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Effect of sildenafil on intestinal adaptation parameters in a rat model of short bowel syndrome

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Abstract. – OBJECTIVE: We aimed to evaluate the effect of sildenafil on the intestinal adaptation in short bowel syndrome (SBS).

MATERIALS AND METHODS: Forty-eight male Wistar-albino rats (weight, 231–390 g) were randomly divided into four groups with 12 rats in each. Group TA had only ileal transection+anastomosis, Group TA+S was given sildenafil after ileal transection+anastomosis, Group RA had a resection of 75% of the small bowel+anastomosis, Group RA+S was given sildenafil after small bowel resection+anastomosis. Sildenafil was injected subcutaneously at 60 mg/kg/day dose throughout 3–21 days postoperatively. Bowel and mucosal weights, villus height, crypt depth, DNA and protein concentrations were determined.

RESULTS: Jejunal bowel weight was lower in TA and TA+S groups than RA and RA+S groups ($p < 0.05$). RA+S group had higher ileal and jejunal mucosal weights than RA and TA+S groups ($p < 0.05$). Villus height was highest in RA+S group both in ileum and jejunum (466.1 ± 38.6 μm and 648.1 ± 65.7 μm, respectively). Jejunal crypt depth was highest in RA+S group (255.1 ± 21.9 μm) compared to other groups ($p < 0.05$). There was no significant difference in ileal and jejunal protein concentration between TA and TA+S groups and in ileal protein concentration between RA ve RA+S groups ($p > 0.05$). Ileal DNA concentration was higher in TA+S group, and jejunal DNA concentration was higher in RA and RA+S groups than TA and TA+S groups ($p < 0.05$).

CONCLUSIONS: Sildenafil has a positive effect on intestinal adaptation parameters, particularly in jejunum in a rat SBS model. Thus, its role in the treatment of SBS should be further investigated with clinical studies.

Key Words:
Short bowel syndrome, Intestinal adaptation, Sildenafil.

Introduction

Short bowel syndrome (SBS) is a state of malnutrition and malabsorption that results from decrease in absorption area following extensive resection of small bowel because of various conditions like intestinal ischemia, Crohn disease, or cancer[1,2]. Pathophysiological changes of SBS are caused by the loss of absorptive surface of bowel and fast passage of bowel content, both of which lead to food malabsorption typically presenting with chronic diarrhea, dehydration, electrolyte abnormalities, vitamin deficiency, and malnutrition[3].

The outcome of SBS is usually poor and depends on the extent and location of resection, structural and functional adaptation of the remaining bowel segment, and preservation of ileocecal valve. The medical, surgical and combined treatment methods are used in the treatment of SBS. Additionally, the prevention of diarrhea and fast passage of bowel content are among the therapeutic approaches that can be applied in SBS[4]. Recently, intestinal transplantation has been successfully implemented in SBS patients who do not respond to medical treatment[5]. Total parenteral nutrition, effective antibiotics, and advanced technology in transplantation surgery and intensive care units improve patient survival and quality of life. However, SBS is still associated with high mortality and morbidity rates[6,7]. Therefore, new pharmacological approaches to improve the prognosis and to reduce treatment complications of SBS are being investigated[8].

The adaptability of the absorption surface of the remaining bowel segment is the main determining factor to decrease the malabsorption and malnutri-

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tion caused by SBS[9,10]. Intestinal adaptation starts within 24-48 hours after the resection and includes structural and functional changes in the remaining bowel. The period of adaptation is about 15 days in rats, although it is much longer in humans[11-14]. Indicators of bowel adaptation in SBS have been reported as an increase in the bowel and mucosal weight, DNA and protein content of the mucosa, the villus height, and crypt depth[15,16].

In literature, there is a number of experimental studies testing the effect of different agents to increase the intestinal adaptation in SBS, such as recombinant growth hormone, glucagon-like peptide, insulin, lactoferrin and melatonin[5,17-19]. However, only a few of these agents can be used in clinics. The various alternative surgical and medical treatment methods for SBS are still being investigated[20].

Sildenafil is a commonly prescribed and easy-to-find drug for the treatment of male erectile dysfuntion and has a relaxant effect on the smooth muscles of the arterioles[21]. Previous studies highlighted beneficial effects of sildenafil on gastrointestinal tract such as protection against acetic acid-induced colonic inflammation and indomethacin-induced intestinal and gastric injury[21-24] in addition to its inhibitory effect on gastric emptying and intestinal transit[25,26]. However, the effect of sildenafil in SBS has not been studied so far.

On the basis of the well-known beneficial effects of sildenafil in gastrointestinal tract, we hypothesized that sildenafil may improve adaptive capacity of the remaining bowel in SBS by various pathophysiological mechanisms, thus can be potentially used in clinical treatment of patients with SBS. To test this hypothesis, we aimed to evaluate the effect of sildenafil on intestinal adaptation in a rat model of SBS.

Materials and Methods

Study Design and Groups

This experimental study was conducted at Akdeniz University Animal Laboratory with the approval of Institutional Ethics Committee for Animal Research.

Forty-eight male Wistar-albino rats weighting 231-390 g (mean, 320 g) were used. Rats were kept in individual cages at 12/12-h light/dark cycle and constant temperature, and had free access to standard pellet diet and tap water during 10 days before the experiment. Animals were randomly divided into four experimental groups with 12 rats in each. Group 1 (TA) was control group in which only ileal transection+anastomosis was performed, Group 2 (TA+S) was given sildenafil after ileal transection+anastomosis, Group 3 (RA) had resection of 75% of the small bowel and anastomosis, Group 4 (RA+S) was given sildenafil after small bowel resection+anastomosis.

Surgical Procedure

The rats were fasted for 12 hours prior to surgery, but had free access to water. Following induction of anesthesia by ether inhalation, 50 mg/kg ketamine hydrochloride (Ketalar®, Eczacibasi, Istanbul, Turkey) and 10 mg/kg Xylazine HCl (Alfazyne® 2%, 20 mg/mL, 30 mL, Alfas Int BV, Netherlands) was injected intramuscularly for long-term anesthesia and analgesia.

The abdominal skin was shaved and cleaned with 10% povidone iodine (Batticon® solution, Adek, Turkey). A 3-cm sagittal midline abdominal incision was performed, and the small intestine was exposed in the intra-abdominal cavity. In Group 3 and 4 animals, small bowel mesentery was tied with 3/0 polyglactin (Vicryl®, Ethicon, Somerville, NJ, USA) and 75% of small bowel was resected by transection from 5 cm distal to Treitz ligament and 10 cm proximal to ileocecal valve (Figure 1a). The continuity of the remaining intestine was restored with end-end anastomosis by suturing with 6/0 polypropylene (Prolene®, Ethicon) (Figure 1b). In Group 1 and 2 animals, ileum transection was performed at 10 cm proximal to the ileocecal junction, and continuity was obtained by anastomosis with 6/0 polypropylene individual sutures. Following injection of 10 mL saline (Eczacibaşi-Baxter®, Istanbul, Turkey) into the abdominal cavity, the abdomen was closed with 3/0 polypropylene sutures.

Rats were started standard pellet diet and water at postoperative first day. In the sildenafil groups (Group 2 and 4), sildenafil (Viagra®, Pfizer, Quebec, Canada) was injected subcutaneously at 60 mg/kg/day dose, starting from the postoperative third day. Body weight of rats was measured daily during 21 days after the operation.

Relaparotomy

Twenty-one days after the surgical procedure, all rats were anesthetized by intramuscular injection of 50 mg/kg ketamine HCl and 10 mg/kg xylazine HCl. All of the remaining small intestine from the ileocecal valve to Treitz ligament were removed, bowel weight was measured. The mucosa was scraped using a glass slide, collected, and weighed. Bowel and mucosal weight were calculated per cm of bowel length per 100 g

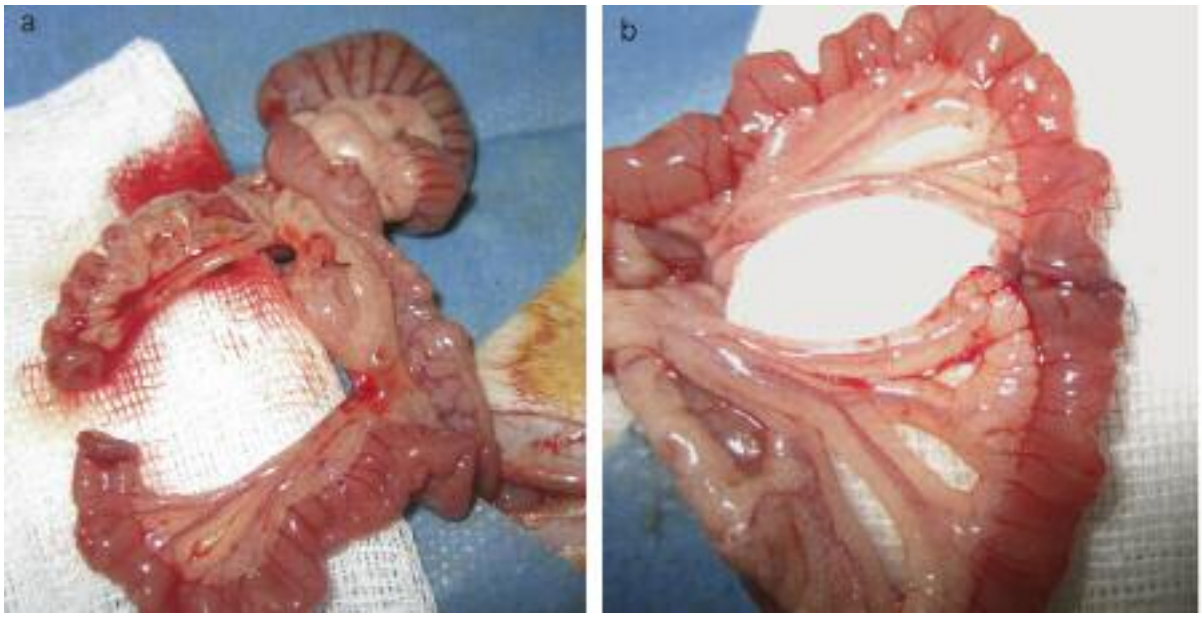

Figure 1. Seventy-five of small bowel was resected by transection from 5 cm distal to Treitz ligament and 10 cm proximal to ileocecal valve ***(a)*** and restored with end-end anastomosis by suturing with 6/0 polypropylene ***(b)***.

of body weight as described previously[27]. For histopathological evaluation, 1-cm sections from jejunum and ileum were taken by keeping the anastomosis line in the middle and put into 10% formalin solution.

Histopatological Evaluation

Three longitudinal samples were cut out from each tissue sample and embedded in paraffin blocks vertically. After taking 5 μm thick sections and they were deparaffinized and routinely stained with hematoxylin and eosin. Prepared samples were evaluated in Antalya Training and Research Hospital Pathology Clinics by a single pathologist blinded to study groups of samples. Villus height and crypt depth were evaluated under Olympus CX 41 light microscope (Shinjuko, Tokyo, Japan) at ↔100 magnification by using ocular micrometer (Figure 2). Images obtained from Olympus DP 20 (CCD, 1600↔1200 UXGA high-definition) photo receiver attached to light microscope were interpreted. Villus height and crypt depth were calculated as the mean of 10 villi and 10 crypts and expressed in μm.

Biochemical Evaluation

Isolation and Concentration of DNA in the Bowel Mucosa Samples

DNA and protein concentration of the mucosa samples was measured at Molecular Biochemistry Laboratory of Akdeniz University Faculty of Medicine Department of Medical Biochemistry Department. DNA was isolated from the intestinal mucosa by AxyPrep MultiSource Genomic DNA Miniprep kit (Catalog No. MS-GDN AP-Mn-50; Axygen Scientific Inc., Union City, CA, USA). Over the 20 mg frozen intestinal tissue, 350 μl PBS and 0.9 μl RNase was added, homogenized at high-rate with a teflon homogenizer. The resulting homogenate was transferred to a 2 mL Eppendorf

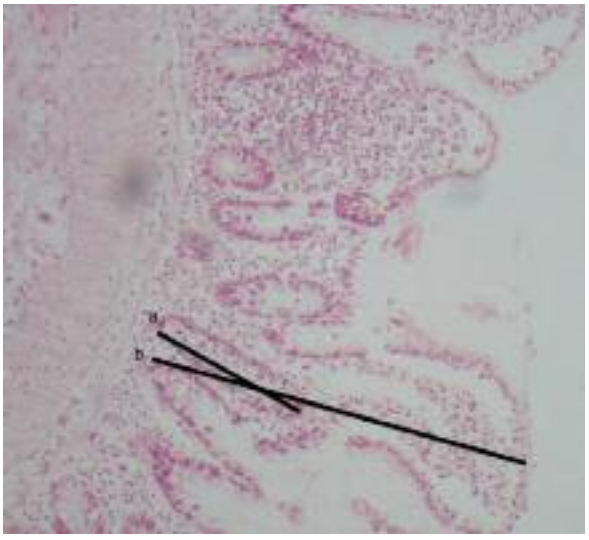

Figure 2. Light microscope image of a bowel sample used to calculate crypt depth ***(a)*** and villus height ***(b)*** (×100, hematoxylin and eosin).

Table I. Total body weight of rats in study groups during follow-up duration of 21 days.

Days	Group 1 (TA) n = 12	Group 2 (TA+S) n = 12	Group 3 (RA) n = 12	Group 4 (RA+S) n = 12	p^a
Day 1	377.5 ± 26.2	301.9 ± 21.2[b]	321.7 ± 25.9	309.2 ± 36.3[b]	0.005
Day 7	341.9 ± 26.2[c]	293.2 ± 20.9[b,c]	299.9 ± 22.6[b,c]	290.2 ± 35.0[b,c]	< 0.001
Day 15	349.4 ± 27.3	301.4 ± 20.3[b]	303.3 ± 25.7[b,c]	295.5 ± 35.0[b,c]	< 0.001
Day 21	357.0 ± 28.2[c]	309.8 ± 20.7[b,c]	308.5 ± 25.9[b,c]	299.9 ± 34.9[b,c]	< 0.001

[a]ANOVA, [b]$p < 0.05$ for comparison with TA (Tukey test), [c]$p < 0.05$ for comparison with Day 1 (paired sample test). TA, transection+anastomosis; S, sildenafil; RA, resection+anastomosis.

tube and vortexed for 1 min after addition of 20 µl proteinase K ve 150 µl buffer C-L. After incubation for 15 minutes at 56°C, 350 µl buffer P-D was added and vortexed. Then, the solution was centrifuged at 12,000↔g and 24°C for 10 min and DNA separation was carried out using the miniprep columns in the kit.

A 990 µl of TE buffer [TE buffer: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA] was added to 10 µl of the isolated DNA the solution (1:100 dilution was provided), which was then read spectrophotometrically at a wavelength of 260 and 280 nm. One unit of absorbance at 260 nm corresponds to 50 µg/ml double-stranded DNA or 150 µM nucleotides. The amount of DNA in the intestinal mucosa was expressed as DNA (µg/g wet tissue)/protein (mg/g wet tissue).

Protein Concentration in the Bowel Mucosa Samples

Bowel samples were homogenized with 200 µl PBS and centrifuged at 15,000↔g and 4°C for 15 min. In the resulting supernatant, the protein concentration was determined spectrophotometrically at 595 nm by protein measurement kit using Coomassie protein reagent (Pierce, Biotechnology, Waltham, MA, USA, Catalog No. 23236). Bovine serum albumin was used as a standard.

Statistical Analysis

Descriptive statistics were used to report the data (e.g., mean, standard deviation, frequency, percentage). The normality distribution of data was determined by Kolmogorov-Smirnov test. ANOVA and post-hoc Tukey test were used to analyze the quantitative data. Paired sample *t*-test was applied for repeated measurements. Statistical analysis was performed using the SPSS software package for Windows (Statistical Package for Social Sciences, version 20.0, SPSS Inc., Chicago, IL, USA). Statistical level of significance was set at $p < 0.05$.

Results

Monitoring Body Weight

Body weight of rats in TA group was significantly higher than TA+S and RA+S groups initially (Table I, Figure 1). Body weight of rats in TA group stayed higher than the weights of rats in

Table II. Comparison of intestinal adaptation paramaters in study groups.

		Group 1 (TA) n = 12	Group 2 (TA+S) n = 12	Group 3 (RA) n = 12	Group 4 (RA+S) n = 12	p^a
Ileum	Bowel weight (mg/cm/100 g)	34.6 ± 3.2	44.4 ± 5.6[b]	58.2 ± 8.1[b,c]	64.0 ± 5.3[b,c]	< 0.001
	Mucosal weight (mg/cm/100 g)	9.9 ± 1.7	13.4 ± 3.2[b]	14.3 ± 3.4[b]	19.3 ± 2.4[b,c,d]	< 0.001
	Villus height (µm)	333.4 ± 44.2	389.5 ± 50.3[b]	388.2 ± 34.5[b]	466.1 ± 38.6[b,c,d]	< 0.001
	Crypt depth (µm)	144.1 ± 19.6	154.0 ± 9.8	154.9 ± 8.9	141.1 ± 7.5	0.051
Jejenum	Bowel weight (mg/cm/100 g)	41.2 ± 6.5	45.7 ± 4.5	63.4 ± 4.7[b,c]	68.5 ± 4.6[b,c]	< 0.001
	Mucosal weight (mg/cm/100 g)	13.1 ± 2.3	21.7 ± 3.2[b]	24.6 ± 3.2[b]	34.2 ± 2.5[b,c,d]	< 0.001
	Villus height (µm)	378.9 ± 42.3	469.8 ± 47.1[b]	539.8 ± 41.7[b,c]	648.1 ± 65.7[b,c,d]	< 0.001
	Crypt depth (µm)	161.1 ± 17.8	187.5 ± 28.6[b]	203.9 ± 15.1[b]	255.1 ± 21.9[b,c,d]	< 0.001

[a]ANOVA, [b]$p < 0.05$ for comparison with TA (Tukey test), [c]$p < 0.05$ for comparison with TA+S (Tukey test), [d]$p < 0.05$ for comparison with RA (Tukey test). TA, transection+anastomosis; S, sildenafil; RA, resection+anastomosis.

Table III. Mucosal protein and DNA concentrations in study groups.

		Group 1 (TA) n = 12	Group 2 (TA+S) n = 12	Group 3 (RA) n = 12	Group 4 (RA+S) n = 12	p^a
Ileum	Protein (mg/ml)/g	14.3 ± 3.1	14.4 ± 3.6	15.2 ± 2.4	17.9 ± 1.7[b,c]	0.022
	DNA (μg/ml)/mg	279.9 ± 19.0	333.2 ± 59.1[b]	313.3 ± 35.7	280.4 ± 23.8[c]	0.006
Jejenum	Protein (mg/ml)/g	9.7 ± 2.8	15.3 ± 2.9[b]	18.7 ± 4.0[b]	27.1 ± 4.6[b,c,d]	< 0.001
	DNA (μg/ml)/mg	300.8 ± 43.3	325.9 ± 47.7	356.0 ± 36.4[b]	383.9 ± 30.3[b,c]	< 0.001

[a]ANOVA, [b]p < 0.05 for comparison with TA (Tukey test), [c]$p < 0.05$ for comparison with TA+S (Tukey test), [d]$p < 0.05$ for comparison with RA (Tukey test). TA, transection+anastomosis; S, sildenafil; RA, resection+anastomosis.

other three groups throughout the study ($p < 0.001$ for each time point, Figure 1). While body weight of all rats showed a tendency to decrease until 7 days postoperatively, it increased steadily afterwards (Table I, Figure 3). There was no difference between TA+S, RA, and RA+S groups in terms of body weight change throughout the study (Figure 3).

Intestinal Adaptation Parameters

Bowel Weight

TA group had significantly lower ileal bowel weight than the TA+S, RA, and RA+S groups ($p < 0.05$, Table II). Ileal bowel weight of TA+S group was lower than that of RA and RA+S groups ($p < 0.05$). Jejunal bowel weight was significantly lower in TA and TA+S groups compared to RA and RA+S groups ($p < 0.05$). However, there was no difference between RA and RA+S, and TA and TA+S groups in terms of ileal and jejunal bowel weight ($p > 0.05$, Table II).

Mucosal Weight

Ileal and jejunal mucosal weights were significantly lowest in TA group (9.9 ± 1.7 and 13.1 ± 2.3, respectively mg/cm/100 g, Table 2). RA+S group had higher ileal and jejunal mucosal weights than both RA and TA+S groups ($p < 0.05$ for all). Ileal and jejunal mucosal weight showed no difference between TA+S and RA groups ($p > 0.05$, Table II).

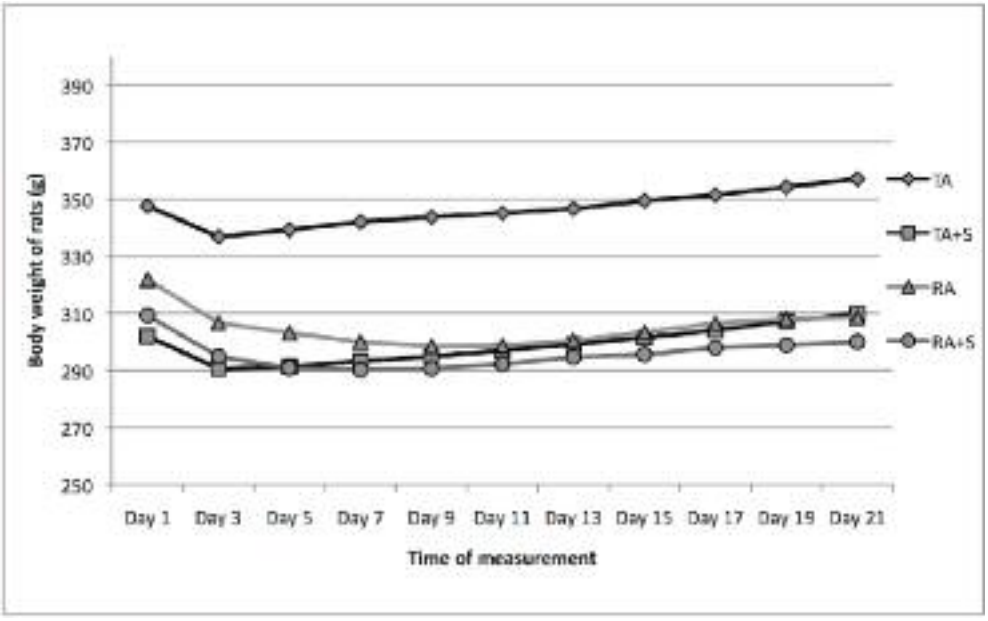

Figure 3. The change in total body weight of rats in study groups during study duration of 21 days. TA, transection+anastomosis; S, sildenafil; RA, resection+anastomosis.

Villus Height

Villus height was significantly lowest in TA group and highest in RA+S group both in ileum (333.4 ± 44.2 and 466.1 ± 38.6 μm, respectively) and jejunum (378.9 ± 42.3 and 648.1 ± 65.7 μm, respectively) (Table II). Ileal and jejunal villus height showed no difference between TA+S and RA groups ($p > 0.05$, Table II).

Crypt Depth

Ileal crypt depth was similar among four groups ($p = 0.051$, Table II). However, jejunal crypt depth was significantly lowest in TA group while it was highest in RA+S group compared to other groups (161.1 ± 17.8 and 255.1 ± 21.9 μm, respectively) (Table II).

Protein Concentration

Ileal protein concentration was significantly lower in TA and TA+S groups compared to RA+S groups ($p < 0.05$, Table III). Jejunal protein concentration was recorded lowest in TA group (9.7 ± 2.8 [mg/mL]/g) than other three groups. Rats in RA+S group demonstrated significantly higher jejunal protein concentration than both TA+S and RA groups ($p < 0.05$, Table III). There was no significant difference in ileal and jejunal protein concentration between TA and TA+S groups and in ileal protein concentration between RA ve RA+S groups ($p > 0.05$, Table III).

DNA Concentration

Ileum DNA concentration was higher in TA+S group than TA and RA+S groups ($p < 0.05$, Table III). Jejunal DNA concentration was significantly lower in TA and TA+S groups compared to RA and RA+S groups ($p < 0.05$, Table III). There was no other significant difference between study groups with regard to ileal and jejunal DNA concentrations (Table III).

Discussion

The survival of patients with SBS is closely associated with adaptation of the remaining intestine. So far, there have been several agents experimentally studied to increase intestinal adaptation in SBS[5,28]. However, many of these substances are not used clinically. Therefore, the search for new substances to improve intestinal adaptation still remains the need for clinical use. Sildenafil has well-known beneficial effects in gastrointestinal tract[21-24]. Furthermore sildenafil delays gastric emptying and intestinal transit in rats when given intravenously[25] or injected directly into the duodenum[26]. Sildenafil was also shown to have inhibitory effect on esophageal peristalsis and tonus of lower esophageal sphincter in cats[29], myorelaxant and antispasmotic effect on rat duodenum[30], and inhibitory effect on small intestinal motility[31]. In contrast, Bianco et al[32] reported the prokinetic effect of sildenafil, which improved gastric emptying in two patients with diabetic gastropathy. In the present study, we evaluated the effect of sildenafil on intestinal adaptation parameters after ileal transection+anastomosis or small bowel resection+anastomosis in rats. The parameters we evaluated in the present study were commonly used parameters in SBS modeling studies. Among these parameters, postoperative weight gain indicate an improvement in dehydration and electrolyte imbalance in short-term and in overall clinical condition in long-term. In studies using SBS rat model, 6-20% weight loss was reported postoperatively[33-35]. In our study, rats with SBS, i.e. groups RA and RA+S had approximately 4% and 3% weight loss in postoperative three weeks without significant difference between groups.

Bowel and mucosal weight is another common parameter used to evaluate intestinal adaptation in SBS. SBS rats demonstrate a significant increase in jejunal and ileal bowel and mucosal weight, which is an indicator of intestinal adaptation[15]. Sukhotnik et al[15] reported that subcutaneous insulin application causes higher bowel and mucosal weight of ileum and jejunum compared to control group of no treatment and improves intestinal adaptation in a rat model of SBS. In our study, we also recorded significantly increased bowel and mucosal weight of both jejunum and ileum in SBS groups (RA and RA+S groups) compared to transection+anastomosis groups. However, sildenafil had no significant effect on jejunal and ileal bowel weight in rats with SBS. On the other hand, sildenafil significantly increased jejunal and ileal mucosal weight in both SBS and transection groups.

Changes in villus height and crypth depth were also investigated to evaluate the intestinal adaptation in several SBS studies[16,36-38]. Collantes Perez et al[36] reported hypertrophy in the remaining bowel in an experimental model of SBS. Sukhotnik et al[15] also showed an increase in villus height and crypt depth in jejunum and ileum of rats after SBS. Sukhotnik et al[15], furthermore, indicated that insulin injection increased crypt depth in jejunum and ileum, but had no effect on villus he-

ight. In another study by Sukhotnik et al[39], the jejunum villus height and the ileal crypt depth was found to be significantly increased after SBS. In this report, transforming growth factor-alpha increased jejunal and ileal villus height without significant effect on crypt depth. In our work, the SBS group treated with sildenafil sildenafil (RA+S) had incrased villus height compared to the SBS group not given sildenafil (RA). Sildenafil also increased both the jejunal and ileal villus height and crypth depth in rats with transection+anastomosis. The jejunal crypt depth was increased in sildenafil-treated groups in both SBS and transection rats. However, in terms of ileum crypt depth, there was no statistically significant difference between study groups.

The concentration of DNA and protein is another marker of mucosal adaptation after SBS – both were reported to increase significantly in both ileum and jejunum after SBS[15,16]. In our study, protein concentration increased only in jejunum in sildenafil-treated SBS and transection groups compared to those not treated with sildenafil. The increase in ileal protein concentration did not reach statistical significance. DNA concentration in ileum was found to be increased only in sildenafil-treated resection group, but sildenafil did not produce any difference in SBS groups. DNA concentration in the jejunum has not changed with the administration of sildenafil in any group.

Conclusions

In the present experimental SBS model, sildenafil, a smooth muscle myorelaxant, improves intestinal adaptation evaluated by bowel and mucosal weight, protein and DNA concentration, villus height, and crypt depth particularly in the jejunum. Promising effect of sildenafil in this experimental rat model of SBS will form a basis for further clinical studies and evaluation of its use in the clinical management of patients with SBS.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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