Abstract: The aim of this study was to investigate the effects of splenic artery ligation on the phagocytic functions and morphology of the spleen in rabbits in which secondary hypersplenism was induced by splenic vein ligation (SVL).

Thirty New Zealand-type rabbits were included in this study. The rabbits were separated into three groups of 10 subjects each. The first group underwent laparotomy, and the second and third groups underwent laparotomy + splenic vein ligation. At the end of the fourth week, the first and second groups underwent laparotomy while the third group underwent laparotomy + splenic artery ligation. At the end of the eighth week, 1 cc of blood was taken from all rabbits to determine hematological parameters. The rabbits were administered with 0.1 mCi/kg of Tc-99m sulfur colloid, and retention rates in the liver and spleen were determined. The spleens of the rabbits were removed and examined histopathologically.

Median liver/spleen retention rates were found to be 26.2 count/pixel in the sham group, 29.1 count/pixel in group II and 55 count/pixel in group III. The retention rate in the spleen was significantly lower in group III (p = 0.0001). Leukocyte levels were similar among the groups while erythrocyte and thrombocyte levels were significantly lower in group II than in the other two groups (p = 0.0001). Histopathologically, wide congestion was observed in group II while extended fibrosis and necrosis were observed in the spleens of group III.

Although the application of SAL in secondary hypersplenism cases reduces the severity of hypersplenism, it fails to improve the phagocytic function of the spleen.

Key Words: Hypersplenism, Splenic vein ligation, Splenic artery ligation, Phagocytosis, Fibrosis.

Introduction

Although splenectomy is a preferred treatment modality in patients with primary hypersplenism, attempts to improve the underlying pathology are given priority in patients with secondary hypersplenism (1). Conservative methods have gained still more importance in the treatment of benign splenic pathologies since the role and importance of the spleen in the immune system became better understood (1-5).

Selective arterial embolization and splenic artery ligation (SAL) are employed in some centers in the treatment of secondary hypersplenism for the prevention of the destruction of blood elements, although they are not very commonly practiced (2,3,6). Despite the fact that there is currently increasing interest in procedures that protect the spleen, there are also studies reporting that such spleens fail to perform previous functions and that they lose their phagocytic functions (7-9).

The aim of this study was to investigate morphological changes in the spleen and determine the phagocytic capacities of the spleen following the SAL procedure in rabbits with induced hypersplenism.

Materials and Methods

This study was performed in the Selçuk University Experimental Medical Research Center in Konya with the permission of the Selçuk University ethics committee. Animal control oversight rules in accordance with Turkish law were followed in this study.

Thirty New Zealand-type rabbits with an average weight of 3550 ± 370 g were used. The rabbits were
divided into three groups containing 10 rabbits each. The animals were left hungry on the morning of the experiment, and were operated on under ketamine HCl anesthesia, administered intramuscularly at a dose of 80 mg/kg.

A midline laparotomy was performed after shaving the abdominal wall and cleaning it with betadine solution. The sham procedure was performed in group I and the splenic vein was ligated in groups II and III. The splenic vein was ligated from the splenic hilum in order to reduce collateral development to a minimum. Ligation was done using 3/0 silk, and the abdomen was closed with 2/0 continuous suture. Following the above procedures, the rabbits were placed in their cages at 22 °C ambient temperature, and were fed with rabbit diet for a period of 4 weeks.

Group 1: Laparotomy + sham procedure
Group 2: Laparotomy + splenic vein ligation
Group 3: Laparotomy + splenic vein ligation

At the end of the fourth week, the subjects were re-operated on under ketamine HCl anesthesia applied intramuscularly at the same dose. After opening the abdomen, the sham procedure was performed in groups I and II, and the splenic artery was ligated in group III. The splenic artery was ligated from the splenic hilum. The rabbits were returned to their cages, and were fed in the same manner for a period of 4 weeks.

Group 1: Laparotomy + sham procedure
Group 2: Laparotomy + sham procedure
Group 3: Laparotomy + splenic artery ligation

At the end of the eighth week, 1 cc blood samples were taken from the subjects and placed into glass tubes containing Na-EDTA. Afterwards, 0.1 mCi/kg Tc-99m sulfur colloid was injected intravenously into the rabbits; 15 min after the injection, imaging was performed in an anterior and posterior position using a low-energy, general purpose collimator (low energy all purpose—LEAP). Liver/spleen ratios were calculated by obtaining the geometrical averages of counts calculated from the front and rear views of the liver and the spleen (10). The rabbits were then sacrificed and their spleens removed for histopathological examination. The extracted spleens were kept in formalin solution and stained with hematoxylin-eosin and examined under a light microscope by a pathologist who was blinded to the composition of the groups. Hematological parameters were measured on the blood samples on the same day using an auto-analyzer (Technicon H-1, USA).

Statistical analyses were performed using Mann-Whitney U test and Kruskal-Wallis test, and the value of p < 0.05 was considered statistically significant.

Results

The leukocyte levels of the groups were similar to each other at the end of the eighth week (p = 0.968); however, the erythrocyte and thrombocyte levels of the second group were significantly lower than the levels in the first and third groups (p = 0.0001 and p = 0.0001 respectively). This decrease was interpreted as an indication of the successful induction of hypersplenism with splenic vein ligation (SVL). The similarity of the parameters in the SAL group to those in the sham group was interpreted as an improvement in hypersplenism following SAL (Table 1).

The medians of scintigraphic measurement ratios (liver/spleen) in the groups were, Group I: 26.2 count/pixel (20.0–36.3), Group II: 29.1 count/pixel (21.1–32.1) and Group III: 55.0 count/pixel (47.5–61.4). The average value in Group III was significantly higher than in the other groups (p = 0.0001), (Table 2). Accordingly, it was concluded that retention in the spleen decreased and that the liver/spleen ratio increased (Figure 1).

The histopathological examination revealed that the spleens of the rabbits in the control group maintained their normal structures. Examination of the spleens in group II revealed that veins and sinusoids were filled with thrombus and that there was a wide congestion (Figure 2). It was noted that trabeculae became thicker in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte, x10^3</td>
<td>9.5 ± 1.6</td>
<td>9.4 ± 1.9</td>
<td>9.6 ± 1.6</td>
<td>0.968</td>
</tr>
<tr>
<td>Erythrocyte, x10^6</td>
<td>5.2 ± 0.7</td>
<td>3.2 ± 0.5</td>
<td>4.7 ± 1.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Thrombocyte, x10^5</td>
<td>4.64 ± 0.4</td>
<td>2.98 ± 0.6</td>
<td>4.26 ± 0.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 1. Hematological parameters at the end of eight week.
spleen in group III, and that, in addition, there was an increase in fibrotic tissue, along with the presence of partially necrotized regions (Figures 3 and 4).

Discussion

The most frequent cause of secondary hypersplenism is portal hypertension developing due to liver cirrhosis. Stasis of the blood in the spleen as a result of resistance to venous blood flow of the spleen constitutes the underlying cause of the developing pathologies (1,6).

Experimental secondary hypersplenism may be induced in rats by ligating the spleen vein from the splenic hilum (5). It is reported in the literature that hypersplenism develops after ligation of the splenic vein in order to prevent acute splenic sequestration in sickle cell anemia, and also after splenic vein thrombosis in normal sized spleens (11,12). In addition, it is possible to induce hypersplenism successfully by injecting a gelatin sponge into the peritoneal cavity (13).

The first aim in the treatment of secondary hypersplenism is the correction of the underlying pathology. However, it is not always possible to improve primary pathology, and in such cases splenectomy becomes the only modality available (1). However, following better understanding of the importance and role of the spleen in the immune system, interest in spleen-protecting methods has grown. The most

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Liver/Spleen ratio of scintigraphic retention of the groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>440 /18²: 55.0 count/pixel (380/8:47.5 - 430/7:61.4)</td>
</tr>
</tbody>
</table>

1Liver scintigraphic retention value, ²Spleen scintigraphic value.
frequently used methods in this respect are splenic artery embolization and SAL (2-5,13).

SAL has started to attract attention again recently in the treatment of portal hypertension and the hypersplenism connected to it. It is applied in some centers with some limitation. However, clinical and experimental studies report controversial results (2,5,7,14).

In our study, SAL was applied at the end of the fourth week to rabbits that had developed thrombocytopenia and erythrocytopenia following SVL application (Group III). Following the procedure, it was observed that cell counts reached normal levels at the end of the eighth week, whereas cell counts in the SVL-applied group remained at low levels. In contrast with the increase obtained in the cell counts, one of the most controversial issues is the development of infarction in the spleen (15). It also claimed that it is not possible to remedy with the SAL procedure structural deformations in the spleen subjected to prolonged congestion (8,9,15).

Clayer et al. reported that phagocytic cells in the spleen could not perform normal phagocytosis following SAL, and that the spleen failed to recover its lost immune functions (8,9). Some investigators reported that the sensitivity of the spleen to pneumococci increased in hypersplenism in rats despite the fact that the spleen had been protected with SAL (7). Auto-transplantation of splenic tissue was recommended as an alternative to splenectomy (16), but it was shown that regenerated
splenic pulp contained significantly less white pulp and marginal zone, and the spleen did not have the phagocytic ability of normal splenic tissue (8,9,17,18). Decreased regrowth of the lymphoid compartments of the spleen was shown to be responsible for immune dysfunction (8,17). We noted congestion after splenic vein ligation and necrosis and fibrosis after splenic artery ligation; in this way spleen lost its lymphoid tissue.

Partial splenic embolization restored hypersplenism and blood tests returned to normal; this might be due to decreased spleen volume (18,19). Watanabe et al. (17) showed that infarction developed in splenic parenchyma after splenic arterial ligation and partial splenic embolization, and de-vascularization caused a reduction in spleen volume. It was shown that protective procedures did not restore immune responses such as the production of antibodies and phagocytic cell growth (18-20). The authors concluded that this result might be due to infarcted white pulp and lower T-cell production. Our histopathological findings correlate with their results.
It was not possible to assess all these claims in our study. Nevertheless, the development of fibrosis and the observation of partially infarcted lesions in the spleens of SAL-applied rabbits are among the findings worth considering. In addition, in the subjects with previously induced hypersplenism with SVL and of which hypersplenism was remedied by SAL, the scintigraphic examination made using Tc-99m sulfur colloid showed that radioactive retention in the spleen decreased significantly. This decrease may be interpreted as a loss of phagocytic properties due to changes in the spleen, such as fibrosis and necrosis (17,20).

In conclusion, it is possible to induce successful hypersplenism using SVL. The hypersplenism formed this way in our study continued for a period of 8 weeks. Blood cell counts, which decreased following SVL, increased again after the SAL procedure, but the spleen lost its phagocytic functions. Questions on this issue may be answered by carrying out further investigations using the same model.

Correspondence author:
Celalettin VATANSEV
Department of General Surgery,
Faculty of Medicine, Selçuk University,
42080, Akyokuş, Konya - TURKEY
E.mail: cvatansev@hotmail.com

References