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RAPID COMMUNICATION

Effects of recombinant human growth hormone on enterocutaneous fistula patients

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Abstract

AIM: To explore the effects of recombinant human growth hormone (rhGH) on intestinal mucosal epithelial cell proliferation and nutritional status in patients with enterocutaneous fistula.

METHODS: Eight patients with enterocutaneous fistulas received recombinant human growth hormone (10 μg/d) for 7 d. Image analysis and immunohistochemical techniques were used to analyse the expression of proliferating cell nuclear antigen (PCNA) in intestinal mucosal epithelial cells in biopsy samples from the patients who had undergone an endoscopic biopsy through the fistula at day 0, 4 and 7. Body weights, nitrogen excretion, serum levels of total proteins, albumin, prealbumin, transferrin and fibronectin were measured at day 0, 4 and 7.

RESULTS: Significant improvements occurred in the expression of PCNA in the intestinal mucosal epithelial cells at day 4 and 7 compared to day 0 (24.93 ± 3.41%, 30.46 ± 5.24% *vs* 12.92 ± 4.20%, $P < 0.01$). These changes were accompanied by the significant improvement of villus height (500.54 ± 53.79 μm, 459.03 ± 88.98 μm *vs* 210.94 ± 49.16 μm, $P < 0.01$), serum levels of total proteins (70.52 ± 5.13 g/L, 74.89 ± 5.16 g/L *vs* 63.51 ± 2.47 g/L, $P < 0.01$), albumin (39.44 ± 1.18 g/L, 42.39 ± 1.68 g/L *vs* 35.74 ± 1.75 g/L, $P < 0.01$) and fibronectin (236.3 ± 16.5 mg/L, 275.8 ± 16.9 mg/L *vs* 172.5 ± 21.4 mg/L, $P < 0.01$) at day 4 and 7, and prealbumin (286.38 ± 65.61 mg/L *vs* 180.88 ± 48.28 mg/L, $P < 0.05$), transferrin (2.61 ± 0.12 g/L *vs* 2.41 ± 0.14 g/L, $P < 0.05$) at day 7. Nitrogen excretion was significantly decreased at day 7 (3.40 ± 1.65 g/d *vs* 7.25 ± 3.92 g/d, $P < 0.05$). No change was observed in the body weight.

CONCLUSION: Recombinant human growth hormone could promote intestinal mucosal epithelial cell proliferation and protein synthesis in patients with enterocutaneous fistula.

Key words: Recombinant human growth hormone; Enterocutaneous fistula; Intestinal; Epithelial cell; Proliferating cell nuclear antigen

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INTRODUCTION

From the mid 1980s, recombinant human growth hormone (rhGH) has been applied clinically. Growth hormone is a peptide hormone which stimulates proliferation and differentiation of many kinds of cells. It also has anabolic effects on the modulation of energy and substance metabolism. Previous animal experiments[1-3] have demonstrated it could promote the structural repair of the intestinal mucosa in short bowel rats, but few studies have made direct observations of the effects of rhGH on intestinal mucosa in human. The objective of this study was to explore the effects of rhGH on intestinal mucosal proliferation and nutritional status in patients with enterocutaneous fistula.

Table 1 General state of the patients

Age (Yr)	Sex	Weight (kg)	Distance from ligament of tretiz (cm)	Rest energy expenditure (KJ)	Enteral nutrition energy (KJ)
65	F	52	40	4276	6276
42	M	68	80	5648	8368
30	M	80	100	6192	9205
32	M	61	120	4384	6276
18	M	48	60	4322	6276
72	F	56	90	5773	8368
38	M	77	135	6158	9205
27	F	49	85	4359	6276

MATERIALS AND METHODS

Study protocol

Eight patients (Table 1) with enterocutaneous fistula were injected with rhGH (10 U/d) for 7 d. Intestinal mucosa biopsies were performed by endoscopy through the fistula at 20 cm proximal to the fistula at day 0, 4 and 7. All the patients gave informed consent to participate in the study. This study was approved by the Ethical Committee of Jinling Hospital, Nanjing University. Biological tests revealed no signs of inflammation, metabolic disturbances or hepatic, renal and cardiac dysfunction before the patients were enrolled into the study. The subjects had a mean body mass index of 14.37 kg/m^2 (range 11.09-17.65 kg/m^2). For all the subjects, enteral nutrition (Peptisorb, Nutricia, Holland) was prescribed and taken by nasogastric or nasointestinal tube to maintain the metabolic balance. The formula contained 1 kcal/mL, and the total calories given according to the energy expenditure was determined by indirect calorimetry (MedGraphics, USA). Endoscopic biopsies were fixed in formalin for histological assessment.

Recombinant human growth hormone (rhGH)

rhGH (Saizen) was provided by Serono China Pte. Ltd, China. The dose of rhGH was 10 U/d administered once a day (8:00 pm) as a subcutaneous injection to an upper limb, beginning on day 1 and continued for 7 d.

Immunohistochemical staining

To assess the degree of cell proliferation, an immunohistochemical technique based on the proliferating cell nuclear antigen (PCNA) was used. Sections from tissue samples were dewaxed, taken through alcohol and then immersed for 10 min in 25% phosphate-buffered saline in methanol with 0.3% hydrogen peroxide to block endogenous peroxidase activity. Sections were subsequently taken to water and immunostained using the Vectastain ABC peroxidase kit (Vecta Laboratories, Burlingame, CA). 0.4% diaminobenzidine (DAB, Aldrich Co.) was employed as a chromogen and a light haematoxylin counterstain was used. Counts were carried out in 30 crypts per preparation under microscope (40 ×), using an automatic image analysis system (HPLAS-1000, Tongji qianping Ltd). A proliferation index was determined based on the ratio between PCNA-positive cells and the total number of cells per longitudinal crypt section at the base of the crypt. This index is equal to the quotient of the number of proliferating cells and the total number of cells multiplied by 100.

Table 2 Changes of intestinal mucosal villus height, PCNA and nitrogen excretion before and after treatment with rhGH

	The days when treated with rhGH		
	0	4	7
Villus height (μm)	210.94 ± 49.16	500.54 ± 53.79^b	459.03 ± 88.98^b
PCNA labelling index (%)	12.92 ± 4.20	24.93 ± 3.41^b	30.46 ± 5.24^b
Nitrogen excretion (g/d)	7.25 ± 3.92	4.64 ± 1.95	3.40 ± 1.65^a

All values are expressed as mean ± SD. $^aP < 0.05$, $^bP < 0.01$ *vs* day 0.

Mucosal height

Sections from tissue samples were fixed in 4% paraformaldehyde, dehydrated with alcohol and then paraffin-embedded. The formatted specimens were cut by sliding microtome and stained with hematoxylin and eosin. Samples were analyzed with the automatic image analysis device (HPLAS-1000, Tongji qianping Ltd), using a microscope at 10 ×. The total mucosal height from the base of the crypt to the villous tip was measured (10 measures per preparation, in the 10 highest villi of each sample, and the base of the crypts measurement reached the muscularis mucosae).

Biochemical assays and nitrogen excretion

Serum albumin, prealbumin, transferrin and fibronectin concentrations were determined by automatic biochemical analysis device (Beckman Coulter, USA).

Daily urinary and fecal nitrogen excretion was determined by the Kjeldahl method at day 0, 4 and 7.

Statistical analysis

Data were analyzed using a statistical software package for Windows (SPSS version 10.0, SPSS Inc, Chicago, IL, USA). All variables of each group were described by common statistical methods. Results are presented as mean ± SD. One-way ANOVA for repeated measures was performed in order to evaluate the differences among the three states of the study. The level of significance was set at P value of 0.05 or less.

RESULTS

Villus height and proliferative activity

Compared with the baseline, significant improvement occurred in the intestinal mucosal villus height at day 4 and 7 (both $P < 0.01$), which was accompanied by the increase of proliferative activity of epithelial cells assessed by the PCNA labelling index (both $P < 0.01$) (Table 2, Figure 1).

Nitrogen excretion, body weight and serum levels of protein

Nitrogen excretion was significantly decreased at day 7 (P

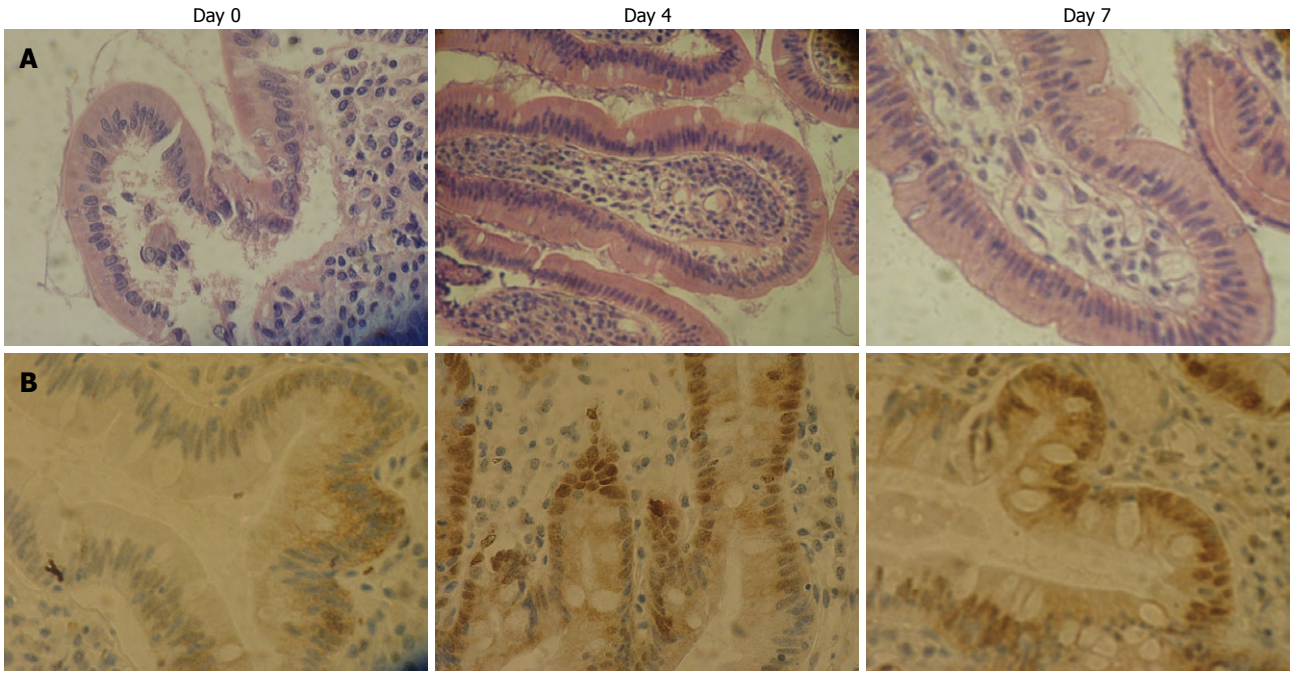

Figure 1 Villus height and proliferative activity. Significant improvements occurred in villus heights and in the expression of PCNA on the intestinal mucosal epithelial cells at day 4 and 7 ($P < 0.01$). A: Villus heights; B: PCNA labelling index.

Table 3 Changes in body weight and serum proteins before and after treatment with rhGH

	The days when treated with rhGH		
	0	4	7
Body weight (kg)	37.39 ± 12.48	38.64 ± 12.84	39.13 ± 12.19
Body mass index (kg/m^2)	14.37 ± 3.28	15.21 ± 3.41	15.40 ± 3.24
Total proteins (g/L)	63.51 ± 2.47	70.52 ± 5.13^b	74.89 ± 5.16^b
Albumin (g/L)	35.74 ± 1.75	39.44 ± 1.18^b	42.39 ± 1.68^b
Prealbumin (mg/L)	180.88 ± 48.28	231.38 ± 52.31	286.38 ± 65.61^a
Transferrin (g/L)	2.41 ± 0.14	2.49 ± 0.12	2.61 ± 0.12^a
Fibronectin (mg/L)	172.5 ± 21.4	236.3 ± 16.5^b	275.8 ± 16.9^b

All values are expressed as mean ± SD. $^aP < 0.05$, $^bP < 0.01$ *vs* day 0.

< 0.05) (Table 2). Serum levels of total proteins, albumin and fibronectin were significantly increased at day 4 and 7 (both $P < 0.01$). The levels of prealbumin and transferrin were increased at day 7 ($P < 0.05$) (Table 3). No change was observed in the body weight.

DISCUSSION

Previous studies have shown that GH stimulates bowel growth[1-4]. Administration of GH improves gut mucosal structure in animals with short bowel syndrome[5-7] and may promote the structural repair of the graft[8,9]. Experiments *in vitro* have also demonstrated that GH is involved in the regulation of crypt cell proliferation in the human small intestine[10-13].

The therapeutic efficacy of rhGH has been suggested by results of animal studies. In the present study the effects of rhGH in situ on the intestine of patients with enterocutaneous fistula were observed. Our results revealed that significant improvement occurred in the intestinal mucosal villus height at day 4 and 7, which was accompanied by the increase of proliferative activity of epithelial cells assessed by the PCNA labelling index.

Nutrient malabsorption often occurs in patients with gastrointestinal fistula[14-17], and it causes body weight loss, barrier damage, followed by bacterial translocation from the gastrointestinal tract to the mesenteric lymph nodes, and even blood. Administration of glutamine and growth hormone synergistically reduces bacterial translocation in sepsis[18,19]. Hormonal therapy with GH can improve weight gain in a rat model of severe short bowel syndrome. This improvement in weight gain was associated with an increase in nutrient transport at the cellular level and variable increases in villus size[20,21]. GH treatment increased [^{14}C] glucose and [^{3}H] palmitic acid plasma concentration after oral nutrient tolerance tests[22]. Clinical trials also showed that GH could promote positive nitrogen balance and protein synthesis[23-25]. However, there are some conflicting results: no improvement was observed in the absorption of total energy, carbohydrate, fat, nitrogen, or wet weight of stool or stool electrolytes compared with baseline and placebo measurements[26-28]. In the present study the body weights of the eight patients were maintained at normal level. All the patients showed positive nitrogen balance and the nitrogen excretion was significantly decreased at day 7. Serum levels of total proteins, albumin and fibronectin were significantly increased at day 4 and 7. And the levels of prealbumin and transferrin were increased at day 7.

GH stimulated the formation and deposition of collagen in both skin incisional wounds and in colonic anastomoses in rats[29,30].

After the trial all the eight patients underwent surgery to close the fistula and they recovered very well, and no fistula recurred.

In summary, our study shows that rhGH can promote intestinal mucosal epithelial cell proliferation and protein synthesis in patients with enterocutaneous fistula.

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COMMENTS

Background

In some medical literature, hormonal therapy with GH has been shown to improve weight gain in a model of severe short bowel syndrome. This improvement in weight gain was associated with an increase in nutrient transport at the cellular level and variable increases in villus size. But there are some conflicting results: no improvement was observed in the absorption of total energy, carbohydrate, fat, nitrogen, or wet weight of stool or stool electrolytes compared with baseline and placebo measurements.

Research frontiers

This study has been carefully designed to investigate whether recombinant human growth hormone (rhGH) could increase the proliferative activity of epithelial cells and nutrient absorption in human. The results showed that rhGH could promote intestinal mucosal epithelial cell proliferation and protein synthesis in humans.

Innovations and breakthroughs

Few studies have made direct observations of the effects of rhGH on intestinal mucosa in humans. In this study, the effects of rhGH on intestinal mucosa proliferation were directly observed. Intestinal mucosal biopsies were performed by endoscopy through enterocutaneous fistula.

Applications

This study suggests that rhGH may reasonably be applied in a clinical setting.

Peer review

Although this is a very interesting study, it is just a preliminary observation. It should be verified in the future. Authors should comment on possible adverse effects of this drug.

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