Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Spleen function after preservation in a physiological solution

*Argos Soares de Matos Filho, MD, PhD, and Andy Petroianu, MD, PhD**

Department of Surgery, School of Medicine of the Federal University of Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Article history:
Received 17 December 2014
Received in revised form
26 May 2015
Accepted 29 May 2015
Available online 5 June 2015

Keywords:
Spleen
Autogenous splenic implant
Trauma
Damage control
Lactated Ringer solution

ABSTRACT

Background: The purpose of this study was to evaluate the morphology and function of implanted autogenous spleen tissue after 24 h of preservation in a physiological solution.
Material and methods: Thirty-five male rats were divided into seven groups ($n = 5$): group 1, without surgical procedure; group 2, total splenectomy; group 3, total splenectomy and immediate implant of autogenous spleen tissue; group 4, total splenectomy and preservation of the entire spleen in lactated Ringer solution at room temperature for 24 h, followed by spleen sectioning and implantation; group 5, total splenectomy, followed by spleen sectioning and preservation in lactated Ringer solution at room temperature for 24 h and subsequent implantation of the slices; group 6, total splenectomy and preservation of the entire spleen in lactated Ringer solution at 4°C for 24 h, followed by spleen sectioning and implantation; and group 7, total splenectomy, the spleen was sliced and preserved in lactate Ringer solution at 4°C for 24 h, followed by implantation of the slices. After 90 d, scintigraphic studies using sulfur colloid labeled with 99mTc of the liver, lungs, spleen, implants, and a blood clot were performed. Hematological (erythrogram, leukogram, and platelets) and histologic studies were carried out.
Results: The autogenous splenic implants regenerated in all animals that received those implants preserved at 4°C and immediately after excision. The scintigraphic study showed a better phagocytic function in groups 1, 3, 6, and 7. No difference was observed in the hematological study.
Conclusions: Spleen tissue preserved in lactated Ringer solution at 4°C for 24 h maintains its vitality and capacity to recover hematological and phagocytic functions.

1. Introduction

Damage control abdominal surgery primarily focuses on the control of active hemorrhage and addressing any gastrointestinal spillage, followed by a temporary abdominal closure to facilitate reexploration at a later time. This addresses any immediately life-threatening pathology but defers more prolonged exploration and definitive reconstructions until the patient has been adequately resuscitated and "normalized." After 24–72 h, a second surgical process is performed to repair the damage [1–4]. Total splenectomy is usually necessary to treat splenic damage, as it is a procedure that can save the life of the patient; however, it may be followed by complications due to asplenism. In this case, the total removal of the spleen is still the standard operation for most trauma settings. Conservative treatment, such as partial or subtotal splenectomy or autologous spleen implants, may represent an option to avoid the well-known complications of asplenism, especially

* *Corresponding author*. Department of Surgery, School of Medicine of the Federal University of Minas Gerais, Avenida Afonso Pena, 1626 – apto, 1901, Belo Horizonte, MG 30130 005, Brazil. Tel./fax: +55 31 3274 7744 and +55 31 8884 9192.
E-mail address: petroian@gmail.com (A. Petroianu).
0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.05.058

in children and the elderly [5–9]. The spleen is responsible for 30% of the immunologic defense system in children and participates in the synthesis of complementary and immunoglobulin factors. This organ removes pyrogenes, foreign bodies, bacteria, fungi and viruses, as well as its role in hematopoiesis and hematological control is relevant [10–12]. The first autologous spleen implants are attributed to Griffini and Tizzioni (1883), who performed this type of surgery in dogs. This procedure was introduced to treat human splenic trauma in 1896, but it has not been widely adopted, as some studies have shown no benefit. However, our experience with splenic auto-implants, which began in 1985 and was used in treatments for portal hypertension, myeloid hepatosplenomegaly, Gaucher disease, leukemia, intense splenic pain, and trauma [13–17], has shown positive results, presenting none of the previously published complications. Therefore, splenic auto-implants represent a suitable option to preserve splenic function when splenectomy is unavoidable. The purpose of the present work was to study the morphology and function of autologous splenic tissue that had been implanted 1 d after the removal of the spleen and preserved in a physiological solution.

2. Materials and methods

This study was approved by the Ethics Committee for Animal Experimentation from Federal University of Minas Gerais (UFMG), registered under the protocol number 265/2008. Thirty-five Holtzman adult male rats (*Rattus norvegicus albinus*) were randomly distributed into seven groups ($n = 5$):

- Sham—without surgical procedure.
- Total splenectomy.
- Immediate autologous implant: total splenectomy and implantation of autologous splenic tissue on the greater omentum immediately after having been removed.
- Total spleen preserved: total splenectomy with the preservation of the full spleen in lactated Ringer solution at room temperature for 24 h; the spleen was then sliced and implanted on the greater omentum.
- Sliced spleen preserved: total splenectomy, followed by sectioning of the spleen into five segments, which were preserved in lactated Ringer solution at room temperature for 24 h; the spleen slices then were implanted on the greater omentum.
- Cold total spleen preserved: total splenectomy, with the preservation of the full spleen in lactated Ringer solution at 4°C for 24 h; the spleen was then sliced and implanted on the greater omentum.
- Cold sliced spleen preserved: total splenectomy, followed by sectioning the spleen into five segments, which were preserved in lactated Ringer solution at 4°C for 24 h; the spleen slices were then implanted on the greater omentum.

The rats were placed in appropriate cages, one per group, and received water and rations *ad libitum*. The rats of groups not sham received intramuscular anesthesia, using a mixture of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg). A total splenectomy was performed under anesthesia via a supraumbilical median laparotomy.

In an immediate autologous implant, the spleen was sectioned into five slices, three of which were immediately sutured on the greater omentum, using continuous 6-0 Prolene sutures (Brasuture Industry, São Paulo, Brazil). The omentum was then folded over itself to recover the splenic segments. The abdominal wall was closed in two layers, using continuous 2-0 silk monofilament sutures. In groups, the total spleen preserved, sliced spleen preserved, cold total spleen preserved, and 7, the spleen was placed in lactated Ringer solution according to the protocol defined for each group. After 24 h, the abdominal cavity was reopened, and the splenic slices were sutured on the greater omentum.

Ninety days after surgery, 1 mL of 99mTc (110MBq/3 mCi)-labeled sulfur colloid solution was intravenously injected. After 20 min, a scintigraphy study of the rat was achieved by means of a gamma camera [13,16,18–21]. Through a median laparotomy, a blood sample was collected from the abdominal vena cava for the hematological and protein studies. Samples of splenic, liver, and lung tissues, as well as a blood clot from the vena cava, were withdrawn. Each tissue was placed in a separate 20-mL plastic cup for the scintigraphic study, and a gamma camera was used to measure the dose of sulfur colloid removed by the tissue or remaining in the blood. A blood sample was collected from the vena cava for hematological study, including erythrogram, leukogram, and platelet counts. A splenic segment from each animal was stored in 10% buffered saline formaldehyde and subsequently processed for a histologic study.

The phagocyte function was estimated based on the radioactive counts per gram of each tissue. The samples were immediately weighed after their removal from the body. The radioactivity of the samples was calculated based on the count per minute (cpm) of the material per gram (g) of tissue [18–21]. This calculation considered the sum of radioactivity from the liver, spleen tissue, lungs, and blood clot samples from the same animal as being 100% of the radioactivity injected into the animal. The following formula [21] was used to calculate the percentage of capture of each tissue:

$$\% = \frac{\text{cpm/g of sample} \times 100}{\text{cpm/g of all samples}}$$

The data were presented as the mean and standard error of the mean. The Kolmogorov–Smirnov normality test, followed by the Bartlett test, was applied to compare the results from the different groups. When the data presented a normal distribution and similar variance, the analysis of variance test was performed, followed by the Tukey–Kramer multiple comparison test. When these values did not present a normal distribution, the Kruskal–Wallis test was used, followed by the Dunn–Bonferroni multiple comparison test. The results were considered significant for a confidence interval of greater than 95% ($P < 0.05$).

3. Results

All rats tolerated the surgical procedure and rapidly recovered to normal activity. Intraoperative or postoperative