Ischemia With Preconditioning in Wistar Rats Maintains Mitochondrial Respiration, Even With Mild Hepatocellular Disturbance

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ABSTRACT

Introduction. In hepatectomy or liver transplantation, preconditioning is a procedure indicated to protect the organ from ischemia-reperfusion injury (I-R).

Objective. Evaluate the effect of preconditioning after hepatic I-R in Wistar rats, through mitochondrial respiration, liver histology, and profile.

Method. Twenty male Wistar rats, weighing on average 307.1 g, were anesthetized with sodium thiopental (25 mg/kg) intravenously and xylazine hydrochloride (30 mg/kg) intramuscularly. The animals were divided into 2 groups: the preconditioning group (PCG), which contained 10 animals, and the hepatic pedicle was isolated and submitted to clamping with microvascular clamp (10 minutes of ischemia and 10 minutes of reperfusion, followed by 30 minutes of ischemia and 30 minutes of reperfusion); and the simulated operation group (SOG), which contained 10 animals submitted to manipulation of the hepatic pedicle and observation for the same length of time, with blood collected for transaminase dosage measurements, and liver biopsy for evaluation of mitochondrial respiration and histologic liver analysis and after sacrificed under anesthesia. The project was approved by the Ethics Committee on Animal Experimentation CEEA/UNICAMP under protocol number 3905-1.

Result. The PCG mitochondria showed the same respiration level as the SOG, when stimulated with the addition of adenosine diphosphate or carbonyl cyanide p-trifluoromethoxyphenylhydrazone. In the respiratory control ratio and resting of velocity of respiration the groups behaved in a similar way. The PCG presented high aspartate and alanine transaminases ($P < .03$) and about 60% of sinusoidal congestion and venous congestion in the histologic analysis when compared with SOG.

Conclusion. We found that ischemia with preconditioning in Wistar rats can lead to mild histologic and biochemical dysfunction without leading to impairment of mitochondrial respiration.

TO control blood loss during surgery or hepatic transplantation, portal triad occlusion is used [1]. Blocking and unblocking resulting from hepatic ischemia and reperfusion (I-R) causes I-R lesion, which impairs liver regeneration, and is one of the main causes of morbidity and mortality during hepatic surgery and transplantation [2–6].

An alternative to minimize these lesions from I-R is ischemic preconditioning, in which a brief I-R period is applied before prolonged ischemic blockade [3,5,7]. In hepatectomy or liver transplantation, ischemic preconditioning is a procedure indicated as a protector of I-R lesions [2,3,7].

However, I-R injury is a complex phenomenon and has not been clearly explained; it leads to increased production

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of reactive oxygen species and microvessel occlusion, compromising the mitochondrial function that is responsible for cellular bioenergetics (which can trigger cell death) [2–13]. Thus the study of mitochondrial respiration compared with enzymatic alterations and hepatic histology to evaluate ischemic preconditioning in Wistar rats is of great value.

METHODS

This intervention study's procedures were performed in a random way at the Nucleus of Experimental Medicine and Surgery (NMCE), at the Faculty of Medical Sciences of the State University of Campinas (FCM Unicamp). The surgical interventions were performed at the Laboratory of Experimental Liver Transplantation, FCM Unicamp. The preparation of the blades for the histologic analysis was carried out in the Laboratory of Pathological Anatomy, Unicamp, and the biochemical tests were carried out at the Laboratory of Clinical Pathology of the Hospital de Clínicas of Unicamp (Laboratory of Pathological Anatomy, Unicamp). The mitochondrial tests were processed in the Bioenergetics Laboratory, FCM Unicamp of the NMCE. The project was approved by the Ethics Committee on Animal Experimentation CEEA/UNICAMP under protocol number 3905-1.

Twenty healthy adult male Wistar rats with an average weight of 307 ± 43 g, aged 8 weeks, of the lineage *Rattus norvegicus albinus*, Rodentia, Mammalia were used. These were provided by the Center for Bioterism of Surgery, Unicamp and kept in the vivarium of the NMCE, with a maximum of 5 animals in each cage, under controlled ambient conditions of luminosities and temperature, in daylight cycles. Animals received standard diet and water ad libitum until the end of the research.

The animals were randomly divided into 2 study groups for surgical intervention of animals under normal conditions; after 8 hours fasting, animals were weighed and anesthetized with thiopental sodium 25 mg/kg (Cristália Pharmaceutical Chemical Ltda, Campinas, Brazil) intravenously, via the caudal vein with a sterile intravenous infusion device (23 gauge) and xylazine hydrochloride 30 mg/kg (Rhobifarma Pharmaceutical Ltda, Hortolândia, Brazil) intramuscularly. Anterior abdominal wall trichotomy with shear thinner Oster Golden A 5-model 5-5-55J (Madison, Wisc, United States) and abdominal skin antisepsis with 10% iodinated polyvinylpyrrolidone solution (Johnson Wax Ltda, Rio de Janeiro, Brazil) were performed. The animals were immobilized in dorsal decubitus on an appropriate surgical board. The abdomen was opened by U incision [10]. Oxygen was given in a continuous flow of 2.0 L/min, supplied in a semiopen plastic bell, favoring breathing throughout the experiment. After 40 minutes of experimentation, 1.0 mL of 0.9% saline solution was administered into the peritoneum (one time only).

In the ischemic preconditioning group (PCG) of 10 animals, the hepatic pedicle was isolated and submitted to clamping with vascular microclamp for 10 minutes of ischemia and 10 minutes of reperfusion, followed by 30 minutes of ischemia and 30 minutes of reperfusion.

In the simulated operation group (SOG), the 10 animals were submitted to manipulation of the hepatic pedicle at equivalent times to the I-R period of the ischemic group.

After the experiment, blood samples were collected for transaminase dosages and hepatic biopsies of the left lobe were carried out to evaluate mitochondrial respiration and histologic analysis. In both groups the sacrifice of the animals was performed through cardiac section, with the animals still anesthetized.

Blood samples were centrifuged (Centrifuga Fanem-206-R, Sao Paulo, Brazil) at 2500 revolutions per minute for 10 minutes; the serum was separated into microtubes, kept on ice and sent to the Laboratory of Pathological Anatomy Unicamp, for serum dosages of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These were performed by a kinetic enzymatic method with automated equipment (Modular P800 Evo Manufacturer: Hitachi High, Technologies Corporation, Tokyo, Japan).

For the histologic analysis of the liver stained in hematoxylin and eosin and Masson's trichomone, the reading of the slides was performed by a single pathologist in a blind study.

For the mitochondrial evaluation, a biopsy measuring 2.0 × 1.0 × 2.0 cm was taken from the left lobe, which was placed in 5 mL of preservation solution containing 2.77 mmol/L CaK_2 EGTA, 7.23 mmol/L K_2 EGTA, 6.65 mmol/L $MgCl_2.6H_2O$, 15 mM Na_2pospho-creatine, 20 mmol/L imidazole, 0.5 mmol/L dithiothreitol and 50 mmol/L MES in 1.0 L with pH 7.1 in a beaker and maintained at 4°C in a Styrofoam cooler with crushed ice. This biopsy was processed using a precision automatic cutter (Chopper) to make homogenous cuts in microscopic pieces.

To measure the oxygen consumption of the biopsies, a high-resolution Oroborus (Innsbruck, Austria), with magnetic stirrer and regular temperature of 37°C was used. Two randomly chosen samples were collected (mean weight, 4.0 and 6.0 mg) and inserted into the vats of the apparatus together with 2.1 mL of MIRO5 reaction medium solution containing 0.5 mmol/L EGTA, 3 mmol/L $MgCl_2.6H_2O$, 60 mmol/L K-lactobionate, 20 mmol/L taurine, 10 mmol/L KH_2PO_4, 20 mmol/L HEPES, 110 mmol/L sucrose, and 1 g/L bovine serum albumin in 1.0 L with pH 7.2. Then mitochondrial respiratory activity was analyzed by the sequential addition of 20 μL of pyruvate, 20 μL malate, 20 μL of 42 mmol/L adenosine diphosphate (ADP) solution, 2 μL of 1 mg/mL oligomycin solution, and 0.8 μL of 1 mmol/L solution of carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP).

Descriptive analysis was used for ordinal variables and the nonparametric Mann-Whitney test for continuous variables. The level of significance was 5%. The program Sigma Stat version 3.5 (Systat Software, Chicago, Ill, United States) was used.

RESULTS

No deaths were observed during the experiment. In the mitochondrial evaluation, the PCG had the same level of respiration when compared with SOG, both when stimulated with the addition of ADP and with the addition of FCCP. In the resting state with addition of oligomycin, there was also no significant difference in the velocity of mitochondrial respiration between PCG and SOG. The respiratory control as the ratio FCCP to oligomycin did not show a significant difference ($P > .05$) when the groups were compared (Fig 1). The serum values of AST and ALT showed significant increases of ($P < .03$) in PCG when compared with the SOG (Table 1).

Histologic evaluation of the liver of the PCG rats showed that 60% of the animals had variable degrees of sinusoidal congestion and venous congestion, but these changes were not observed in the SOG (Fig 2).

DISCUSSION

In this study we evaluated the effect of preconditioning after hepatic I-R through mitochondrial respiratory activity, biochemical evaluation, and liver histology in Wistar rats.