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ORIGINAL RESEARCH

Morphological Adaptation in Adult Short Bowel Syndrome Undergoing Intestinal Rehabilitation

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ABSTRACT

Objectives: To observe the adaptive changes of the intestinal mucosa in patients with short bowel syndrome (SBS) who received intestinal rehabilitation therapy and further evaluate the feasibility of the magnifying endoscopy for monitoring intestinal adaptation. *Method*: Ten patients with SBS received growth hormone (GH) (0.05 mg/kg/day), oral glutamine (30 g/day), plus enteral nutrition (EN) for 4 weeks. Intestinal absorptive function, mucosal morphology, proliferating, and apoptotic status were investigated before and after treatment. *Result*: All patients completed the treatment. Intestinal absorptive function and villus height were significantly improved after treatment ($p < .05$). Furthermore, the proliferation levels of the small bowel were increased ($p < .05$). In addition, we also found that magnifying endoscopy can well describe the microscopic changes of intestinal mucosa. *Conclusion*: Treatment with GH, glutamine, and EN has positive effect on intestinal mucosal morphologic adaptation in patients with SBS. Furthermore, magnifying endoscopy for monitoring intestinal adaptation is practicable and reliable.

Keywords: growth hormone; glutamine; nutrition; magnifying endoscopy; short bowel syndrome

INTRODUCTION

Short bowel syndrome (SBS) is a group of problems related to malabsorption of fluid, electrolyte, and nutrients, occurring in people who underwent extensive intestinal resection [1]. Patients with SBS usually require long-term home parental nutrition (HPN) until intestinal adaptation occurs. However, in adult population, the incidence of HPN-associated complications is high [2, 3].

Although previous investigations have demonstrated that the intestinal rehabilitation therapy (IRT), which includes growth hormone (GH), glutamine, and a modified diet, contributes greatly to the compensatory effect of residual small bowel [4–6], the direct evidence for the microscopic changes of intestinal mucosa is limited. Magnifying endoscopy, which has been designed to improve the quality of images and the ability to distinguish details, has been extensively used for clinical diagnosis [7–9]. However, none of them focus on evaluating adaptive changes of intestinal mucosa.

Therefore, the aim of this study is to observe the adaptive changes of intestinal mucosa in short bowel patients and further evaluate the feasibility of the magnifying endoscopy for monitoring intestinal adaptation.

MATERIALS AND METHODS

Patients

Patients enrolled in the study were identified by physicians and the inclusion criteria were as follows: over 18 years of age; the length of remnant small bowel ≤ 100 cm; without any surgical resection of the stomach, duodenum, or pancreas; normal liver, kidney function and cardiovascular status; no clinical suspicion of active inflammatory bowel disease or fistulas; and no history of radiation enteritis. The total length of residual small bowel was derived from operative reports and confirmed by radiographic examinations.

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Patients with a history of malignancy, diabetes mellitus, or those in an unstable condition such as sepsis, acute gastroenteritis, pneumonia, or previous exposure to GH were excluded.

Those patients who met these screening criteria underwent a complete history and physical examination. Each eligible patient signed a detailed informed consent form before the study, which was approved by the Ethics Committee of Jinling Hospital and was performed in compliance with the Helsinki Declaration of 1975, as revised in 1983.

Study Protocol

On admission, since most short bowel patients were characterized by malnutrition and/or dehydration, PN began early in most of them. Moreover, intravenous albumin and furosemide were given if necessary to reduce excessive water in the interstitial tissue and retrieve water–electrolyte disturbances. Once patients' tissue edema regressed, enteral-nutrition (EN)-enriched diet fiber [Nutrition Fibre, Nutricia Pharmaceutical Co., Ltd. (China); 15 g Dietary Fibre in 1000 ml, 1.0 kcal/ml] was initiated and combined PN and EN support was given. EN was delivered to all the patients via continuous nasogastric tube feeding. To avoid EN-related gastrointestinal complications, the initial speed was controlled at 20 ml/hr by a peristaltic pump. If the EN was well tolerated, the speed gradually increased to 50–80 ml/hr, and the concentration of EN also gradually increased from 500 to 1000 kcal/day and to 1500 kcal/day. Along with the increase of EN, PN gradually decreased. The total goal for daily nonprotein energy intake was determined by indirect calorimetry.

When EN was added and the patients were confirmed positive nitrogen balance by Kjedahl's method, IRT was initiated and continued for 4 weeks. Recombinant GH (Serono, Inc., Switzerland) 0.05 mg/kg/day was administered subcutaneously. Concurrent glutamine (Ajinomoto, Japan) supplementation was also provided at a dose of 30 g/day by the oral route.

Antidiarrhea agents were also given when EN was initiated and maintained throughout the study. In addition, other drugs, such as Vitamin D_3, calcium, and antiacids agents were delivered if necessary.

Intestinal Absorption Function

Intestinal absorptive capacity was determined through 72-hr enteral nitrogen balance test as described previously before and after the IRT [10]. For the enteral nitrogen balance test, the patients were fed with standard enteral formula for 3 consecutive days. For each day, all oral intake and stool outputs were recorded, and a sample of 2 ml was taken for nitrogen determination. The percentage absorption of nitrogen is calculated as [(oral intake − intestinal output)/oral intake] × 100%.

Magnification Endoscope and Mucosal Biopsy Specimens

Immediately before and after the treatment, endoscopic examination was performed in all the patients with a magnifying endoscopy (CF-H260, Olympus Corporation, Japan) which has the capability of magnifying images up to 70-fold. The procedures of examinations were performed by an experienced gastroenterologist who was aware of the indication (to obtain intestinal biopsy specimens) and clinical information but blind to laboratory data. The distal 10 cm of residual small bowel was evaluated for microscopic changes of intestinal mucosa, and a series of photographs was obtained during the whole process by the gastroenterologist.

On the basis of endoscopic findings, biopsy specimens were taken from each patient for histologic analysis. Samples were placed in formalin for standard processing (hematoxylin and eosin staining). They were then evaluated by a pathologist who was aware of clinical information but blind to the endoscopic

TABLE 1 Characteristics of the patients with SBS enrolled in the study

Patient ID	Sex/age (year)	Jejunum/ileum (cm)	Ileocecal valve intact	Colon-in count (%)[a]	Diagnosis	Delay since last surgery (year)
1	M/40	100/0	N	84	AWD	1
2	M/18	15/10	Y	100	SBV	1.5
3	M/38	100/0	N	84	IO	1
4	M/61	65/15	Y	100	MI	<1
5	M/32	35/15	Y	100	AWD	<1
6	M/33	70/10	Y	100	IO	<1
7	M/47	40/0	N	84	MI	<1
8	F/39	65/5	Y	100	MI	<1
9	M/31	35/0	N	100	SBV	<1
10	M/38	40/0	N	84	MI	<0.5

Note: MI, mesenteric infarction; SBV, small bowel volvulus; IO, intestinal obstruction; AWD, abdominal wall defect.
[a]According to Cummings et al. [11].

TABLE 2. Intestinal absorptive capacity during pretreatment and post-treatment

	Pretreatment	Post-treatment	p values
Absorptive rate of nitrogen (%)	57.97 ± 3.00	78.4 ± 0.93	.000

Note: Values are expressed as mean ± *SEM*.

findings. A total of 10 well-oriented intestinal crypt villus units in each section were quantitated by gastrointestinal pathologists using a graded scale.

Detection of Ki-67 and Caspase-3 by Immunohistochemistry

Expression of Ki-67 and caspase-3, the biological markers for cell proliferation and apoptosis, respectively, were evaluated by the standard avidin–biotin complex method. To evaluate and compare the results of immunohistochemistry, immunostaining results were evaluated on a semiquantitative scale including staining intensity and the percentage of positive cells. The qualitative intensity of immunostaining of the positive cells was graded as weak (+), moderate (2+), and strong (3+). In addition, the extent of immunostaining was scored into four categories according to the percentage of the immunostained positive cells: <25% (1+), 25%–50% (2+), 50%–75% (3+), and >75% (4+). A combined immunoreactivity score was calculated by multiplying the score of the percentage and the intensity, resulting in a combined score that ranged from 0 to 12.

Statistics

Paired student's t test was used for statistical analysis by using SPSS 16.0 (SPSS, Inc., USA) software. A p value $< .05$ was considered statistically significant. All data are expressed as means ± standard error of measurement (*SEM*).

RESULTS

Characteristics of Patients Enrolled in the Study

The baseline characteristics for each patient are listed in Table 1. Ten patients (9 men, 1 women; mean age 38 ± 4 years, range 18–61 years) with SBS were eligible for this study and the average length of residual intestine was 62 ± 8.7 cm (range, 25–100 cm). The causes of SBS were small bowel volvulus ($n = 2$), intestinal obstruction ($n = 2$), mesenteric infarction ($n = 4$), and trauma ($n = 2$). Five had ileocecal valves and intact colon. All patients received bowel rehabilitation therapy <2 years after massive intestinal resection. No patients were withdrawn during the study.

TABLE 3. Measurement of intestinal mucosal morphology in all patients with SBS

	Pretreatment	Post-treatment
Villus height (μm)	267.96 ± 34.52	368.32 ± 74.61*
Crypt depth (μm)	96.33 ± 22.73	103.53 ± 21.23

Note: Values are in micrometers expressed as means ± *SE*.
*$p < .05$ compared with pretreatment.

Intestinal Absorption Function

Values on nitrogen absorption are showed in Table 2. Compared with pretreatment, intestinal absorptive capacity was significantly improved at the conclusion of the treatment ($p < .05$).

Morphological Data

Villus height was significantly ($p < .05$) increased compared with that of pretreatment. Although there was an increase in crypt depth of intestinal mucosa, this was not statistically significant ($p > .05$; see Table 3 and Figure 1). The proliferation rate of intestinal mucosa was evaluated by Ki-67 as a marker of proliferative cells (see Figure 2) and caspase-3, as a surrogate of apoptosis (see Figure 3). The results obtained by immunohistochemistry indicate that Ki-67 expression after treatment was up-regulated significantly ($p < .05$); while that of caspase-3 had no significant change at the conclusion of treatment ($p > .05$; see Figure 4).

In addition, mucosal morphology of remnant intestine observed by magnifying endoscopy revealed that the villa height, diameter, and density during post-treatment are remarkably increased compared with those of pretreatment (see Figure 5).

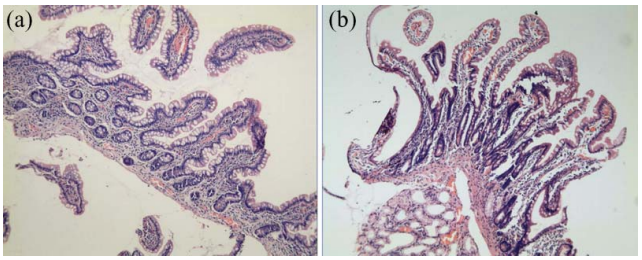

FIGURE 1 Morphologic changes of small bowel mucosa (HE × 100). (a) pretreatment; (b) post-treatment.

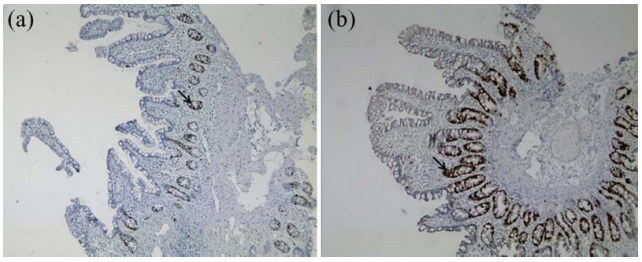

FIGURE 2 Ki-67 protein expression in the small bowel mucosa (original magnification ×100). (a) pretreatment; (b) post-treatment.

DISCUSSION

SBS occurs when the functioning intestinal mass is lessened below the amount necessary for adequate digestion and absorption of nutrition and water–electrolyte [1]. To meet the basic nutritional requirements of body, patients with SBS often require long-term HPN, which is expensive and associated with certain complications [2, 3]. However, the residual intestine has an inherent ability to adapt morphologically and functionally after massive intestinal resection. To enhance intestinal adaptation and regain nutritional autonomy, maximum exposure of all of the available intestinal surface area to nutrition and other trophic factors, such as GH and glutamine, in early stage is indispensable.

The gastrointestinal tract is highly responsive to the trophic effect of GH, which has been shown to directly stimulate protein synthesis and the growth of the intestinal mucosa by increasing cell proliferation and collagen deposition [12, 13]. Moreover, several groups have demonstrated that there is an increase in absorption per unit length of carbohydrates, proteins, water, and electrolytes following GH administration in SBS models [14–16]. Glutamine is the primary fuel source for enterocytes, which has been shown to be trophic to the bowel and to enhance nutrient absorption [17–19]. It is also necessary for cell signal transduction pathways when gut enterocytes are exposed to trophic factors [20]. In addition, GH could enhance intestinal uptake of glutamine [21]. Therefore, a combination of GH and glutamine may have a synergistic and additive effect on gut adaptation.

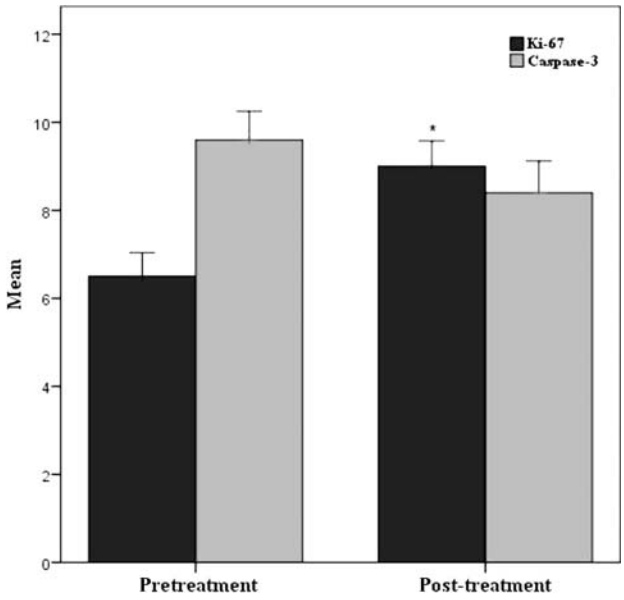

FIGURE 4 Immunohistochemical analysis of Ki-67 and caspase-3 protein expression in the intestinal mucosa. $^{*}p < .05$ compared with pretreatment.

Evidence supporting the use of GH, glutamine, and EN in SBS includes the fact that IRT stimulating structural and functional adaptation of intestine in rodents underwent extensive intestinal resection [5, 6, 22]. However, the direct evidence for these changes in humans is limited. Although the functional changes have been documented in patients with SBS, the changes in villus/crypt architecture described in animals have not been reproduced by most studies.

In the current study, we have shown that the intestinal absorptive function has been significantly increased compared with that of pretreatment. In accordance with functional changes, the microscopic changes of intestinal villus/crypt architecture described in rodents have also been reproduced in our study. Although no significant difference in the expression of caspase-3 was observed during the treatment, the Ki-67 expression in the intestinal mucosa was significantly increased

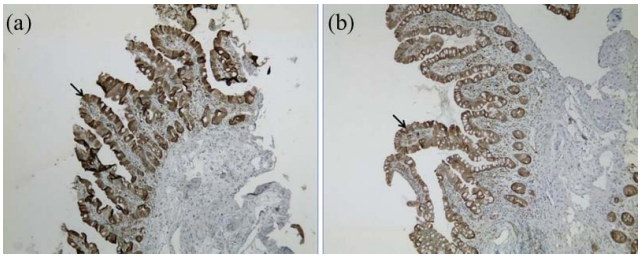

FIGURE 3 Caspase-3 protein expression in the small bowel mucosa (original magnification ×100). (a) pre-treatment; (b) post-treatment.

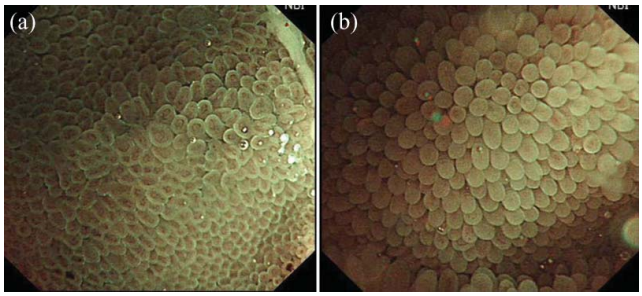

FIGURE 5 Magnifying endoscopic images of the intestinal mucosa. (a) pretreatment; (b) post-treatment.

compared with that of pretreatment. Therefore, there is an increase in both the epithelial cell proliferation rate in the crypts and the migration rate of the cells upward to the villus tip, resulting in elongated villi and increased mucosal absorptive area, which was further validated by endoscopic findings.

Although magnifying endoscopy has been widely applied to clinical diagnosis, none of them focus on evaluating intestinal adaptive of short bowel patients [7–9]. To our knowledge, we are the first who have proposed the idea and applied it in clinical practice [23]. As is well known, magnifying endoscopy is useful in that it more accurately detects subtle changes of mucosa than standard endoscopy. Furthermore, magnifying endoscopy could display three-dimensional imaging of the intestinal villi, which is more vivid than standard endoscopy and histopathology. Currently, despite many obvious advantages of the instrument, it still has certain slight technical limitations. For instance, for accurate focusing, a constant distance must be maintained between the object and the lens. In our study, magnifying endoscopy could well describe the microscopic changes of intestinal mucosa, which is in conformity with pathological findings. And this further demonstrates that direct evaluation of the intestinal adaptation by magnifying endoscope is feasible.

In summary, our data suggest that patients with SBS who received IRT presented in the remaining intestine a significant adaptive process characterized by an enlarged absorptive surface with an increased proliferative rate. In addition, our study also indicates that magnifying endoscopy for monitoring intestinal adaptation is practicable and reliable. However, the magnifying endoscopic feature and grade hyperplasia of intestinal mucosa should also be identified. So a series of studies is necessary to be done for the diagnosis of mucosal morphologic adaptation in SBS patients by magnifying endoscopy.

Declaration of Interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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