Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Analysis of liver fragment subjected to autologous transplant at rat's retroperitoneum

Mirla Fiuza Diniz, BSc, Sávio Lana Siqueira, PhD, Tiago Soares Baumfeld, MD, Luis Felipe Cintra Pereira, MD, Filipe Garcia Moreira, MD, Gustavo Meirelles Ribeiro, MD, and Iure Kalinine Ferraz de Souza, PhD*

School of Medicine, Federal University of Ouro Preto (UFOP), Ouro Preto, Brazil

ARTICLE INFO

Article history:
Received 14 January 2015
Received in revised form
22 May 2015
Accepted 11 June 2015
Available online 17 June 2015

Keywords:
Hepatic regeneration
Autotransplantation
Experimental surgery

ABSTRACT

Background: To investigate the regeneration process of autologous implants of liver on the retroperitoneum.

Methods: Thirty male Fisher rats were used divided in to group 1 (G1): studied 60 d after surgery; group 2 (G2): studied 90 d after surgery; group 3 (G3): studied 180 d after surgery; and group C (GC): animals without surgery. Hepatic fragment was processed for histologic and biochemical analysis.

Results: There was inflammatory infiltrate, diffuse hydropic degeneration, necrosis, and moderate fibrosis that reduced in direct relation to the postsurgical time. The concentration of albumin was different between GC and G1 and between G1 and G3 ($P = 0.0007$). The Catalase (CAT) was related to the time of surgery with GC being different when compared with G1, G2, and G3 ($P < 0.0001$). The oxidative stress measured through the thiobarbituric acid reactive substances lipid peroxidation was different between the GC and the G2 groups ($P = 0.0381$).

Conclusions: The analysis made showed hepatic regeneration in the fragment subjected to autologous transplant at the retroperitoneum.

1. Introduction

Since its first realization [1], organ transplant revolutionized medical history and saved thousands of patients. An example of that is liver transplant, which drastically improved prognosis, morbidity, and mortality of patients with terminal stage hepatic disease [2]. In the last 50 y, transplant has been the only treatment for patients with terminal stage hepatic disease [3].

Liver transplant has many complications, especially when it is from a living donor [4]. The most frequent complications are those related to transplant rejection and to the biliary tract. Of transplant patients, 10%–40% develop biliary complications associated with a mortality rate of 8%–15% [5,6]. To alleviate the high demand for liver transplants, the interest in liver cell therapy has been increasing continuously in recent years [7].

The necessity of intervening in the evolution of tumors and/or chronic diseases, especially those related to the hematopoietic system, led to a new therapy, the autologous transplant [8]. To avoid homologous transplant complications, among other benefits, studies on hepatic regeneration are necessary [9,10]. Thus, our aim was to investigate the regeneration on autologous transplant of liver tissue on rat's retroperitoneum, evaluating the viability and functionality of selftransplanted hepatic tissue.

* *Corresponding author*. Campus Morro do Cruzeiro, School of Medicine, Federal University of Ouro Preto (UFOP), Ouro Preto 35400 000, Brazil. Tel./fax: +5503199837286.

E-mail address: saviolanasiqueira@gmail.com (S.L. Siqueira).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2015.06.029

2. Materials and methods

2.1. Animals

Animal care and experimental procedures were approved by the Ethics Committee on Animal Use at the Federal University of Ouro Preto (UFOP), under protocol number 2010/047, and followed the rules established by the Brazilian Society of Laboratory Animal Science. During all experiments, animals were kept at room temperature (21°C ± 2°C) and under controlled light cycles at the vivarium of the School of Nutrition (UFOP), with food and drinking water *ad libitum*. Thirty Fisher rats, of the species *Rattus norvagius*, were used, all male, with an approximate weight of 350 g and an approximate age of 90 d. The rats were divided into three groups of 10 animals each: group 1 (G1) was composed of animals that were studied 60 d after surgery; group 2 (G2) was composed of animals that were studied after 90 d after surgery; group 3 (G3) was composed of animals that were studied 180 d after surgery. Group C (GC) was composed of five control animals.

2.2. Surgical procedure

Under an anesthesia of ketamine (80 mg/kg) and xylazine (7 mg/kg), the animal, after trichotomy and antisepsis, was subjected to a median supraumbilical longitudinal laparotomy of about 3–4 cm long, extending to the xiphoid process. It was then made the mobilization of the liver's left lobe, so the selection of the fragment (5 mm × 5 mm) to be biopsied could be made. After a simple stitch repair with a catgut 4-0 suture, at the vascular root of the segment, a wedge incision in the ischemic portion (triangular, with the base toward the hepatic edge) was made, observing a complete excision of it. Right after that, starting from the repair stitch, a Greek running suture was made to get the liver's edges closer together. The retroperitoneum was exposed, with the externalization of the animal's intestines. After visualization of the right kidney, a small incision in the retroperitoneum (root of the mesentery) was made, near the kidney's lower pole. The piece, already treated, was placed through the surgical orifice and a running suture was made in the retroperitoneum with a catgut 4-0 thread. At this stage, the anatomic structures were carefully observed, due to the delicacy of the organs and of the renal capsule, as well as to the possibility of ligature of the renal hilum and of the ureter.

2.3. Euthanasia and tissue collection

The animals' euthanasia occurred by overdose of anesthetics with sodium pentobarbital (100 mg/kg, intramuscular), after the respective postsurgery time of each group. After the administration of the anesthetic, animals were perfused with 0.9% saline solution, and the liver's right lobe, of the control animals, and the hepatic fragment selftransplanted (mean weight was 70 ± 5.2 mg) were collected from animals submitted surgery. The organs were each divided into two samples. The first sample was fixed in buffered formalin solution for at least 48 h as to be used in the histologic analysis, and the second sample was immediately frozen at −80°C as to be used in the biochemical analysis.

2.4. Histologic staining and morphometric analysis

After fixation, the tissue samples of the liver's right lobe and the hepatic fragment selftransplanted were processed according to routine histologic techniques and embedded in paraffin blocks. Paraffin blocks were sectioned using a microtome into 4-μm-thick sections, placed onto glass slides, and fixed for histologic staining. Staining was performed using hematoxylin-eosin and Masson trichrome stains.

All morphometric analyses were performed at the Multiuser Laboratory of the Research Center for Biological Sciences, Federal University of Ouro Preto. To count the number of inflammatory cells present in the hepatic lobes, measure the different liver areas, quantify the collagen fibers and the glycogen deposition, and determine the hepatic capsule thickness; we randomly obtained 20 images from histologic slides that were prepared from the liver sections. These slides were scanned using the Leica Application software and analyzed using the Leica Q-Win Plus software (Leica Microsystems, Inc, Buffalo Grove, IL) [11].

2.5. Biochemical analysis

The liver homogenates were used to assess the tissue-specific activities of antioxidant defense enzymes and oxidative damage, comparing the transplanted liver biopsy with liver fragments *in situ*. Catalase (CAT) activity was measured by the ability to convert hydrogen peroxide into water and molecular oxygen and detected as a decrease in absorbance at 240 nm [12]. The total protein content of the samples was verified by the Lowry technique [13]. The thiobarbituric acid reactive substances (TBARS) assays to quantify malondialdehyde levels were performed by heating the samples in the presence of thiobarbituric acid and measuring the absorbance of the supernatant at 532 nm [14]. Albumin was measured by immunonephelometry at 630 nm [15].

2.6. Statistical analysis

The descriptive statistical analysis and inferences were made by the Prism 5.0 software (GraphPad Software, Inc, La Jolla, CA). All data were represented with mean and standard deviation, being the evaluation of the data parametric distribution made with the normality test. For comparison of the three groups, the analysis of variance was used for parametric samples and the Kruskal–Wallis one-way analysis of variance for nonparametric data, followed by *post hoc* Dunn test. The statistical significance was 5% ($P < 0.05$).

3. Results

3.1. Histologic analysis

The histologic analysis was based on four parameters that evidence the process of hepatic regeneration: inflammation,