Cardiac Effect of Ischemic Preconditioning and Heparin Following Intestinal Ischemia and Reperfusion in Rats

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ABSTRACT

To study the role of heparin and ischemic preconditioning (IPC) in cardiac injury after intestinal ischemia (I) and reperfusion (R), 54 rats underwent 60 minutes of I, which was produced by occlusion of the superior mesenteric artery, and/or 120 minutes of R. The IPC group had the I procedure stimulation for 5 minutes and R for 10 minutes. The control group was subjected to sham surgery only, and the other groups were injected with saline solution (SS; 0.1 mL) or heparin (100 IU/kg) via the inferior cava vein 5 minutes before I and 5 minutes before R and 55 minutes after the R begins in I-R groups. In all animals, cardiac samples were stained with hematoxylin and eosin for optical microscopy analysis, and other sample was processed for lipid peroxidation determination. In I-R groups, both heparin and IPC showed significant protection compared to the SS group; conversely, in animals subjected only to I, no protection was observed. Moreover, when heparin was associated with IPC, I-R protection was compromised and the ischemic injury increased. Data showed that IPC and heparin attenuated cardiac dysfunction caused by intestinal I and I-R, but when used in association did not show beneficial effects.

INTESTINAL ISCHEMIA (I) followed by reperfusion (R) leads to activation of circulating leukocytes that trigger a local followed by a systemic microcirculatory inflammatory response [1], oxygen free radical production [2], and multiple-organ failure as a final event. Therefore, intestinal I-R injury not only has local impact but also compromises distant organs. Horton and White [3] showed cardiac contractile depression as early as 2 hours after I-R in small intestine after superior mesenteric artery occlusion.

Ischemic preconditioning (IPC), which involves a brief period of I and R preceding the sustained I of the organ [4], increases expression of intestinal aldose reductase to remove the reactive oxygen species, promotes down-regulation of nuclear factor-kB, reduces leukocyte-mediated tissue injury, and improves macrohemodynamics and intestinal microcirculation, inducing systemic beneficial effects [5]. The heart could be protected by subjecting a remote organ or tissue, such as kidney, small intestine, liver, or limb, to short episodes of I-R, a phenomenon known as remote IPC [6], and current evidence suggests that humoral mediators are released by the remote organ into circulation and exert their effects on the heart [7].

Several therapeutic innovations for the prevention of intestinal I-R-induced injury have been studied, showing varied effects. Administration of heparin attenuated intestinal dysfunction caused by I and I-R [8] and decreased remote organ injury on the lung and heart after infrarenal aorta clamping in a rat model [9]. These effects are associated with the ability of heparin to down-regulate tumor necrosis factor-α (TNF-α)-induced leukocyte rolling, adhesion and migration into gut tissues [10], as well as the activity and release of leukocyte enzymes [11] and apoptosis [12].

The mechanism that both IPC and heparin protect remote organs in I-R injury, and their associated effects in local and distant response to I-R injury are not totally clear. Therefore, this study was designed to evaluate the effect of

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these associated strategies on rat heart damage after intestinal I or I followed by R.

METHODS

The experimental protocol was submitted and approved by UNIFESP Ethics Committee on Animal Research, protocol number 0362/09. Adult, male Wistar rats ($n = 54$) were used; they weighed between 250 and 350 g and were obtained from the Center for Experimental Models Development in Medicine and Biology–Federal University of São Paulo. The animals were kept under standard conditions of light, humidity, temperature, and nutrition, and 12 hours before the experiment the supply of solid food was restricted.

After weighing, animals were anesthetized with a combination of xylazine (10 mg/kg) and ketamine (60 mg/kg) intramuscularly. Midline laparotomy was performed, and the superior mesenteric artery was dissected under surgical microscope (10-fold increase) using microsurgical instruments.

The animals were divided randomly into 9 groups with 6 rats in each group: control group, sham surgery; I + SS group, animals treated with saline solution + intestinal I; I-R + SS group, animals treated saline solution + intestinal I and R; I + heparin group, animals treated with heparin + intestinal I; I-R + heparin group, animals treated with heparin + intestinal I and R; I + SS + IPC group, animals treated with saline solution + intestinal IPC + intestinal I; I-R + SS + IPC group, animals treated with saline solution + intestinal IPC + intestinal I and R; I + heparin + IPC group, animals treated with heparin + intestinal IPC + intestinal I; I-R + heparin + IPC group, animals treated with heparin + intestinal IPC + intestinal I and R.

Control group animals underwent only laparotomy without I and R. In the other groups, the superior mesenteric artery was isolated and repaired with 4-0 cotton thread. Vascular occlusion was obtained by applying microsurgical vascular clamp.

I and I-R Protocol

Rats were submitted to 60 minutes of I (groups I + SS; I + heparin; I + SS + IPC; I + heparin + IPC) followed by 120 minutes of R (groups I-R + SS; I-R + heparin; I-R + SS + IPC; I-R + heparin + IPC).

IPC Protocol

Groups treated with IPC (I + SS + IPC; I + heparin + IPC; I-R + SS + IPC; I-R + heparin + IPC) were subjected to 5 minutes of I and 10 minutes of R before the prolonged I, by clamping the superior mesenteric artery.

Heparin Protocol

Heparin (100 IU/kg) or saline solution at the similar volume (0.1 mL) was administered 5 minutes before I and 5 minutes before R and after 55 minutes of R in I and R groups. Heparin has been studied in our laboratory looking at other organs with this same dose [8,9,12]. The dose was chosen based on a previously work published by Hirsh et al [13]. They report the half-life of heparin increases from approximately 60 minutes with a bolus of 100 U/kg, justifying our protocol with 3 doses, during the experiment.

Following the procedures, animals were euthanized under anesthesia to surgically remove heart tissue samples for histologic analysis and quantification of thiobarbituric acid reactive substances (TBARS).

Histologic Analysis

Samples of cardiac tissue excised for morphologic examination were fixed in 10% formalin and embedded in paraffin and sections of 3 μm were done. The slides were stained with hematoxylin and eosin and qualitatively evaluated under an optical microscope (Zeiss Axio Image A2, Oberkochen, Germany) by an experienced pathologist kept blind to the different groups. At least 10 fields of each slide were randomly chosen and analyzed. The cardiac histologic score was based on the scale from 0 to 5, as follows: 0 for injury missing, 1 for light injury (contraction bands and scattered pyknosis), 2 for mild injury (contraction bands, and pyknosis karyorrhexis), 3 for moderate injury (contraction band, pyknosis, and hypereosinophilia karyorrhexis), 4 for moderate to intense injury (contraction band, pyknosis, hypereosinophilia, karyorrhexis and vacuolization), and 5 for intense injury (all others, and presence of edema or inflammation) [14].

TBARS Analysis

Cardiac tissue sample was frozen at −80°C. After the tissue samples were homogenized in 1 mL of 1.15% KCl with sonicator (Polytron PT3100) and used for determination of malondialdehyde (MDA). The cell membrane lipid peroxidation was determined by the method of TBARS, which measures the amount of MDA derivative of lipid peroxidation, and the value was expressed in nanomoles per milligram of protein (nmol/mg protein). For this purpose, after homogenization, aliquots were centrifuged at 10,000 revolutions per minute for 20 minutes at 4°C (Eppendorf Centrifuge 5804, Hamburg, Germany). Sodium dodecyl sulfate (100 mL) was added to 8.1% 750 μL of 20% acetic acid and 750 μL of thiobarbituric acid to 8%. The mixture was heated for 50 minutes at 95°C. After the set period, samples were analyzed 200 μL of 532-mn spectrophotometer (Multiscan Ex, MTX Labsystems, Va, United States). All analyses were performed in duplicate.

Statistical Analysis

Data are presented as mean ± standard deviation. Analysis of variance test was used for comparison among groups. Statistical significance were considered for $P \leq .05$.

RESULTS

Morphologic analysis showed that cardiac tissue suffered injury because of intestinal I (2.0 ± 1.3) and I-R (3.5 ± 0.5) compared to control group (grade 0; $P < .001$). It was also observed after R that heparin (1.3 ± 0.5) or IPC (1.7 ± 0.7) modulates this lesion ($P < .001$; $P < .007$, respectively; Fig 1). However, the association of both strategies showed deleterious effect, as it can be observed in Table 1. Similarly, lipidic peroxidation was reduced by IPC or heparin, but not when these strategies were associated (Table 1).

DISCUSSION

These results suggest that IPC and heparin attenuated cardiac dysfunction caused by intestinal I and I-R, but when used in association did not show beneficial effects.

Intestinal I-R is a life-threatening clinical complication that can lead to systemic inflammatory response syndrome for the translocation of inflammatory mediators, bacteria, and endotoxin from injured intestines and by immune system activation [15], but the exact pathophysiologic mediators