Ex Situ Regeneration of Liver Remnants Hypothermically Preserved for 24 Hours

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ABSTRACT

Introduction. After partial hepatectomy (PH), the liver remnant (LR) shows a regenerative response, always keeping a percent relationship with the host. This process has been well described in the literature, but several aspects still need to be understood. There are no studies on hepatic LR regeneration during hypothermic preservation. Thus, the objective of the present study was to analyze LR regeneration after PH under conditions of hypothermal preservation.

Materials and Methods. Twenty adult Wistar rats were divided into 4 experimental groups: PHS (70% PH); PHP (70% PH of an organ perfused and preserved for 24 hours); PWL (perfused whole liver preserved for 24 hours); and NPWL (nonperfused whole liver). The liver was perfused with 250 mL Celsior solution with a catheter connected to a 1.30-cm-high liquid column. Hepatic tissue samples were submitted to immunohistochemical analysis for the evaluation of protein Ki67 expression, related to the mechanism of cell proliferation, to analysis of micro-RNA expression (miR-21 and miR-16) by real-time polymerase chain reaction, and to analysis of mitochondrial function. Nonparametric statistical analysis was used ($P < .05$).

Results. Ki67 analysis revealed that the PHP group showed 17.41% cell proliferation in LR ($P < .01$) compared to PHS (42.22%), PWL (11.43%), and NPWL (11.98%). miR-16 expression (proapoptotic) was found to be higher in the NPWL group compared to all others (PHS, PHP, and PWL), with a statistically significant difference between the NPWL group and the PHS and PHP groups.

Conclusion. The animals submitted to PHS and PHP presenting greater Ki67 expression showed low miR-16 expression, indicating a low apoptotic index. In summary, the LR showed ex situ regeneration even under hypothermal conditions. There are no similar data in the literature surveyed.

THE LIVER HAS AN EXTRAORDINARY CAPACITY to regenerate after various types of injuries, including partial resections of the organ [1–4]. After partial hepatectomy (PH), the liver remnant (LR) shows a regenerative response, always keeping a percent relationship with the host; through the portal vein, under conditions of portal hypertension, the LR receives the hepatotrophic factors responsible for hepatic trophism [4–6]. This process has been well described in the literature [1,4,7,8], but several aspects still need to be understood. There are no studies on hepatic LR regeneration ex situ during hypothermal preservation after liver resection, a condition in which the LR loses its bodily and hemodynamic relation with the host and is kept under conditions favorable to the maintenance of its tissue, physiologic, and biochemical vitality, which, however, are theoretically adverse to LR regeneration [9–12]. Thus, the objective of the present study was to analyze LR

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regeneration after PH under conditions of hypothermal preservation.

METHODS

Twenty adult Wistar rats were divided into 4 experimental groups: PWL (perfused whole liver preserved for 24 hours), NPWL (nonperfused whole liver). PHS (70% PH, with the LR maintained in situ); PHP (70% PH of an organ perfused and preserved for 24 hours).

The surgical procedure was carried out in a closed room with a controlled temperature of 23°C. All animals were submitted to general anesthesia with xylazine (Dopaser, Gepec, Belo Horizonte, Brazil)/ketamine (ketalar, Pfizer, São Paulo, Brazil) at the dose of 80/16 mg/kg administered intramuscularly. The anesthetized animal was placed in dorsal decubitus on a wooden support, with its paws fixed in extension. Bilateral subcostal laparotomy was then performed for identification of the abdominal organs, followed by bilateral thoracotomy for animal perfusion.

Hepatic perfusion was performed by hydrostatic pressure after puncture of the left ventricle with a catheter on a 22-caliber needle (Abocath, Becton Dickinson Ind, Cirúr Ltda, São Paulo, Brazil) connected to a 1.30-cm-high liquid column containing 250 mL of the perfusion solution, generating a pressure of approximately 90 mm Hg in order to perfuse the organ but without producing excessive pressure. Next, the right atrium was opened and, after perfusion was started, the intrahepatic vena cava was opened. The liver was perfused with 250 mL Celsior solution, perfusion was stopped, and the perfusate was retained in the hepatic parenchyma [13].

At the end of perfusion, total or partial 70% hepatectomy was performed and the organ was stored at 4°C. The animals were sacrificed with a lethal dose of the same anesthetic.

Hepatic tissue samples were submitted to immunohistochemical analysis for the evaluation of protein Ki67 expression related to the mechanism of cell proliferation [14], and to analyze micro-RNA expression (miR-21 and miR-16), the cDNA was synthesized using the High Capacity c-DNA archive Kit (Applied Biosystems, Foster City, CA, USA). Real-time polymerase chain reaction analysis of cDNA was performed in an ABI Prism 7500 Sequence Detection System using TaqMan Reaction Master Mix (Applied Biosystems), in accordance with the manufacturer's instructions. The U6 was used as endogenous controls (housekeeping) for miRNA reactions. The data were analyzed using the ABI-7500 Sequence Detection System software, and the variation of expression among samples was calculated by the $2-\Delta\Delta Ct$ method [15].

Preparation of Mitochondria

Isolation of liver mitochondria was performed by differential centrifugation as previously described [16]. Mitochondrial protein was determined by Coomassie method (Coomassie plus, Thermo Fischer Scientific Inc., Rockford, IL, USA).

Oxygen Consumption Assays

Oxygen consumption was analyzed polarigraphically with a homemade oxygraph constructed at Physics Institute of São Carlos (USP) equipped with Clark oxygen electrode. Succinate (5 mmol/L) was used as oxidizable substrate in 1.4 mL of medium with 125 mmol/L sucrose, 65 mmol/L KCl, 1 mmol/L $MgCl_2$, 2 mmol/L KH_2PO_4, 0.1 mmol/L EGTA, and 10 mmol/L Hepes-KOH, pH 7.4, and 2 mg of mitochondrial protein were used. State 3 respiration was induced with 400 mmol MgADP, and state 4 respiration was determined after phosphorylation of additional adenosine diphosphate (ADP). The ratio between state 3 and state 4 rates (respiratory control ratio [RCR]), which represents coupling between electrons transport and oxidative phosphorylation, was determined [17].

Mitochondrial Swelling

Transition of the inner mitochondrial membrane permeability was determined spectrophotometrically, at 540 nm, by decreasing the optical density (ΔDO) in medium containing 20 mmol/L calcium and phosphate 1 mmol/L [18].

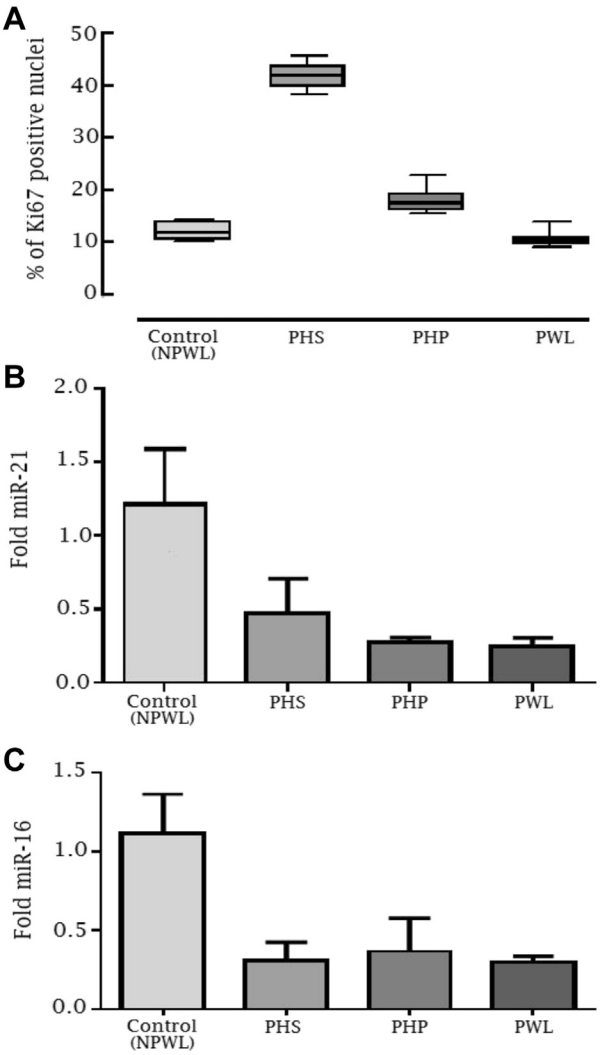

Fig 1. **(A)** Percentage of Ki67-positive nuclei in the different groups studied (relative to the control). **(B)** Fold miR-21 levels in the different groups analyzed: NPWL vs PHS, PHOP, and PWL groups ($P < .05$). **(C)** Fold miR-16 levels in the different groups analyzed: NPWL vs PHS, PHP, and PWL groups ($P < .05$). NPWL, nonperfused whole liver; PHS, 70% partial hepatectomy; PHP, 70% partial hepatectomy of an organ perfused and preserved for 24 hours; PWL, perfused whole liver preserved for 24 hours.