Effect of Splenectomy to Short Bowel Syndrome in Rats

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Abstract The aim of this study was to determine the effect of splenectomy in the short bowel syndrome. Twenty-four Wistar-albino rats weighing between 210 and 375 g were used. They were divided into three groups. In group A, short bowel syndrome (SBS) was created by 75 % bowel resection. In group B, SBS and splenectomy was performed. In group C, after transecting the bowel, it was anastomosed. Before and 45 days after the procedures, all rats were weighed. In all three groups, the first and final weight of the rats, the final bowel weight and length, the ileal and jejunal crypt depths, the villus height, the luminal diameter, the bowel wall thickness, and the number of apoptotic cells and mitosis per 100 crypt cell were compared. Periportal fibrosis, infiltration, bile stasis, and bile duct proliferation were detected in liver samples. The rat intestinal length and weight was the least in group B while the jejunal crypt depth was higher in group B than in group A and it was exactly the opposite for the jejunal and ileal villus heights. The ileal and jejunal luminal diameter, the ileal bowel wall thickness, the jejunal and ileal apoptotic cell number, the jejunal mitosis, and the periportal fibrosis were highest in group B. Adding splenectomy to an SBS model has a negative impact on bowel adaptation.

Keywords Experimental · Short bowel syndrome · Splenectomy · Bowel adaptation · Rat

Introduction

Short bowel syndrome (SBS) is a reduction in functioning bowel length, which is most often a result of surgical resection. Risk factors in the neonatal period include necrotizing enterocolitis, small bowel atresia, and gastroschisis. With the increasing survival of preterm infants, there is an increase in incidence [1]. Intestinal adaptation is a natural compensatory process that occurs following extensive intestinal resection, whereby structural and functional changes in the intestine improve nutrient and fluid absorption in the remnant bowel. In animal studies, postresection structural adaptations include bowel lengthening and thickening and increases villus height and crypt depths [2]. In SBS—in addition to total parenteral nutrition, which enhances survival—a variety of drugs that improves adaptation, such as growth factors, trophic hormones, glucagon (like peptide-2), and somatostatin was introduced into clinical practice [3–5]. In the current literature, there are no studies indicating that the spleen has a comprehensive task other than hematopoiesis. Lately, performing a total splenectomy for trauma and for immunologic, hematologic, and oncologic diseases, procedures protecting the spleen, like splenorrhaphy or partial splenectomy, are recommended [6]. Formerly, the spleen could be considered a neglected organ. To date, it has been deemed an ancillary organ in portal hypertension or an organ localization in lymphoproliferative diseases, even though it has been given significant attention in infectious diseases for some time. Now, it is thought to be a metabolic asset, central in regulating the immune system and involved in endocrine function [7]. Zoli et al. in a series of 20 patients with ulcerative colitis, visceral

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myopathy, and mostly Crohn's disease demonstrated that long-term intravenous nutrition for intestinal failure causes splenic hypofunction and increases the risk of infection. However, this splenic hypofunction depends on intravenous nutrition therapy and especially to long-chain triglyceride emulsions. So in SBS or in intestinal pathologies, splenic functions are impaired, but in this study, in contrary to the study of Zoli et al., we want to investigate the effect of spleen on bowel adaptation. And for this purpose, we performed splenectomy in one group after creating SBS [8]. This study is the first in the current literature that investigates the effect of splenectomy on the intestinal adaptation in the experimental short-bowel model.

Materials and Methods

Twenty-four Wistar-albino rats, with weights ranging from 210 to 357 g, were used. The rats were divided into three groups so that each group included eight subjects. In this study, the following procedures were performed:

- Group A: 75 % small bowel removal
- Group B: 75 % small bowel removal and splenectomy
- Group C: small bowel incision and anastomosis

Before the surgical procedures, all rats were weighed and the weights were recorded. The rats in groups A and B were anesthetized by 50 mg/kg intramuscular ketamine hydrochloride administration then, after appropriate site clearance, a midline incision was made and 75 % percent SBS was created by removing short-bowel segments between 15 cm proximal to the ileocecal valve and 5 cm distal to the Treitz's ligament and anastomosing the two open intestinal ends. For the rats in group B, splenectomies were performed after ligating the splenic hilar vessels. In the control group (group C), after the rats were anesthetized in the same way, the short bowel, 15 cm proximal to the ileocecal valve, was transected and reanastomosed. In all groups, intestinal anastomoses were made by a single layer with 6/0 prolene, and mesenteric defects were repaired with 6/0 vicryl. In group B, splenic hilar vessels were ligated with 5/0 vicryl and the abdomens of all rats were closed with 4/0 silk. Following the procedures, the rats were kept in individual cages and were allowed to drink water after 1 hour and to eat a normal diet after 24 h. All subjects were weighed and anesthetized in the same way, 45 days after the initial procedures. After midline incisions, the small bowels of all rats, from the ileocecal valve to the Treitz's ligament, were removed, weighed, and measured. For histopathological evaluation, in group A and B, a 1-cm length of the jejunal and ileal bowel biopsies were taken distal to the anastomotic line

and preserved in 10 % formalin. In the control group, these biopsies were taken 15 cm proximal to the ileocecal valve and 5 cm distal to the Treitz's ligament. In addition, liver biopsies were taken from all rats and preserved in 10 % formalin. All tissue samples were evaluated by the same pathologist in the Department of Pathology.

From the parenchyma of tissues subjected to routine procedures, 4- μ m sections were prepared. These sections were HE (hematoxylin and eosin) stained and examined under an Olympus BX51 light microscope. For the examination of apoptosis under a light microscope, the sections were immunohistochemically stained at BD TriPath Imaging with caspase 3 (Diagnostic Biosystems). Morphological measurements were done with a ZEISS Imager M2 microscope. In all rat specimens, both ileal and jejunal crypt depths, villus height, luminal diameter, bowel wall thickness, and the number of apoptotic cells and mitosis per 100 crypt cells were determined. Periportal fibrosis, bile stasis, periportal infiltration, and bile duct proliferation were detected in the liver samples and coded as 0, 1+, 2+, or 3+.

All data was recorded in an IBM SPSS, version 20, statistical program. The groups were compared for the bowel weight and length, the jejunal crypt depth, the jejunal and ileal villus heights, the luminal diameter, the bowel wall thickness, and the number of mitosis per 100 crypt cells and ileal apoptotic cells per 100 crypt cells with the one-way ANOVA test. The groups were compared for the ileal crypt depth and the number of jejunal apoptotic cells per 100 crypt cells with the Kruskal Wallis test because their variances were not homogeneous. The groups were compared for periportal fibrosis, bile stasis, periportal infiltration, and bile duct proliferation with the chi-square test.

Results

The mean values of the bowel weight and length, the jejunal and ileal crypt depth, the villus height, the luminal diameter, the bowel wall thickness, and the number of apoptotic cells and mitosis per 100 crypt cells of all three groups are shown in Tables 1 and 2. The intestinal weight and length was significantly lower in group B. While the ileal and jejunal villus height was significantly higher in group A (Fig. 1), it was significantly lower in group B when compared with the other two groups. In group B, the ileal and jejunal luminal diameter, the jejunal crypt depth (Fig. 2), the and ileal bowel wall thickness (Fig. 3) were significantly higher when compared with the two other groups. In group B, the jejunal and ileal number of apoptotic cells per 100 crypt cells and the jejunal number of mitosis per 100 crypt cells were significantly higher when compared with the other two groups. When liver

Table 1 The mean values of bowel weight and length, ileal crypt depth, villus height, luminal diameter, bowel wall thickness, and number of apoptotic cells and mitosis per 100 crypt cells of all three groups

Groups	Intestinal weight (g)	Intestinal length (cm)	Ileal crypt depth (μm)	Ileal villus height (μm)	Ileal luminal diameter (μm)	Ileal bowel wall thickness (μm)	Ileal number of apoptotic cell/100 crypt cell	Ileal number of mitosis/100 crypt cell
A	9.3 $\pm$ 2.2	54.6 $\pm$ 11.9	36.6 $\pm$ 3.4	132.2 $\pm$ 22.7*	657.6 $\pm$ 111.6	32.2 $\pm$ 5.6	0.1 $\pm$ 0.4	3.8 $\pm$ 0.7
B	6.2 $\pm$ 2.2*	44.5 $\pm$ 9.6*	44.1 $\pm$ 6.5	87.8 $\pm$ 16.1	745.9 $\pm$ 117.1*	41.4 $\pm$ 11.4*	2.0 $\pm$ 1.3*	5.0 $\pm$ 1.1
C	11.0 $\pm$ 0.8	103.7 $\pm$ 7.5	46.3 $\pm$ 10.5	100.5 $\pm$ 13.8	509.2 $\pm$ 117.5	31.4 $\pm$ 3.9	0.6 $\pm$ 0.5	4.0 $\pm$ 1.4

*Statistical significant difference in one-way ANOVA test ($p < 0.05$)

specimens were taken into consideration in the short-gut groups (groups A and B), periportal fibrosis, bile stasis, periportal infiltration, and bile duct proliferation were higher than the control group (group C). Only periportal fibrosis in group B was statistically significantly higher than the other two groups.

Discussion

In rat SBS, changes in the rest of the intestine begins in the first 1–2 days and reaches the highest level at 12–15 days [9]. In this study, with the intestinal weight, length, villus height, and crypt depth and with apoptosis and mitosis count, an attempt to calculate the current adaptation mechanisms was undertaken.

In animal studies, postresection structural adaptations include bowel lengthening and thickening and increase the villus height and the crypt depth [2]. In this study, the intestinal weight and length was found to be lowest in group B. While the jejunal and ileal villus heights was found to be highest in only SBS-created group A, in group B, in which splenectomies were performed in rats with SBS, these values were found even lower than that of the control group (group C). These findings suggest that performing splenectomies on rats with SBS has a negative effect on intestinal adaptation. While luminal diameter and bowel wall thickness was higher in group B compared with other groups, bowel weight was lower because the length was less than the others.

Even if it is still controversial, as a step of adaptation, apoptosis has been determined to be increased in SBS. Reducing this process with a variety of treatments has a positive contribution to adaptation [10]. Mitosis is known to be increased in SBS based on adaptation [5]. Both apoptosis and mitosis numbers were increased in SBS-created groups A and B, while this increase was more in the splenectomy-added group B. These results suggest that cell turnover increases when splenectomies are performed on rats with SBS.

The ileum is an essential site for the absorption and recycling of bile salts; therefore, in short gut syndrome, there is a depletion in the bile salt pool, which changes the bile salt metabolism in the liver [11]. This may be the cause of hepato-gastrointestinal disease, which is seen in the short gut syndrome called IFALD (intestinal failure liver disease), which is mostly correlated with parenteral nutrition in which liver biopsies show cholestasis, bile duct proliferation, periportal inflammation, and fibrosis [12]. In our study, in the liver biopsies of the short-gut groups, there was an increase in bile duct proliferation, periportal fibrosis, bile stasis, and periportal infiltration compared with the control group's liver biopsies. However, only the increase in periportal fibrosis in splenectomized rats was statistically significant. Because these rats did not receive parenteral nutrition, this increase can be explained by the change of bile salt metabolism in livers, which is caused by SBS. Furthermore, this change may be influenced by the absence of a spleen.

Table 2 The mean values of jejunal crypt depth, villus height, luminal diameter, bowel wall thickness, and number of apoptotic cells and mitosis per 100 crypt cells of all three groups

Groups	Jejunal crypt depth (μm)	Jejunal villus height (μm)	Jejunal luminal diameter (μm)	Jejunal bowel wall thickness (μm)	Jejunal number of apoptotic cell/100 crypt cell	Jejunal number of mitosis/100 crypt cell
A	39.7 $\pm$ 6.9	139.4 $\pm$ 16.8*	295.4 $\pm$ 123.4	32.2 $\pm$ 6.3	0.1 $\pm$ 0.4	4.4 $\pm$ 0.9
B	52.2 $\pm$ 10.1*	110.5 $\pm$ 26.2	493.5 $\pm$ 114.7*	32.3 $\pm$ 5.4	1.3 $\pm$ 0.5**	5.0 $\pm$ 0.5*
C	48.3 $\pm$ 9.3	116.9 $\pm$ 19.2	400.7 $\pm$ 127.8	30.7 $\pm$ 4.3	0.6 $\pm$ 0.7	2.9 $\pm$ 1.0

*Statistical significant difference in one-way ANOVA test ($p < 0.05$); **statistical significant difference in Kruskal Wallis test ($p < 0.05$)

Fig. 1 Measurement of jejunal villus height in a rat in group A

There are other studies investigating whether the spleen has an effect other than hematopoiesis, but there is no such study in the SBS model. An interesting hypothesis relates the spleen to the activity of gut-associated lymphoid tissue (GALT) [7]. The dysfunction of GALT is known to predispose inflammatory bowel diseases (IBD) and for its role in T cell activation and trafficking in the gut. Moreover, the frequency of IgM memory B cells is decreased in IBD subjects, establishing a relationship among GALT and the spleen in humans [13]. Animal studies have also shown that splenocytes can differentiate into pancreatic islets and ductal epithelial cells when injected into non-obese diabetic mice; thus, splenocytes may be useful in the treatment of type 1 diabetes [14, 15]. Subsequently, Chong et al. questioned the origin of these stem cells [16]. The aim of this review is to let clinicians appreciate the new role of metabolic and endocrine functions of the spleen with a special emphasis on obesity and non-alcoholic fatty liver

Fig. 2 Measurement of jejunal villus height in a rat in group B

Fig. 3 Measurement of ileal bowel wall thickness in a rat in group B

diseases in the context of the available literature. Moreover, understanding the spleen function could be important in developing appropriate prevention strategies in order to counteract the pandemic of obesity. In another study, splenic hypofunction was proved in a group of 20 patients, with eight of them having Crohn's disease, who developed SBS because of undergoing numerous operations and had long-term intravenous nutrition with pitted red cell increase and reduced tuftsin activity, when compared with 50 control patients [8]. It may be that the components of parenteral nutrition other than long-chain triglycerides are involved in this process, although, so far, they have not been able to hypothesize what these could be. However, their study suggests that it may be worthwhile monitoring splenic function in patients on long-term IVN and to consider the need for vaccination against encapsulated bacteria in these patients. Tuftsin also stimulates the bactericidal activities of phagocytic cells; its activity falls after splenectomy and is low in sickle cell anemia, AIDS, and coeliac disease [8]. On the contrary, our study was conducted to investigate the effect of splenectomy on SBS. We show that splenectomy impairs intestinal adaptation in SBS. This effect can be attributed to the impairment of splenic molecules like tuftsin, to disruption of phagocytic cell release, and to factors that cannot be demonstrated yet.

Conclusions

This study is the first that shows that splenectomy has a negative effect on adaptation in the experimental SBS model. Therefore, the spleen must be considered not only a member of hematopoietic systems but also an organ that has an effect on the intestinal system. To understand the mechanism of this effect, more detailed immunological, biochemical, and hormonal studies must be performed.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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