Subtotal splenectomy preserving the lower pole in rats: technical, morphological and functional aspects[1]

Esplenectomia subtotal com preservação do pólo inferior em ratos: aspectos técnicos, morfológicos e funcionais

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ABSTRACT

Purpose: To assess the possibility of preserving the lower pole of the spleen, supplied by the inferior lobar vessels and segmental vessels, or by vessels of the gastrosplenic ligament, in subtotal splenectomy; to study the viability and function of the lower pole of the spleen. **Methods**: Thirty-six male Wistar rats were used in this study. Said animals weighed 273-390 g (355.2 ± 30.5 g), and were randomly distributed into three groups. Group 1 comprised ten animals which were submitted to exploratory laparotomy with spleen manipulation (sham operation). Group 2 comprised 16 animals which were submitted to total splenectomy. Group 3 comprised ten animals which were submitted to subtotal splenectomy, preserving the lower pole of the spleen. Blood was collected from all animals before and 90 days after surgery to measure the levels of cholesterol and triglycerides. The animals were sacrificed 90 days after surgery. Spleens and remaining spleens were removed for macroscopic and microscopic examination. **Results**: Surgery was performed with no complications in all groups. Six animals died in group 2. Spleens of groups 1 and 2, and lower poles of group 3 were macroscopically viable. Apparent white pulp hyperplasia was observed in group 1. In group 3, slight inflammation and capsular fibrosis were observed at the incision site, as well as diffuse hemosiderosis in the red pulp. Average mass of remaining spleen was 35.84% ± 4.31%. No significant difference was observed between preoperative and late postoperative lipid levels in groups 1 and 3 ($p > 0.05$). Late postoperative lipid levels significantly increased in group 2. **Conclusions**: Preservation of the lower pole of the spleen (supplied by gastrosplenic vessels or inferior lobar vessels and segmental vessels) was possible with subtotal splenectomy. The lower pole was macroscopically and microscopically viable in all cases. Subtotal splenectomy preserving the lower pole prevented changes in lipid levels, which were observed in rats submitted to total splenectomy. Plasma lipid levels in rats submitted to subtotal splenectomy were similar to those observed in sham operated rats.
Key words: Spleen. Splenectomy. Rats.

RESUMO

Objetivo: Avaliar a exequibilidade de preservação do pólo inferior suprido por vasos lobares inferiores e segmentares ou por vasos no ligamento esplenogástrico, na esplenectomia subtotal, e estudar a viabilidade e a função desse pólo. **Métodos**: Foram utilizados 36 ratos machos, Wistar, com peso entre 273 gramas e 390 gramas (M.A 355,2 ±30,5), distribuídos aleatoriamente em 3 grupos : grupo 1- 10 animais submetidos à laparotomia com manipulação do baço (operação simulada); grupo 2- 16 animais submetidos à esplenectomia total ; grupo 3- 10 animais submetidos à esplenectomia subtotal com preservação do pólo inferior. Em todos os animais foi colhido sangue no pré-operatório e no 90º P.O para dosagem do colesterol e frações e triglicérides. Os animais foram mortos após 90 dias e o baço e o remanescente esplênico foram retirados para estudo macro e microscópico. **Resultados**: As operações nos três grupos foram realizadas sem dificuldades. Houve 6 óbitos no grupo da esplenectomia total. Os baços dos grupos 1 e 2 e os pólos inferiores do grupo 3 estavam macroscopicamente viáveis. Houve uma aparente hiperplasia da polpa branca no grupo simulação O pólo inferior apresentou áreas discretas de inflamação e fibrose capsular na área da incisão e hemossiderose difusa na polpa vermelha. O percentual médio de massa remanescente do pólo inferior foi 35,84% ± 4,31%. Não houve alterações significantes nos níveis de colesterol e frações e triglicérides no pós-operatório

tardio em relação ao pré-operatório (P>0,05) nos grupos 1 e 3. No grupo 2 houve aumento significante do colesterol e frações e triglicérides no pós-operatório tardio. **Conclusões**: A preservação do pólo inferior suprido por vasos lobares inferiores e segmentares ou vasos no ligamento esplenogástrico foi exeqüível na esplenectomia subtotal. Esse pólo manteve-se macro e microscopicamente viável em todos os casos. A esplenectomia subtotal com preservação do pólo inferior previne contra as alterações dos níveis de lípides plasmáticos observadas em ratos submetidos à esplenectomia total, e permite a manutenção dos níveis de lípides semelhantes aos do grupo simulação.
Descritores: Baço. Esplenectomia. Ratos.

Introduction

The spleen was considered unnecessary for life for many centuries (Aristotle *apud* Cooper)[1]. Splenectomy was performed by surgeons regardless of the severity of the trauma or disease. Local and systemic complications, however, would result from this surgical procedure. Overwhelming post-splenectomy infection (OPSI) is the most feared complication[2]. King and Shumacker Jr[3] reported that 5 children submitted to splenectomy due to spherocytosis died in consequence of OPSI. Infectious complications resulting from splenectomy have also been observed in experimental animals[4,5]. In addition to these complications, lipid metabolism disorders, which may lead to atherosclerosis[7], have been reported in humans[6] and laboratory animals[7,8,9,10]. For these reasons, procedures that preserve the spleen have been increasingly chosen over total removal of the organ. Some of these surgical procedures include: a) splenorrhaphy[11,12]; b) ligation of the splenic vessels in cases of splenic trauma[13], portal hypertension[14], splenic artery aneurysm[15], and distal pancreatectomy[16], whose consequences to the spleen have been recently described[17]; c) spleen transplantation[18,19]; d) partial splenectomy in humans and experimental animals[20], and subtotal splenectomy, preserving either the upper pole[21] or the lower pole of the spleen[22]. Subtotal splenectomy preserving the lower pole has been recently described in dogs[22] and rats[9]. In a previous study[9], it was observed that the lower pole of the spleen received two major types of vascular supply: vessels of the gastrosplenic ligament and the inferior lobar vessels. Thus, it was realized that ligation of the inferior lobar vessels should be performed in two different ways to preserve vascular supply to the lower pole of the spleen. Unfortunately, no detailed information on the morphology and function of the lower pole is available in the related literature. The purpose of this study was: a) to assess the possibility of preserving the lower pole of the spleen, supplied by the inferior lobar vessels and segmental vessels, or by vessels of the gastrosplenic ligament, in subtotal splenectomy; and b) to study the viability and function of the lower pole of the spleen.

Methods

The use of animals in this study was in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA); the Helsinki Declaration of 1964 and its later versions of 1975, 1983 and 1989; the principles of the International Council for Animal Protection ; and Resolution nº 196/96 of the Brazilian National Health Council. Thirty-six male Wistar rats were used in this study. The animals were donated by the laboratory animal facility of the School of Science, Santa Casa de Misericórdia (EMESCAM), Vitória, Brazil. Said animals weighed 273-390 g and were randomly distributed into three groups. Group 1 comprised ten animals which were submitted to exploratory laparotomy with spleen manipulation (sham operation). Group 2 comprised 16 animals which were submitted to total splenectomy. Group 3 comprised ten animals which were submitted to subtotal splenectomy, preserving the lower pole of the spleen, followed by ligation of the splenic artery and vein. Anesthesia for blood collection was performed using ethyl ether. Animals were placed in a bell jar with a cotton ball soaked with ether. After induction of anesthesia the rats were removed from the jar and submitted to cardiac puncture. A total of 1.2 mL of blood was collected to measure levels of cholesterol (total cholesterol, LDL, HDL and VLDL) and triglycerides. Animals were submitted to surgery approximately three days after blood collection. The following steps were taken: induction and maintenance of anesthesia with ethyl ether; shaving of the anterior abdominal and thoracic walls; antisepsis with 2% iodine and creation of a sterile field to delimit the region where the laparotomy was to be performed; midline incision of the skin and subcutaneous tissue, of approximately 2.5 cm in length (extending from 0.5 cm below the xiphoid process to the pubic symphysis); scission of the linea alba and peritoneum; inspection of the abdominal cavity. Each group was then submitted to a different surgical procedure. Group 1 was submitted to spleen manipulation. Group 2 was submitted to total splenectomy as follows: the stomach was exteriorized from the peritoneal cavity to expose the spleen; three ligatures were placed close to the spleen using 4-0 monofilament suture for the section of the vessels; the spleen was removed and sent for histopathological examination. In group 3, mobilization of the spleen was followed by devascularization of the upper and middle portions of the organ, which was sectioned just below the inferior polar vessels and segmental vessels. The lower pole was preserved (Figure 1a). The cut surface was sutured with two simple interrupted stitches using 5-0 prolene suture. When the inferior lobar vessels and their branches entered the lower pole, they were ligated and sectioned approximately 0.5 cm above the distal surface of the lower pole and approximately 0.5 cm from the hilar surface of the spleen (Figure 1b). Thus, the distal stump of those vessels could be anastomosed to the gastrosplenic ligament vessels to maintain blood supply to the lower pole (Figure 1b). Figure 2 shows the details on the vessels that supply the lower pole. After each procedure, the abdominal wall was sutured in two layers: first, the peritoneum and the muscle aponeurosis, then the skin. Continuous over-and-over suture with 4-0 monofilament was placed.

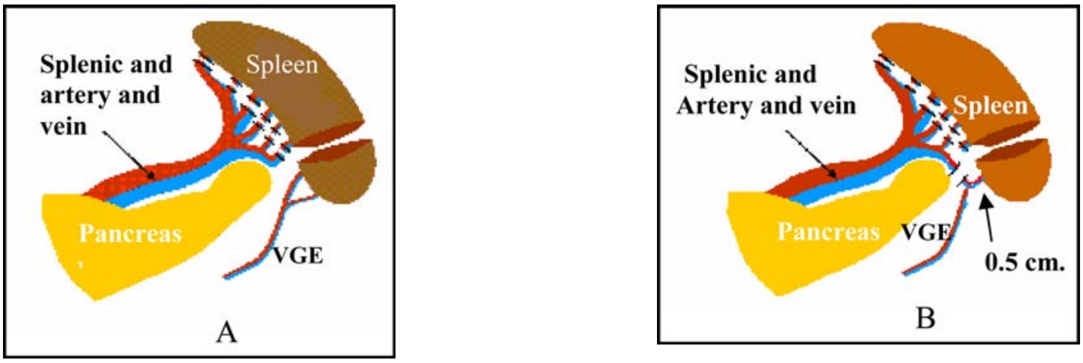

FIGURE 1 - Schematic illustration showing the two possible techniques for subtotal splenectomy, preserving the lower pole. Ligation of the segmental vessels is performed in the upper and middle portions of the spleen. To maintain blood supply to the lower pole, it is necessary: A – to preserve the vessels in the gastrosplenic ligament (GSV) when they supply the lower pole of the spleen; or B – to preserve 0.5 cm of distal stump of the inferior lobar vessels (arrow) to be anastomosed with vessels of the gastrosplenic ligament when the lower pole is mainly supplied by the inferior lobar vessels

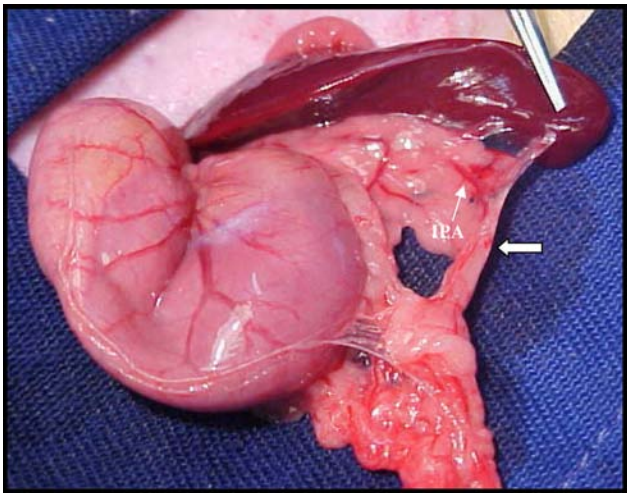

FIGURE 2 - Details on the vascular supply of the lower pole. Note the vessels in the gastrosplenic ligament (arrow) which are anastomosed to the inferior lolar artery (IPA) and will provide blood supply the lower pole after subtotal splenectomy with ligation of the splenic vessels (narrower arrow)

The animals were tagged and placed in collective cages with wood chips on the bottom and metal bars on the top. Each cage housed six animals, which were fed with laboratory animal feed and had water *ad libitum*. The animals were kept in these conditions for 90 days. After this period of time, more blood was collected to measure the levels of cholesterol and triglycerides. The rats were then sacrificed with a lethal dose of ethyl ether. An inverted-U incision was made in the abdominal wall, followed by examination of adhesions and abdominal viscera – particularly the macroscopic appearance of the spleen (group 1) and lower pole (group 3). The spleen of group 1 animals and the lower pole of the spleen of group 3 animals were removed, weighed on a 0.001 g precision balance, photographed and fixed in 4% formaldehyde solution. Specimens were transversely sectioned and three to eight slices (2 mm and 3 mm thick) were obtained from each specimen. The slices were embedded in paraffin and stained with hematoxylin-eosin. Microscopic examination was performed by a pathologist with a binocular microscope. Morphological alterations were analyzed.

Statistical analysis

Descriptive statistics was applied to calculate the mean and standard deviation for the following variables: rat weight, levels of cholesterol and triglycerides. To calculate the mass of remaining splenic tissue in group 3, the weight of the lower pole was divided by the ideal weight of the spleen for each animal, and the result was multiplied by 100. The ideal weight of the spleen was calculated by applying the following formula: weight of the spleen = 1.80 x body weight + 230.49. The mean and standard deviation for the mass of remaining spleen were calculated. Student's *t*-test for related samples was applied to compare preoperative and late postoperative levels of cholesterol (total cholesterol, LDL, HDL and VLDL) and triglycerides. Student's *t*-test for independent samples was applied to compare cholesterol (total cholesterol, LDL, HDL and VLDL) and triglycerides levels between groups. The p values were considered statistically significant when $p \leq 0.05$ (5%).

Results

No complications resulting from anesthesia or surgery were observed. Weight gain was observed in all animals. Mean mass of the remaining spleen (group 3) was 35.84% ± 4.13%. Six animals died in the total splenectomy group (group 2); no animal died in the partial splenectomy group (group 3) or in the sham operated group (group 1). Examination of the abdominal and thoracic cavity of the dead animals, as well as culture of blood, lung tissue and liver tissue, did not show the possible cause of death. The dead animals were excluded from the study and replaced by other ones. In group 1 animals, loose adhesions were observed between the spleen and the stomach, the liver and the abdominal wall. In group 2 animals, more adhesions, which were also more severe, were observed. Infection of the skin and subcutaneous tissue was observed in two animals from group 2. In group 3, adhesions were observed between the remaining spleen and the omentum, the adjacent viscera and the abdominal wall. In this group, partial wound dehiscence was observed in two animals.

The spleen of the animals from groups 1 and 2, and the remaining spleen of the animals from group 3 were macroscopically viable. Vascular adhesions between the greater omentum and the remaining spleen were frequently observed (Figure 3).

Microscopic examination of the specimens revealed normal histological appearance in all cases. Apparent white pulp hyperplasia was observed in specimens from group 1. In group 3 (subtotal splenectomy), slight inflammation of the remaining spleen and capsular fibrosis were observed at the incision site, as well as hemosiderin deposits in the red pulp (Figure 4). Cholesterol and triglycerides levels are shown in graph form (Figure 5). No significant difference was observed between preoperative and postoperative levels in groups 1 and 3, with the exception of HDL, whose postoperative levels increased significantly in group 3 ($p = 0.03$). In addition, postoperative LDL levels decreased in group 3 ($p = 0.06$). In group 2, postoperative levels of cholesterol and triglycerides increased significantly ($p < 0.01$).

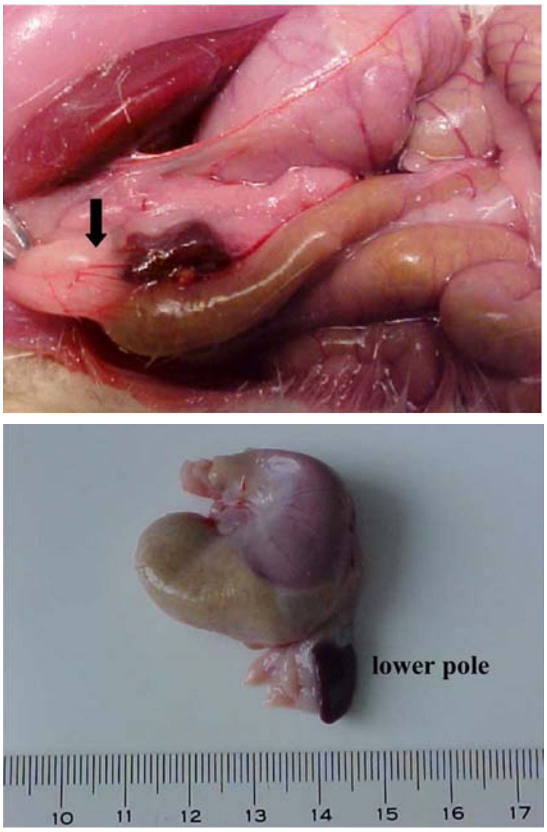

FIGURE 3 - Macroscopic appearance of the lower pole 90 days after subtotal splenectomy. Note: a) vascular adhesions between the greater omentum and the lower pole of the spleen (arrow); b) appearance of the lower pole

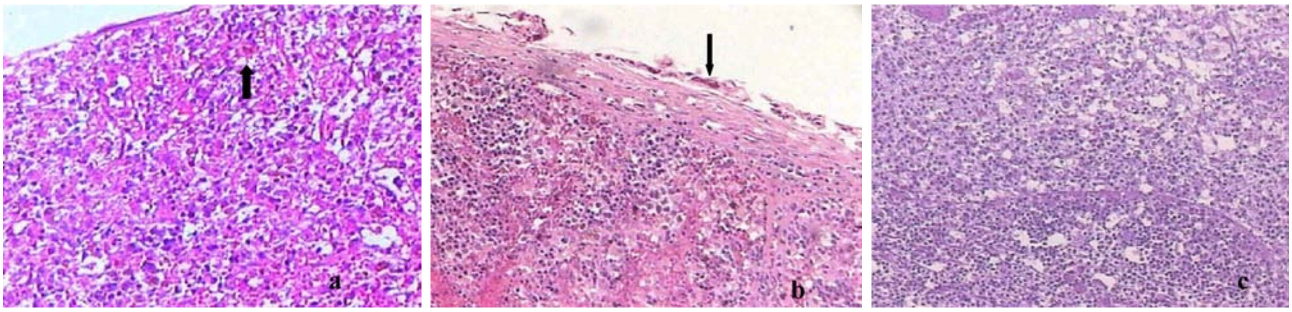

FIGURE 4 - Microscopic appearance of the spleen a) diffuse hemosiderosis (arrow) in animals submitted to subtotal splenectomy, preserving the lower pole; b) capsular fibrosis (arrow) at the incision site in animals submitted to subtotal splenectomy, preserving the lower pole; c) white pulp hyperplasia in the sham operated animals (group 1)

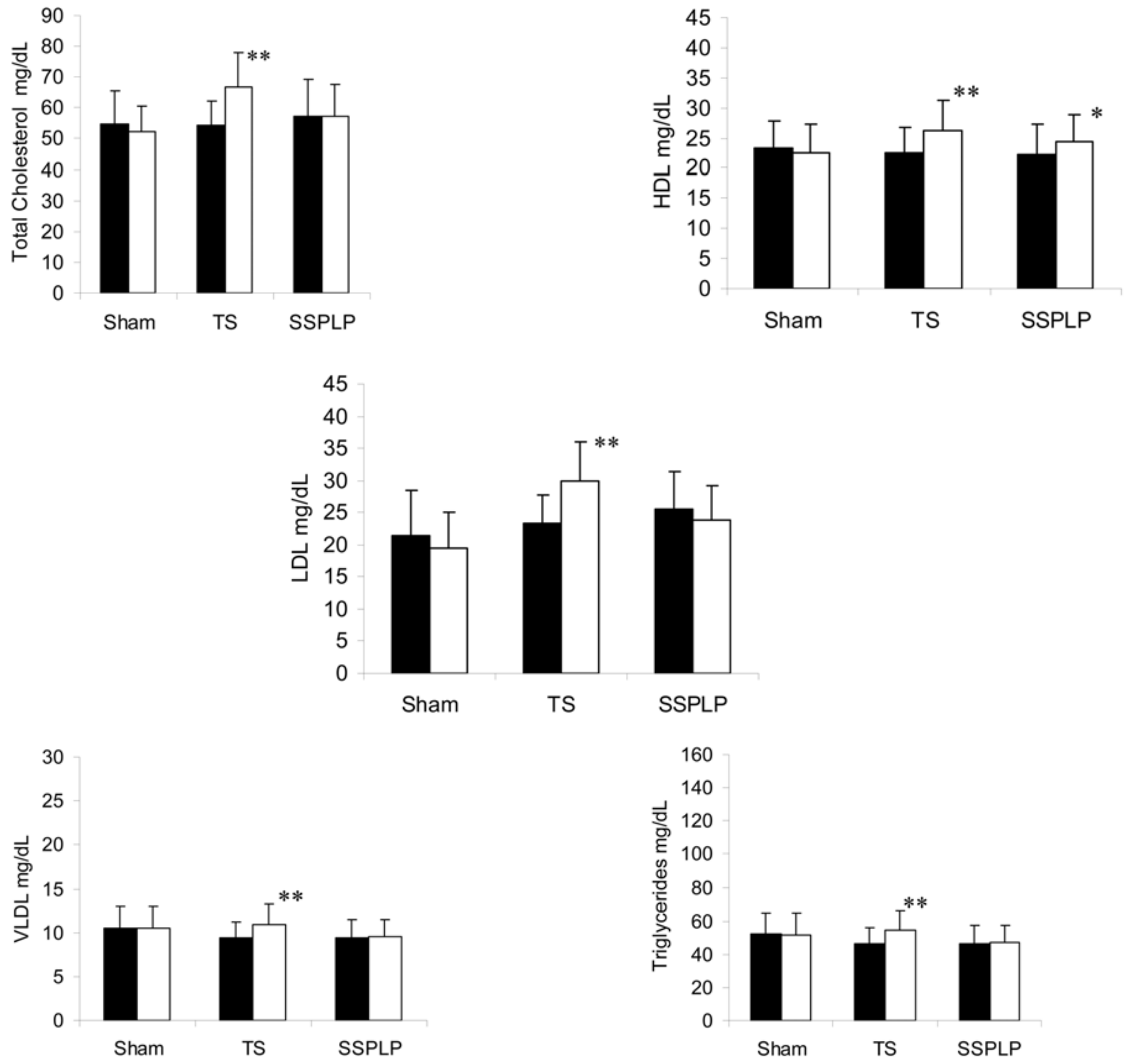

FIGURE 5 - Preoperative (black column) and late postoperative (white column) lipid levels (total cholesterol, HDL, LDL, VLDL, triglycerides) in animals submitted to sham operation (sham), total splenectomy (TS), and subtotal splenectomy preserving the lower pole (SSPLP). Student's *t*-test for related samples. *$p<0.05$; **$p<0.01$. Means (columns) and standard deviations (symbols) are shown on the graph

Discussion

Although its use has been increasingly discouraged, anesthesia with ethyl ether proved safe and effective. However, it is necessary to carefully control the administered dose not to cause drug-induced respiratory depression or death. The animals recovered soon after ether inhalation was interrupted. Spleen manipulation was performed with no difficulty. Subtotal splenectomy preserving the lower pole of the spleen was performed as it was previously done in dogs[22] and rats[9]. It is important to observe where the inferior lobar vessels enter the spleen in order to adequately plan vessel ligation. As we have reported, the inferior lobar vessels should be ligated approximately 0.5 cm above the bottommost portion of the lower pole, and 0.5 cm from the hilum. This should be done to guarantee blood supply to the organ, and consequently, its viability. There is experimental evidence that the spleen can be supplied by vessels that do not originate from the main splenic ones[23,24]. It seems to us that such vessels, in rats and dogs, can be found in the gastrosplenic ligament. Further studies should be conducted in order to shed a light on the matter. Macroscopic examination revealed that the spleen had normal appearance in all groups; microscopic alterations, however, were more evident in group 3. More severe adhesions were observed in groups 2 and 3. This is entirely justifiable because the factors that commonly cause adhesions were more frequent in those groups[25]. In a recent study, Paulo *et al.*[9] performed subtotal splenectomy preserving the lower pole of the spleen in rats. They calculated the ideal weight of the spleen by applying the formula: weight of the spleen = 1.80 x body weight + 230.49. This formula was devised after linear regression analysis of the weights of spleens in function of the body weight of animals in the control group. The mean mass of the remaining spleen was then calculated, and the value obtained was

37.26%±3.77%. In our study, the value obtained was 35.84%±4.13%. Bradshaw and Thomas[26] reported that the larger the remaining splenic tissue the stronger the protection against sepsis. According to them, phagocytosis will not be affected if at least 25% of splenic tissue is preserved. Their report is in accordance with those of most authors[27]. Van Wick *et al.*[28], in an experimental study using rats, reported that 1/3 of the spleen had to be preserved for normal spleen function. An average of 35.84% of splenic tissue was preserved in our study, which may justify the fact that postoperative levels of plasma lipids did not change in group 3. It is possible that subtotal splenectomy may cause ischemia of the lower pole of the spleen. Considering that ischemia induces adhesion formation, which in turn leads to angiogenesis, an ischemic pole could, with time, become well vascularized. In our study, macroscopic and microscopic findings such as vascular adhesions, inflammation areas and capsular fibrosis suggested ischemia. On the other hand, the remaining splenic tissue proved viable (macroscopically and microscopically) 90 days after surgery, and continued to play its role in lipid metabolism. In group 1, when preoperative and late postoperative lipid levels were compared, no significant difference was observed. This shows that sham operation did not affect lipid levels. Analysis of preoperative and late postoperative lipid levels in group 2 showed increased cholesterol and triglyceride levels, which is in accordance with findings reported in the literature[6,7,8,9]. It is important to mention that late postoperative levels of total cholesterol and LDL were significantly higher in group 2 when compared with group 1 ($p < 0.01$). However, no significant difference was observed between the two groups when preoperative lipid levels were compared ($p > 0.05$). This shows that total removal of the spleen increased plasma lipid levels. Caligiuri[29] has recently reported that this increase is due to immunologic factors. In group 3, when preoperative and late postoperative lipid levels were compared, no significant difference was observed. However, we observed an increase in HDL levels ($p = 0.03$) and a decrease in LDL levels, which contributed to the maintenance of cholesterol levels. When preoperative and late postoperative levels of cholesterol and triglycerides were compared between groups 1 and 3, no differences were observed ($p > 0.05$). This shows that the preservation of part of the spleen is important to lipid metabolism. When the spleen is completely removed, lipid metabolism is affected. In this study, the average mass of remaining tissue was 35.84% of the total weight of the spleen. When lipid levels were compared between groups 2 and 3, it was observed that changes in lipid levels were more substantial in the former. This shows that the remaining spleen can satisfactorily maintain its role in lipid metabolism. Further studies will be conducted to investigate: a) other functions of the lower pole of the spleen in the early and late postoperative periods; b) details on the vascular supply of the spleen; c) resistance to infection in animals submitted to subtotal splenectomy preserving the lower pole.

Conclusions

Preservation of the lower pole of the spleen (supplied by gastrosplenic vessels or inferior lobar vessels and segmental vessels) was possible with subtotal splenectomy. The lower pole was macroscopically and microscopically viable in all cases. Subtotal splenectomy preserving the lower pole prevented changes in lipid levels, which were observed in rats submitted to total splenectomy. Plasma lipid levels in rats submitted to subtotal splenectomy were similar to those observed in rats submitted to sham operation.

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