The Trophic Effects of Long Chain Triglycerides on the Atrophic Ileal Mucosa of Rats

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Summary Rats with atrophic intestinal mucosa due to enteral nutrition supplied by an elemental diet (ED) for 4 weeks or more, received a fat-enriched ED containing 10% long chain triglycerides (10% FED) orally. The atrophic ileal mucosa became trophic 4 weeks after administration of the 10% FED. Ornithine decarboxylase activity in the ileal mucosa increased 3 days after the administration of 10% FED. Rats with atrophic intestinal mucosa that had undergone a 70% proximal jejunileectomy, received an oral ED containing 4% long chain triglycerides (4% FED). In the jejunileectomized rats, marked proliferation of the remaining ileum was observed irrespective of diet, when compared with the transected control group. In the transected group, the 4% FED had trophic effects on the ileum, but in the jejunileectomized group, the 4% FED had no significant trophic effect on the remaining ileum. In conclusion, long chain triglycerides had mild trophic effects on ileal mucosa and were effective in the treatment of atrophic intestinal mucosa. However, the trophic effects of fat were apparently masked by the marked proliferation of the ileal mucosa following jejunileectomy.

Key Words long chain triglyceride, elemental diet, ileal mucosa, ornithine decarboxylase activity

Enteral nutrition is often used to keep the bowel at rest in the acute stages of small intestinal diseases. However, long-term enteral nutrition is known to induce mucosal atrophy in the small intestine, which impairs digestion and absorption of nutrients. These mucosal changes are thought to be caused by the reduction in physical stimulation of the intestine in addition to the lack of dietary fiber in enteral nutritional preparations (1, 2). The fat content in nutritional preparations also seems to be an important factor in the maintenance of the intestinal mucosa for the following reasons. Mucosal atrophy in the small intestine is marked after long-term enteral nutrition with an elemental diet (ED, Elental® contains the lowest quantity of fat among the clinical enteral nutrition preparations) (3). Maxton et al. demonstrated the trophic effects of linoleic acid on the normal small intestine of rats.
after 4 weeks of feeding on an amino acid elemental diet (Vivonex HN®) or on Vivonex HN with 50% calorie substitution by linoleic acid (4). Other studies have indicated that orally administered triglycerides, regardless of linoleic acid or saturated fatty acid content, have trophic effects on the normal small intestinal mucosa of rats (5). However, there are no reports which have elucidated the effects of fat on atrophic intestinal mucosa caused by enteral nutrition. If orally administered fat has trophic effects on atrophied gut mucosa, the addition of a small amount of fat to enteral nutritional preparations may reduce the progression of mucosal atrophy. This study was undertaken to clarify the proliferative effects of fat on atrophic small intestinal mucosa. An ED formula containing an increased amount of soybean oil was administered orally to rats with atrophic small intestinal mucosa, and its trophic effects on the intestinal mucosa were evaluated.

In addition, the trophic effects of fat on the intestine after intestinal resection were studied, for the purpose of evaluating whether the effects of fat are enhanced under conditions of rapid proliferation.

METHODS

Forty-eight male Wistar rats aged 4 weeks (Clea Japan Co., Tokyo, Japan) were individually maintained in wire bottom cages. The following 3 types of food were prepared: 1) ED, 2) ED with the addition of 4% soybean oil (4% FED), and 3) ED with the addition of 10% soybean oil, and an equivalent amount of dextrin removed for calorie adjustment (10% FED) (Table 1). Each diet was administered as an oral solution (1 kcal/ml).

Experiment 1. Twenty-four rats were maintained on the ED for 6 weeks and then divided into 2 groups. One group was maintained on the ED (ED group) and the other was fed the 10% FED (10% FED group). The rats were allowed free access to the bottle containing the liquid food, but paired feeding was performed to maintain equal caloric intake for the 2 groups. Six rats in each group were

Table 1. Composition of diet formulae.

|                | ED                     | Fat-enriched ED (FED) |
|----------------|------------------------|-----------------------|
|                | g in 100g ED | mg/ml in solution | g in 100g FED | mg/ml in solution | g in 100g FED | mg/ml in solution |
| Amino acids    | 16.43       | 42.24          | 16.43       | 39.19          | 16.43       | 37.57           |
| Dextrin        | 79.37       | 204.06         | 79.37       | 109.34         | 70.40       | 160.98          |
| Soybean oil    | 0.64        | 1.65           | 4.00        | 9.54           | 10.00       | 22.87           |
| Lecithin       | 0.021       | 0.05           | 0.326       | 0.78           | 0.931       | 2.13            |
| Polysolvent    | 0.025       | 0.06           | 0.069       | 0.16           | 0.159       | 0.36            |
| Minerals, vitamins, others | 3.514       | 9.03           | 3.165       | 7.55           | 2.080       | 4.76            |

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decapitated after 3 days, and the same number of rats after 4 weeks, under anesthesia by diethyl ether. The abdomen was opened, and the intestinal segment from Treitz's ligament to the cecum was removed, rinsed with phosphate-buffered saline (pH 7.6) at 4°C, and was hung with a 10-g weight. A 10-cm ileal segment, from 10 to 20 cm oral to the terminal ileum, was removed, and the mucosa was scraped with a glass slide. Mucosal sampling by scraping method was done by one person both in experiments 1 and 2. After measurement of the wet weight of the mucosa, the tissue was homogenized for 30 s with 10 ml of phosphate-buffered saline (pH 7.2) containing 0.1 mM pyridoxal-5’-phosphate and 5 mM dithiothreitol using an ultradisperser (LK-22, Yamato Scientific Co., Tokyo, Japan). A part of the homogenate was used for the measurement of ornithine decarboxylase (ODC) activity (6). ODC activity was determined by measuring 14CO2 released from L-(1-14C)-ornithine (American Radiolabeled Chemicals Inc., St. Louis, USA). The remaining homogenate was frozen, and stored at −20°C and used for the measurement of protein content by the Lowry method (7) and the measurement of DNA content by the ethidium bromide method (8). Results of measurements were compared between the two groups.

Experiment 2. Twenty-four rats were fed the ED for 4 weeks and then divided into 4 groups. Transection in the small intestine or 70% proximal jejunoileectomy was performed under pentobarbital anesthesia (30 mg/kg body weight). Transection was performed, a jejunal site (below Treitz’s ligament) and ileal site (30 cm oral to the terminal ileum) were transected and sutured. For proximal jejunoileectomy, the intestinal segment between these two sites was removed and the remaining jejunum and ileum were anastomosed by the end-to-end method. After surgery, the rats were fed the ED or the 4% FED. Four weeks after the operation, a 10-cm ileal segment which was 10–20 cm oral to the terminal ileum was obtained, and the wet weight, protein content, and DNA content of the mucosa were measured.

Statistical analysis. A STAT FLEX Statistics Program (Nankodo Co., Tokyo, Japan) was used to analyze the data. Data were expressed as the M±SD. The t-test was used for comparisons between the two groups, and p values <0.05 were considered to be significant.

RESULTS

Experiment 1

Weight gain did not significantly differ between the ED and 10% FED groups (Fig. 1). The wet weight and protein content of the ileal mucosa 4 weeks after the change to the experimental diet were significantly higher in the 10% FED group than in the FD group (Fig. 2). Since the DNA content was also higher in the 10% FED group, the increases in mucosal parameters were apparently due to hyperplastic changes in the ileal mucosa, suggesting that the 10% FED had trophic effects on the ileum.
Fig. 1. Oral intake and body weight changes of the rats.

Fig. 2. Mucosal parameters of the ileum 4 weeks after the change to the ED or 10% FED.

ODC activity was significantly higher in the 10% FED group than in the ED group 3 days after the change to the experimental diet, but did not differ significantly after 4 weeks (Fig. 3). These results suggest that proliferative changes in the ileal mucosa may occur during the early stages following the change to the experimental diet.

Experiment 2
The wet weight of the remaining ileum was significantly higher in the jejunoileectomy group than in the transected group, irrespective of type of diet. In the transection group, the mucosal wet weight was significantly higher in the 4% FED
Fig. 3. ODC activity in the ileal mucosa of rats fed with the experimental diet for 3 days and 4 weeks.

Fig. 4. Mucosal parameters of the remaining ileum after proximal jejunoileectomy or transection.

**DISCUSSION**

In our preliminary experiments, the atrophy in the rat small intestine has progressed for the first 4 weeks of ED feeding, but remained unchanged thereafter. In this study, the ED was given for more than 4 weeks to induce atrophy of the
intestinal mucosa, and the effects of long chain triglycerides on the atrophied ileal mucosa were evaluated. Soy bean oil, which consists of long chain triglycerides, was used as the source of dietary fat in these experiments.

Marked diarrhea was observed for about 1 week after the proximal jejunooileectomy was started on the 10% FED. This diarrhea occurred in spite of increased lecithin as emulsion, suggesting that the reduction of the effective absorptive area was caused by small intestinal resection. Since an evaluation of the trophic effects on the intestinal mucosa was difficult in rats with severe diarrhea, the 4% FED was used instead of the 10% FED in experiment 2. Since the linoleic acid in Elental® accounts for only 0.6% of the calories, rats might become deficient in essential fatty acids after 4 weeks of Elental® (9) feeding. Soy bean oil used in this experiment has about 50% of linoleic acid, so that the administration of 4% FED is sufficient for preventing the rats from essential fatty acid deficiency. Therefore, the trophic effect shown in our experiments may be due to essential fatty acids as well as long chain triglycerides. Furthermore, the increased lecithin and polysolvent are necessary for emulsifying enriched fat in 4 and 10% FED. The trophic effect of FED would be due to long chain triglycerides, although the effect of those additional elements might not be negligible.

The trophic effects of the 10% FED on the ileal mucosa were observed in experiment 1, but body weight did not differ between the two diets. These findings suggest that the trophic effects on the ileum were not secondary to improvements in the general nutritional states, but induced directly in the proliferative system of the intestine. Trophic effects were also apparent in the transected group in experiment 2, and the degree of increase in mucosal mass did not markedly differ between the 10% FED and 4% groups, suggesting that even 4% long chain triglycerides have measurable trophic effects.

In general, it is recognized that ODC activity transiently increases at the early stage when the acceleration of cell proliferation is initiated (6). In experiment 1, ODC activity in the ileal mucosa was higher in the 10% FED group than in the ED group 3 days after the change to the experimental diet. This suggests that the proliferation of the ileal mucosa may partially be promoted via polyamine metabolism. The ODC activity, however, was not markedly increased, compared with the increase observed following jejunectomy (10), and the degree of proliferation was apparently minimal.

The ileum remaining after proximal jejunoileectomy had increased entero-glucagon levels, and demonstrated markedly enhanced proliferation in the mucosa (11). Assuming that orally administered 4% FED is effective for ileal proliferation, the proliferative effects of fat on the remaining ileum after 70% proximal jejunoileectomy should be more marked than after transection. However, fat had no significant trophic effect in the jejunoileectomized rats. These findings suggest that the proliferative effects of fat are not associated with the adequacy of the energy supply. Orally administered fat is known to increase exocrine pancreatic juice and bile (12) and to promote the release of neurotensin (13, 14). These mechanisms
may be related to the trophic effects of the 4% FED on the intestine (15). However, these mechanisms induced by 4% FED may be minimal, and the trophic effects seem to be masked. Thus, it may be difficult to detect proliferative effects in the ileal mucosa after resection.

The mild trophic effects on atrophic ileal mucosa following the oral administration of long chain triglycerides, have clinical applicability. Atrophic intestinal mucosa caused by long-term administration of ED or parenteral nutrition may be prevented by the oral administration of a small amount of fat. In recent years, attention has been focused on the usefulness of medium chain triglycerides (16), and long chain triglycerides tend to be removed from nutritional preparations because of difficulty with digestion and absorption. Long chain triglycerides, however, can be used to maintain the mucosal mass as well as to improve nutritional management.

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