The effectiveness of splenic autotransplantation: an experimental study

Dalak ototransplantasyonunun etkinliği: Deneysel çalışma

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BACKGROUND

The purpose of this study was to establish the effects of spleen autotransplantation on immunity and to examine the viability of autotransplanted spleen tissue.

METHODS

Three groups were assigned and following operations were performed in twenty New Zealand rabbits. 1st group: sham laparotomy, 2nd group: splenectomy and 3rd group: splenectomy and autotransplantation. Scintigraphic methods, hematological-immunological tests and histopathological examination were used to evaluate the effects of splenic autotransplantation.

RESULTS

Histopathological findings showed that eight rabbits had splenic regrowth and two had necrosis of autotransplanted splenic tissue. Preoperative hematological and immunological findings compared with those at sixth week postoperatively. In group 3, postoperative immunoglobulin G, immunoglobulin M and interleukin-1 levels were significantly increased when compared with preoperative levels. Between all groups, postoperative leukocyte counts were found to be significantly higher in group 2 comparing with group 1. In group 2, postoperative platelet counts were significantly higher in comparison to group 1 and 3. No statistically significant difference was detected between the groups for immunoglobulin G, immunoglobulin M and interleukin-1 levels.

CONCLUSION

Conservative treatment is important in patients with splenic injury. However when splenectomy is indicated, splenic autotransplantation into the omentum pouch could be a reliable method for reconstruction of splenic functions and the viability of the implanted spleen tissue can be monitored by scintigraphy and laboratory examination.

Key Words: Reticuloendothelial system; spleen/physiology; scintigraphy; spleen/surgery/transplantation; splenectomy/adverse effects.

AMAÇ

Bu çalışmada dalak ototransplantasyonunun immüniteye etki-şini göstermek ve ototransplantasyon uygulanan dalak doku-sunun canlılığı deneysel olarak araştırılmak amaçlandı.

GEREÇ VE YÖNTEM


BULGULAR


SONUÇ

Dalak yaralanmalarında konservatif tedavi önemlidir. Bununla birlikte splenektomi endikasyonu olan hastalarda omentum içerisinde dalak ototransplantasyonu yapılmasi dalak fonksiyonlarının yeniden kazanılmadası güvendiir bir yöntemdir. Transplantasyon uygulanan dalak dokusunun işlevi ve canlılığı, sintonji ve laboratuar testleri ile takip edilebilir.

Anahtar Sözcüklер: Reticülöendothelial sistem; dalak/fizyoloji; sintonji; dalak/cerrahi/transplantasyon; splenektomi/yen etki.

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The first infection case after splenectomy was published in 1919. However this article did not receive enough attention until King and Schumacker experienced sepsis after splenectomy in five neonates. Diamond has first used the term “Overwhelming Postsplenectomy Infection” (OSPI) in 1969 for a bacteria that caused death of his patients. The microorganisms responsible for OSPI are usually encapsulated bacteria and 48% of these bacteria are pneumococcus.

The susceptibility of splenectomized patients to encapsulated microorganisms is closely associated with elimination of these bacteria in spleen. Reticuloendothelial and mononuclear fagocytic systems within the organ, effective immunoglobulin M (IgM) and opsonin productions are three factors provided by the spleen that protect body from capsulated microorganisms.

Elevated risk of sepsis in OSPI increased the importance of splenic salvage methods in surgery. Recently, 65% of the patients with blunt splenic trauma are followed by non-surgical methods and 98% of them do not require surgery during their clinical observations. In patients where conservative treatment cannot be applied, splenic salvage is tried to be achieved by splenophagy or partial splenectomy. However, in cases where the spleen is completely fragmented, splenectomy is inevitable. In those cases, splenic autotransplantation could be the only alternative to restore splenic functions. Although splenic autotransplantation has a history of twenty years, there is no consensus on its effects. There are a lot of reports arguing that either the splenic autotransplantation is ineffective or it could be at least as effective as splenophagy. There is no consensus on the observation methods regarding the function and viability of implanted tissue.

In this study we aimed to evaluate the effects of spleen autotransplantation on immunity and viability of autotransplanted spleen tissue by scintigraphic methods, hematological tests (leukocyte and platelet counts), immunologic tests (immunoglobulin G (IgG), IgM and interleukin-1 (IL-1) measurements) and histopathological examination.

MATERIALS AND METHODS

New Zealand white rabbits were used in this study. Twenty adult female rabbits weighing 2.5 kg were randomly allocated into three groups. All protocols were approved by Medicine Faculty of Trakya University ethical committee. The animals were maintained on an unrestricted standard diet with free access to tap water for at least 1 week before experiments and postoperative follow up. All operative procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Anesthesia was provided by xylazine hydrochloride 15 mg/kg IM and ketamine hydrochloride 10 mg/kg IM. Ketamine hydrochloride was administered intravenously during operation as needed. A median abdominal skin incision of 6-8 cm was used for surgery.

Three groups were assigned in this study; 1st group (n=5): sham laparotomy, 2nd group (n=5): splenectomy and 3rd group (n=10) splenectomy and splenic autotransplantation. In the third group rabbits underwent splenectomy and the 40% of spleen was sliced into 2x10x10 mm pieces and this pieces were reimplanted into the greater omentum by 4/0 catgut sutures.

Blood samples were collected from the ear of all animals preoperatively and at postoperative sixth week. The whole blood analysis, IL-1, IgG and IgM values were studied. IgG and IgM were measured by nephelometric methods and IL-1 was measured by chemical immunoassay methods.

All rabbits were returned to vivarium and followed-up for six weeks. After six weeks rabbits in 2nd group and 3rd group Technetium-99m (Sn) labeled pyrophosphate and heat denatured red blood cells were administered intravenously and then radionuclide imaging of the splenic tissues was performed. Ten minutes before the scintographies, rabbits were anaesthetized with xylazine hydrochloride 15 mg/kg IM. Static scintigraphic posterior-anterior images were taken with five-minute intervals. On these images, spleen and liver uptake rates were calculated quantitatively. One day after scintigraphy, rabbits in the 3rd group were reoperated through their previous incisions under general anesthesia. Greater omentum and implanted spleen tissues were removed and histologically examined under light microscope.

In order to study the difference between blood tests Kruskal Wallis test was used. When a difference between measurements was calculated, post-
hoc pair wise test was used to identify the different groups. Wilcoxon T test was used to compare preoperative and postoperative hematological and immunological parameters in these three groups. The scintigraphic results were evaluated by Mann-Whitney U-test.

RESULTS

a) Scintigraphic Findings: In the scintigraphic examination, ratio of contrast binding in the 2nd group with respect to the 3rd group (p<0.05) were significantly lower in splenic region than the liver region. So we decided that the tissue with contrast binding in scintigraphy was the implanted spleen (Table 1, Fig. 1, 2).

b) Histopathological Findings: Macroscopically, in the 3rd group, rabbits 9 and 10 presented lower growth rates and fewer adhesions; rabbits 1, 2, 6 and 7 presented growth rates four times greater than the original implanted tissue (Fig. 3); rabbits 3, 4, 5 and 8 presented growth rates 2-3 times greater than the original implanted tissue. In rabbits 1, 5 and 7, implanted tissue and abdominal wall and small intestines and colon showed intensive adhesions (Fig. 4, 5).

Table 1. Scintigraphic uptake rates and means

<table>
<thead>
<tr>
<th>Rabbits</th>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td>1</td>
<td>2.01</td>
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</tr>
<tr>
<td>2</td>
<td>2.32</td>
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<td>3</td>
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<td>4</td>
<td>1.79</td>
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<td>5</td>
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</tr>
<tr>
<td>6</td>
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<td>10</td>
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<td></td>
</tr>
<tr>
<td>Means</td>
<td>1.98</td>
<td>0.52*</td>
</tr>
</tbody>
</table>

A: Uptake rates of spleen incubation region as compared to liver in autotransplantation group; B: Uptake rates of spleen region as compared to liver in splenectomy group; *Significantly low as compared to autotransplantation group (p<0.05).

We found that implanted tissues had necrosis in rabbits 9 and 10 in contrast to other 8 cases. Autotransplanted spleens in these two cases were not successful and they were excluded from the study. For statistical studies, hematological and immunological parameters of eight cases in the 3rd group were used (Fig. 6, 7).

Fig. 1. Scintigraphy of the rabbit 1 in splenic autotransplantation group at postoperative sixth week. (L: Liver; IT: Implanted tissue; LK: Left kidney; RK: Right kidney)

Fig. 2. Scintigraphy of the rabbit 1 in splenectomy group at postoperative sixth week.
platelet counts were prominently higher and IgG and IgM levels were lower than preoperative levels, but no significant difference was found for IL-1 levels (p<0.05, p>0.05 respectively). In the 3rd group, postoperative IgG, IgM and IL-1 levels were significantly increased when compared with preoperative levels (p<0.01, p<0.01, p<0.05 respectively). Between all groups, postoperative white blood cell and platelet counts were found significantly higher in the group-2 as compared with the group-1 (p<0.01). In the group 2, postoperative platelet counts were significantly higher than the 1st and 3rd groups (p<0.01). No statistically significant difference was found between the groups for IgG, IgM and IL-1 levels (p>0.05, Table 2).

The postoperative platelet and white blood cell counts of two rabbits were excluded from the study because of necrosis determined histopathologically was higher than the values in the 3rd group (postoperative white blood cell 15600-15700 /mm³, platelet 873000-939000/mm³ respectively). IgG, IgM and IL-1 levels were lower than those of the 3rd group (postoperative IgG 67.4-59 mg/dL, postoperative IgM 30.8-35.4 mg/dL, postoperative IL-1 5-7 pg/dL, respectively).

**DISCUSSION**

There is no consensus on the effectiveness of splenic autotransplantation, even if clinical and
Experimental studies have been performed for over 20 years. In this study, the greater omentum was used as the seeding region and 40 percent of the splenic tissue was implanted and results were observed 6 weeks after seeding on the basis of other studies.

Even if the best method to demonstrate living tissue is histopathological methods, its clinical use is not always available. Growth of implanted tissue and encapsulation by fibrous tissue were found in 80% of the rabbits (n=8) in our study. However, necrotic splenic tissue pieces were detected in the remaining two rabbits histopathologically. Weber et al., explained this event by decrease in blood supply of omentum due to per-operative long lasting shock. Many alternative studies are available to demonstrate living transplanted tissue by means of imaging techniques (e.g. scintigraphy, computed tomography, ultrasonography). In this study, viability of implanted tissue was monitored scintigraphically. Scintigraphic uptake rates in two rabbits with necrotic spleen tissue were smaller than the others, indicating that scintigraphic uptake rates were important for the follow-up of tissue viability. However, due to small number of cases, statistical examination could not be performed for these two rabbits.

Leukocytosis and thrombocytosis after posttraumatic splenectomy is well documented in the literature. Consistent with the literature, we have found significant leukocytosis and thrombocytosis in splenectomized rabbits. On the contrary, no significant increase was detected in the control and splenic autotransplantation groups (Table 2). These findings were consistent with the other studies in the literature.

IgM, a parameter used for follow-up of spleen functions is the first antibody occurring after antigen stimulus and helps reticuloendothelial system destroying some species of microorganisms. IgG which defends the body against bacteria, virus and toxins is important for secondary immunity. In this study, IgG and IgM levels were assessed to determine effects of autotransplanted spleen on immune response. Bergmann et al., Knezevic et al. and

![Fig. 6. Microscopic view of the splenic tissue surrounded by a fibrous tissue within fat in rabbit 1 in splenic autotransplantation group (H-E x 100).](image1)

![Fig. 7. Microscopic view of the splenic tissue surrounded by a fibrous tissue within fat in rabbit 2 in splenic autotransplantation group (H-E x 100).](image2)

**Table 2.** Preoperative means of hematologic and immunologic parametric values in group 1, group 2 and group 3

<table>
<thead>
<tr>
<th>Group</th>
<th>White blood cell (mm³)</th>
<th>Thrombocyte (mm³)</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IL-1 (pg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
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<tr>
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</tr>
<tr>
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<td>506600</td>
<td>1164000</td>
<td>69.94</td>
</tr>
<tr>
<td>Autotransplantation</td>
<td>7787</td>
<td>9912</td>
<td>522000</td>
<td>497750</td>
<td>69.05</td>
</tr>
</tbody>
</table>

† Significantly high as compared to preoperative levels (p<0.05); # Significantly low as compared to preoperative levels (p<0.05); * Significantly high as compared to preoperative levels (p<0.01); ‡ Significantly high as compared to sham laparotomy group (p<0.05); * Significantly high as compared to sham laparotomy group and autotransplantation group (p<0.01); Pre: Preoperative; Post: Postoperative.
Leemans et al., reported recovery of immunoglobulin levels after splenic autotransplantation in their studies.\textsuperscript{22,24} In our study, similar to these results, lower postoperative IgG and IgM levels in splenectomy group and higher levels of these immunoglobulins in splenic autotransplantation group implicates that splenic autotransplantation has beneficial effects on humoral immunity.

IL-1 is a cytokine secreted by mononuclear fagocytic cells after antigenic stimulus of T lymphocytes. IL-1 provides reproduction of T-helper cells and maturation and differentiation of B lymphocytes. A study performed on 57 splenectomized patients demonstrated impaired levels of IL-1 and IL-2 after splenectomy.\textsuperscript{25} In our study, IL-1 levels were compared to examine the effectiveness of autotransplanted spleen on immune response via cytokines. In addition, usefulness of this parameter was studied in the follow-up of the function of implanted tissue. Postoperative IL-1 levels were lower than the preoperative levels in splenectomy group but no significant difference was found (p>0.05). However, postoperative IL-1 levels were found to be higher than the preoperative levels in autotransplantation group (p<0.05). Significant increase in postoperative IL-1 levels as compared with preoperative levels in splenic autotransplantation group indicated that immunomodulator role of spleen after implantation continues. However this indication should be examined by further investigations. As a result, it is supposed that IL-1 level can be a valuable parameter to demonstrate the function of implanted spleen tissue.

CONCLUSION

Conservative treatment is important in patients with splenic injury. However when splenectomy is indicated splenic autotransplantation into omentum could be a reliable method to restore splenic functions and viability of the implanted splenic tissue can be followed by scintigraphic and laboratory examination.

REFERENCES

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