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The trophic effect of epidermal growth factor on morphological changes and polyamine metabolism in the small intestine of rats

Tomoyuki TSUJIKAWA, Tadao BAMBA, and Shiro HOSODA
Second Department of Internal Medicine, Shiga University of Medical Science, Shiga 520-21, Japan

Summary: This study was undertaken to evaluate the effect of epidermal growth factor (EGF) on the morphological changes and polyamine metabolism in the atrophic small intestinal mucosa of rats caused by feeding elemental diet (ED; Elental®, Ajinomoto, Tokyo) for several weeks. Four-week-old Wistar male rats were given ad libitum ED (1 kcal/ml) for 4 weeks. The body weight increased to the same extent as the control group fed a pellet diet. However, the small intestine became atrophic: the mucosal wet weight of the jejunum decreased to 70%, while that of the ileum decreased to 60%. EGF (10 μg/kg) was subcutaneously injected into these rats every 8 hours. Ornithine decarboxylase (ODC) activities of the jejunal and ileal mucosa rose within 12 hours of the initial EGF administration. Mucosal DNA specific activities tended to increase. Next, EGF (30 μg/kg/day) was intraperitoneally administered with a Mini-osmotic pump for one week. The wet weight, protein and DNA contents of the ileal mucosa increased significantly compared with those of the saline administered controls, while the crypt cell production rate (CCPR) also increased. Histologically, increases in both villus height and crypt depth were confirmed. These findings indicate that EGF causes mucosal proliferation through polyamine metabolism even in the atrophic small intestine of mature rats after ED administration for 4 weeks. *Gastroenterol Jpn 1990;25:328–334*

Key words: *elemental diet; epidermal growth factor; ornithine decarboxylase*

Introduction

A fiber-free elemental diet, an enteral nutritional product, is widely and clinically used not only for pre- and post-operative nutritional control but also for the nutritional treatment of inflammatory bowel disease. In recent years, reports have been published on mucosal atrophy of the small intestine[1] and on changes in the mucosal immunological states associated with prolonged total parenteral nutrition[2]. As a result, the importance of intraluminal nutrient supply has been recognized. However, atrophy of the small intestinal mucosa was also noted in rats fed on ED for a long period after weaning, regardless of any differences in body weight or growth between pellet diet-fed groups and ED-fed groups. Since epidermal growth factor (EGF) was originally isolated from the submaxillary grand of the mouse by Cohen et al[3] in 1962, it has been reported to affect proliferation[4] and differentiation[5] in various cells and organs. As to the small intestine, it is accepted that EGF stimulates the differentiation and proliferation of the small intestine in young rats and mice[6-8]. However, the effect of EGF on mature rats remains unclear[9-11].

In this study, we examined the effect of EGF on the atrophic small intestine of mature rats fed on ED for 4 weeks.

Materials and Methods

1. Experimental animals and diet

Male 4-week-old Wistar rats were used for this

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Address for correspondence: Tomoyuki Tsujikawa, M.D., The Second Department of Internal Medicine, Shiga University of Medical Science, Tsukinowa-cho, Otsu, Shiga 520-21, Japan.

study. After feeding on only ED for 4 weeks, rats were divided into control and EGF groups. Elental® (Aminomoto Inc., Tokyo, Japan; ED) consisting of (g/kg) 176 amino acids, 794 dextrin (mean molecular weight 900), 6 soybean oil, 24 vitamins and minerals was dissolved in water to yield 1 kcal/ml and was given ad libitum with a water-feeding bottle. The animals were housed in wire-bottomed cages in an animal room maintained at 22°C with a 12-hour period of light (from 8 a.m. to 8 p.m.)

2. EGF

EGF used in the experiment was extracted from the mouse submaxillary gland (mEGF: Toyobo Co., Ltd. Osaka, Japan).

3. Administration of EGF and preparation of specimens

EGF was dissolved in a saline solution and injected subcutaneously at a dose of 10 μg/kg every 8 hours in order to keep a constant EGF blood level. Three distinct groups were determined according to the period of EGF administration: 12, 24 and 48 hours. All the rats were anesthetized with pentobarbital and then decapitated. For the control group a saline solution was used. Even during EGF injection, ED was given ad libitum. One hour before sacrifice, 18.5 kBq/g body weight of (methyl-^{3}H) thymidine (740-1,480 GBq/mmol, ICN Biomedicals, Inc., Costa Mesa, USA) was intraperitoneally injected to all the rats. The small intestine between the ligament of Treitz and ileocecal valve was rapidly excised and washed in chilled 0.9% NaCl solution. The bowel was suspended under uniform tension from a 10g weight. Two 10-cm segments of the bowel were identified, one starting 2cm distal to the ligament of Treitz (jejunum) and one 2cm proximal to the ileocecal valve (ileum). Mucosal scrapings were obtained from the segments using glass slides on a chilled plate. After measurement of the mucosal wet weight, the mucosa was homogenized with 10 strokes of a teflon pestle at 10ml in chilled 0.03M sodium phosphate buffer (pH 7.2) with 0.1mM pyridoxal-5'-phosphate, 5mM dithiothreitol, 0.1mM ethylenediamine tetraacetic acid disodium salt and 0.01% Tween 80, and then sonicated for 20 seconds. Aliquots of the homogenate were frozen at −70°C until measurement.

Following this, EGF administration with a Mini-osmotic pump (Model 2001, Alza Corp., Palo Alto, USA) during a period of one week was chosen, as rats had become weak because of the large number of injections. Furthermore, intraperitoneal insertion was chosen, since hematoma lesions were observed after subcutaneous insertion. In this way, a 50μg dose of EGF poured into the pump could be administered at an average daily dose of 30 μg/kg. In the control group, a saline solution was poured into the pump.

One week later, specimens were prepared in the same way as above mentioned. For the measurement of the crypt cell production rate (CCPR), rats were injected intraperitoneally with 1.0 mg/kg body weight of vincristine sulfate (Shionogi Pharmaceutical Inc., Osaka, Japan) at either 30, 50, 70, 90 or 110 min before being killed.

4. Measurement of the activities of ornithine decarboxylase (ODC)

ODC activity was measured with the method of Luk et al[13]. Briefly, 300μl aliquots of the homogenate were incubated for 1h at 37°C with 0.4μl of 0.03M sodium phosphate buffer (pH7.2) containing 0.1mM pyridoxal-5'-phosphate, 6mM dithiothreitol, 0.8mM 1-ornithine, and 46.25 kBq/ml 1-(1-^{14}C) ornithine HCl (1.48-2.22 GBq/mmol, American Radiolabeled Chemicals Inc., St. Louis, USA) in a test tube with a flat bottom. During the incubation, ^{14}C-CO_2 was trapped in a filter paper soaked in hyamine hydroxide. The reaction was stopped with 1ml of 10% trichloroacetic acid (TCA), and the test tube was allowed to stand for an additional hour. Then, the ODC activity was measured by determining the radioactivity of the filter paper. An ACS II (Amersham Inc., Arlington Heights, Ill) scintillator and a Packard TRI-CRBB 1500 liquid scintillation counter were used.

The protein content was measured with Lowry's method[14]. The activity of ODC was expressed as specific activity per milligram protein.

5. Measurement of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and crypt cell production rate (CCPR)

DNA was measured with the ethidium bromide method[15], and the radioactivity of ^{3}H-thymidine in 500μl homogenate was measured. The value of radioactivity was divided by DNA content in order to calculate DNA specific activity. After continuous EGF administration for one week, nucleic acid was extracted from the homogenate with the Schmidt-Thanhauser-Schneider method[16,17]. DNA was measured with diphenylamine method[18], while RNA was measured with orcin method[18]. CCPR was measured next. Specimens from the jejunum and ileum were fixed in Carnoy's fluid and stained with Feulgen reagent. After microdissection, the number of arrested metaphases in 10 crypts was counted in each specimen. The slope of the line fitted to the least-squares linear regression was considered to be the CCPR.

6. Measurement of polyamine

Polyamine was measured with a high-performance liquid chromatography, after specimens at 12 and 48 hours after EGF administration were deproteinized with TCA.

7. Preparation of histological specimens

After EGF administration for one week, the jejunum and ileum were fixed in 4% formalin, embedded in paraffin, and cut into 4μm-thick segments. After hematoxylin-eosin staining, villus height and crypt depth were measured.

8. Statistical analysis

The measurement was repeated 2 times for each specimens. The data were expressed as means±SEM unless otherwise indicated. Student's t-test was used for a statistical comparison between the control and the EGF groups, and $P<0.05$ was considered significant.

Results

1. Changes in the small intestinal mucosa after ED administration for 4 weeks

ED was given ad libitum to 4-week-old rats for 4 weeks. After 4 weeks, there was no difference in food intake or weight gain between the pellet-fed group and the ED-fed group (**Fig. 1**). The ED group showed a more significant decrease in small intestinal length and mucosal wet weight than the pellet diet group ($P<0.01$). In particular, the wet weight of the jejunal mucosa of the group fed with ED decreased to 60% of that of the pellet-fed group (**Fig. 2**).

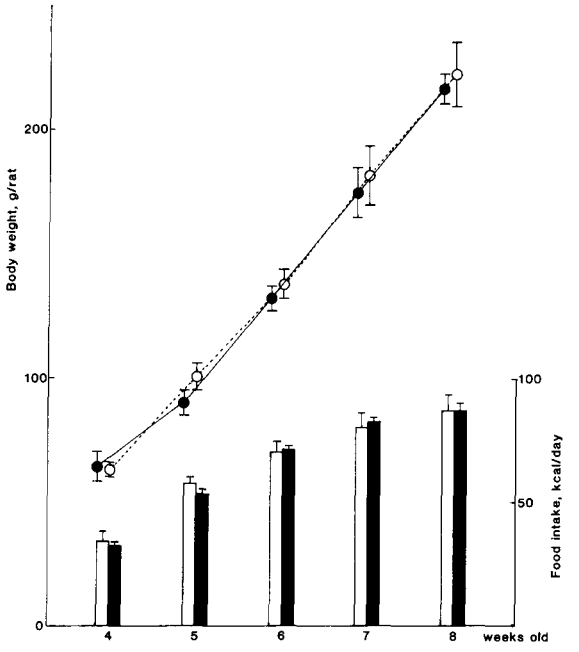

Fig. 1 Comparison of food intake and body weight change of rats fed ED and pellet diet for 4 weeks. Data were shown as mean±SD of 8 subjects. There was no significant difference in food intake and weight gain between ED and pellet diet groups. ○···○, □; Pellet diet-fed groups, ●—●, ■; ED-fed groups.

2. ODC activity in the small intestinal mucosa after the subcutaneous injection of EGF

The ODC activity in the jejunal mucosa was 144.14±24.11 (CO_2 pmols/h/mg protein) 12 hours after EGF injection, showing a significant rise ($P<0.05$) in comparison to the control group (59.26±19.59). Even 24 hours after EGF injection, the ODC activity was significantly higher ($P<0.05$). However, there ws no difference between the EGF group and the contorl group after 48 hours (72.81±15.34 vs. 76.54±15.36). The

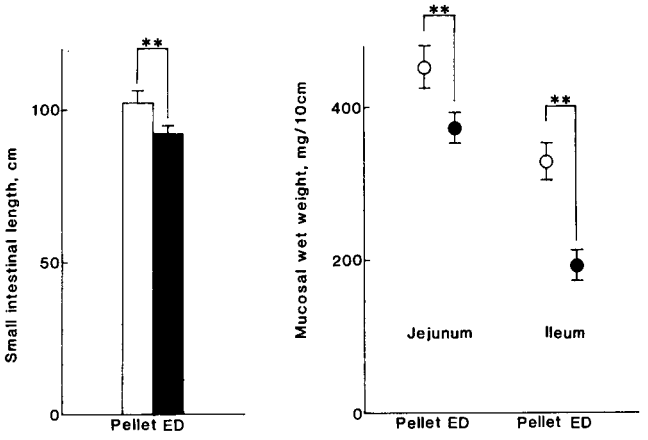

Fig. 2 Comparison of the small intestinal length and mucosal wet weight of rats fed ED and pellet diet after 4 weeks. Data were shown as mean±SD of 6 subjects. Significant differences were found in small intestinal length and mucosal wet weight between ED and pellet diet groups (**P<0.01).
□, ○; Pellet diet-fed groups, ■, ●; ED-fed groups.

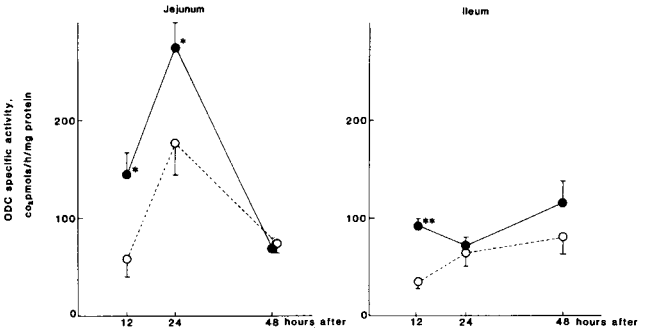

Fig. 3 Effect of EGF injection on ODC specific activity in the small intestinal mucosa of rats fed ED. Data were shown as mean±SEM of 4 subjects. There was a significant difference in ODC activity between control and EGF groups (*P<0.05, **P<0.01).
○···○; Control groups, ●—●; EGF injected groups.

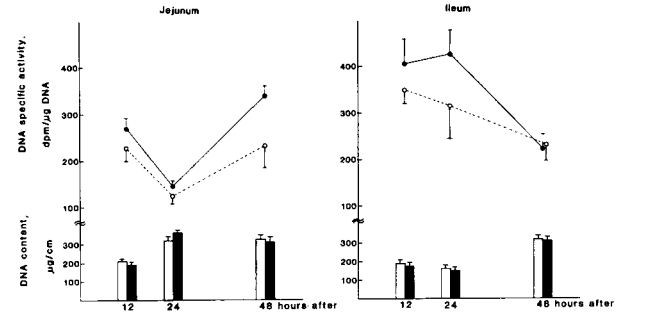

Fig. 4 Effect of EGF injection on DNA content and DNA specific activity in the small intestinal mucosa of rats fed ED. Data were shown as mean±SEM of 6 subjects. There was no difference in DNA content and DNA specific activity between control and EGF groups. ○···○, □; Control groups, ●—●, ■; EGF injected groups.

ODC activity of the ileal mucosa increased to 91.77±7.24 12 hours after EGF injection, significantly (P<0.01) higher than in the control group (33.90±4.83). Nevertheless, there was no difference between the two groups 24 and 48 hours after subcutaneous EGF injection (**Fig. 3**).

3. Changes in DNA-specific activity and DNA content in the small intestinal mucosa after the subcutaneous injection of EGF

The DNA-specific activity in the jejunal mucosa tended to rise 48 hours after EGF injection, while that in the ileal mucosa tended to do so 24 hours after EGF injection. However, there ws no difference in the DNA content of the jejunum and ielum between the two groups until 48 hours after EGF injection (**Fig. 4**).

4. Changes in polyamine content

Putrescine, spermidine, and spermine in the jejunal mucosa all increased 12 and 48 hours after EGF injection. In particular, putrescine levels in this group increased three times more than those in the saline control group by 48 hours after EGF injection. The same tendency was seen in the ileal mucosa: 48 hours after EGF injection, putrescine increased about four times compared with the saline injection group, though putrescine levels were rather low 12 hours after EGF injection (**Table 1**).

5. Changes in the small intestinal mucosal parameters after continuous intraperitoneal administration of EGF for one week

After the insertion of the Mini-osmotic pump, no sign of intraperitoneal infection was noted in either the EGF group or the control group. The gain in body weight decreased slightly in both the control group and the EGF group, as compared with that at one week before pump insertion. There was no difference between the two groups

Table 1 Effect of EGF injection on polyamines concentration (nmols/mg protein) in the small intestinal mucosa of rats fed ED

		Group	n	Putrescine	Spermidine	Spermine
Jejunum	12h	Control	6	0.27±0.08	6.55±0.37 *	2.87±0.17 *
		EGF	6	0.49±0.05	9.16±1.28	3.89±0.66
	48h	Control	6	0.36±0.14 **	7.24±1.42	3.34±0.62
		EGF	6	1.03±0.10	9.97±0.78	4.50±0.36
Ileum	12h	Control	6	0.49±0.29	4.02±0.79	2.17±0.53 *
		EGF	6	0.24±0.15	5.78±0.40	3.36±0.23
	48h	Control	6	0.17±0.12 *	6.77±0.70	3.94±0.30
		EGF	6	0.69±0.13	8.01±0.89	4.95±0.61

Data were shown as mean±SEM of 6 subjects. *P<0.05, **P<0.01 compared to control

Table 2 Effect of continuous intraperitoneal administration of EGF (50μg/rat/week) on the small intestinal mucosal parameters of rats fed ED

Mucosal parameters		Jejunum		Ileum	
		Control	EGF	Control	EGF
Mucosal wet weight	(mg/10cm small bowel)	337±19	375±23	197±9	240±17*
Protein content	(mg/cm small bowel)	4.27±0.14	4.68±0.18	2.44±0.07	3.10±0.20*
DNA content	(μg/cm small bowel)	207±13	252±9*	199±20	161±14**
RNA content	(μg/cm small bowel)	513±28	516±21	226±11	285±28

Data were shown as mean±SD of 6 subjects. *P<0.05, **P<0.01 compared to control

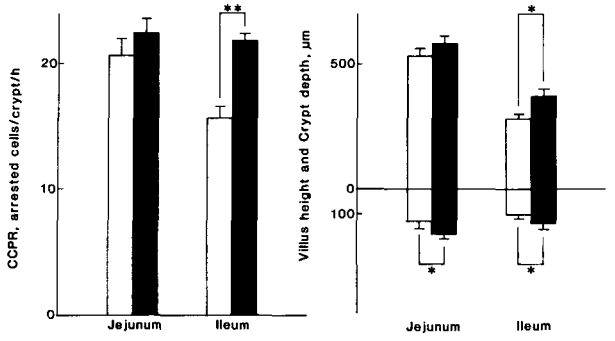

Fig. 5 Effect of continuous intraperitoneal administration of EGF (50 μg/rat/week) on CCPR (n=5) and villus height or crypt depth (n=3) in the small intestine of rats fed ED. Data were shown as mean±SD of 5 or 3 subjects. Significant differences were found in CCPR and the villus height and crypt depth between control and EGF groups (*P<0.05, **P<0.01). □; Control groups, ■: EGF groups.

in the body weight gain after one week (1.0±1.5 vs. 3.5±3.7 g: n=6), although energy ingested decreased to 80% in the EGF group.

In the jejunum, mucosal wet weight and protein content increased in the EGF group. DNA content increased significantly more in the EGF group than in the control group (253.3±9.0 vs. 206.6±13.5 μg/cm: P<0.05). In the ileum, mucosal wet weight, protein content, and DNA content increased significantly. The increase in the EGF group was significant (P<0.05), compared with that in the control group (**Table 2**).

No significant differences in RNA contents were demonstrated between the EGF and the control groups.

In both the jejunum and ileum in the EGF group, CCPR tended to rise. Especially in the ileum, CCPR rose significantly more (P<0.01) in the EGF group (22.2±4.3 arrest/crypt/h) than in the control group (18.3±2.9).

Histological examination revealed that in the jejunum, crypt depth had elongated significantly in the EGF group and that in the ielum, both villus height and crypt depth had increased significantly (P<0.05) (**Fig. 5**).

Discussion

After mature rats were fed with only ED for 4 weeks, the wet weight of the jejunal mucosa decreased to about 70%, and that of the ileal mucosa to about 60%.

It was reported that the small intestinal mucosa becomes atrophied because of a lack of dietary fiber[19] or digestive fluid secretion[20].

Reports on the effect of EGF on the small intestinal mucosa of rodents show that most of the studies employed immature animals within 3 weeks post natal[6-8]. In rats, the small intestine had not maturated completely 3 weeks after birth, the activities of brush border enzymes (ALP and sucrase) are low, while the activity of lactase is high. Morphologically, supranuclear vacuoles are seen in the ileal villus enterocyte[21]. EGF can induce precocious maturation in the immature rat's small intestine, while increasing maltase and sucrase activity[7]. Further, it is accepted that EGF leads to an increase in DNA content while exerting its mucosa-proliferating effect.

On the other hand, there have been no observations of mucosal wet weight increase after an increase in ODC activity in the mature rat with EGF administration. In recent years, Johnson et al.[22,23] have biochemically and immunohistologically demonstrated an increase in ODC activity in the small intestinal mucosa after EGF injection to mature rats. However, the effect of EGF occurred under non-physiological conditions, because the rats were fasted for at least 48 hours in their series of experiments and EGF was injected at a high dose of 150 μg/kg. On the other hand, we gave ED to mature rats for 4 weeks and demonstrated that EGF at a relatively low dose (10 μg/kg/8 hr) could have a trophic effect on the atrophic small intestinal mucosa under physiological feeding condition. In order to keep the rats in a physiological situation, ED was given ad libitum to rats during the injection period. This explains why ODC activity changed to a certain extent in the EGF group as well as in the control group.

It has been ascertained that the EGF receptor in the small intestine is located in the brush border membrane[24] and that EGF is resistant to degradation in the gastric fluid[25]. Therefore, it is suggested that EGF is secreted into saliva, pancreatic juice and bile juice and thus affects EGF receptors. According to some reports, on the other hand, the trophic effect of EGF on the small intestinal mucosa was noted even after intravenous administration[26], subcutaneous administration[27], or intraperitoneal administration[22]. In this experiment also, this trophic effect was noted after the subcutaneous or intraperitoneal administration of EGF. Martin et al.[11] demonstrated that EGF was intraluminally perfused in the small intestinal portion of adult rats accompanied by an increase in ODC activity. At the same time, they observed a rise in ODC activity even in the small intestinal portion which was not perfused with EGF. After EGF was subcutaneously or intraperitoneally administered, therefore, it is considered that its trophic effect on the small intestine is the result of the action of EGF on the receptor subsequent to the secretion of EGF from the blood into the digestive fluid or the direct action of EGF on the intestinal serosal side or the action of EGF through other mediators.

For the trophic action through the adaptation of the residual small intestine after jejunectomy, polyamine metabolism in the mucosal epithelium of the small intestine plays an important role. First ODC activity rises, followed by putrescine levels in the cell[13]. Therefore, polyamine levels were measured in respect to the trophic action following EGF administration.

Martin et al[11] reported that after the continuous perfusion of EGF into the lumen of the small intestine, ODC activity had not risen 12 hours afterwards but rose abruptly 24 hours later. Further, Fitzpatrick et al.[22] reported that after a one-time intraperitoneal injection of EGF, ODC activity reached a peak in the upper and lower small intestine 48 hours later, and the activity of another polyamine-synthetic enzyme, i.e., S-adenosylmethionine decarboxylase (SAMDC) reached a peak in the upper small intestine 4 hours later, while the activity in the lower small intestine rose 2 hours later. A definitive value has not been established for the period from EGF administration to the rise in ODC activity.

In this experiment, EGF was intermittently administered at intervals of 8 hours. Next, ODC activity and polyamine levels were measured over the time. After EGF injection, ODC activity in the jejunum rose 12 hous later. Polyamine levels, especially putrescine levels, rose slightly in the jejunum 12 hours later, almost in parallel with ODC activity, and had risen significantly 48 hours later. In the ileum, ODC activity and putrescine levels tended to rise later than those in the jejunum. These time lags in increase in ODC activity and putrescine levels as well as the differences in the site of the small intestine seem to be ascribable to the effect of food-ingestion and the intermittent injection of EGF at intervals of 8 hours. The distribution of EGF receptors might vary with differences in the site of the small intestine.

After the intermittent injection of EGF, the ODC activity in the upper small intestine was higher than in the lower intestine. However, after continuous EGF administration for one week, the trophic effect tended to be higher in the ileum than in the jejunum. This fact may be related to more remarkable mucosal atrophy in the ileum following ED administration and to ODC activity and the levels of putrescine and spermidine levels in the ileum, which tended to rise even 48 hours after EGF injection.

These findings indicate that EGF exerts its trophic effect not only on the atrohic small intestinal mucosa of weaning rats but also on that of mature rats after ED administration for 4 weeks. It is also suggested that polyamine metabolism may be involved in the mechanism of mucosal proliferation.

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