagic shock, and markedly reduce the systemic consequences. The protective effect was accompanied by an increased expression of heme-oxygenase-1 and attenuated by a specific inhibitor suggesting that the inflammatory response was mediated in part by heme-oxygenase-1 in this model[19].

Intestinal ischemic preconditioning improved blood gas parameters and attenuated neutrophil sequestration, ameliorated pulmonary microvascular permeability and local inflammation in pulmonary tissue. The arachidonic acid cascade is an important factor in the ischemia reperfusion injury, and the ischemic preconditioning partly modulates this pathway[20].

TNF is another target for the ischemic preconditioning effect as shown by Peralta *et al*.[21] Following hepatic ischemia reperfusion, it happens a systemic release of TNF by Kuppfer cells which is reduced by the ischemic preconditioning, preventing lung and liver injury[21].

Intestinal ischemic preconditioning promotes local and distant organs protection, as verified by Gho *et al*.[22] in the heart and by Bashir *et al*.[23] in the spinal cord, showing reduction in oxidative stress and inflammation.

These studies enhanced the benefits of the ischemic preconditioning in health animals, and the present work showed for the first time that ischemic preconditioning has remote protective effects in alloxan induced diabetic rats.

Conclusion

In diabetic rats, the lung was protected by remote ischemic preconditioning in the intestinal or hepatic ischemia-reperfusion injury.

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