Effects of recombinant human growth hormone on enterocutaneous fistula patients

Guo-Sheng Gu, Jian-An Ren, Ning Li, Jie-Shou Li

Guo-Sheng Gu, Jian-An Ren, Ning Li, Jie-Shou Li, The Research Institute of General Surgery, Clinical School (Jinling Hospital), School of Medicine, Nanjing University, Nanjing 210002, Jiangsu Province, China

Author contributions: Li N and Li JS designed research; Gu GS and Ren JA performed research; Gu GS wrote the paper.

Supported by National Natural Science Foundation of China, No. 30571797 and National Natural Science Foundation of Jiangsu Province, No. BK2006719

Correspondence to: Jian-An Ren, MD, The Research Institute of General Surgery, Jinling Hospital, 305 Zhongshan East Road, Nanjing 210002, Jiangsu Province, China. guguoshengde@yahoo.com.cn

Telephone: +86-25-80860437 Fax: +86-25-84803956

Received: September 9, 2008 Revised: November 7, 2008 Accepted: November 14, 2008 Published online: November 28, 2008

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.

© 2008 The WJG Press. All rights reserved.

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

Peer reviewers: Yoshiharu Motoo, MD, PhD, FACP, FACG, Professor and Chairman, Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan; Adrian Reuben, Professor of Medicine, Department of Medicine, Medical University of South Carolina, Division of Gastroenterology and Hepatology, 210 Clinical Sciences Building, PO Box 250327, 96 Jonathan Lucas Street, Charleston SC 29425, United States

Gu GS, Ren JA, Li N, Li JS. Effects of recombinant human growth hormone on enterocutaneous fistula patients. World J Gastroenterol 2008; 14(44): 6858-6862 Available from: URL: http://www.wjgnet.com/1007-9327/14/6858.asp DOI: http://dx.doi.org/10.3748/wjg.14.6858

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.
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/m² (range 11.09-17.65 kg/m²). 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.

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 haematoxylin 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 mucosa).

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/m²)        | 14.37 ± 3.28    | 15.21 ± 3.41    | 15.40 ± 3.24     |
| Total proteins (g/L)           | 63.51 ± 2.47    | 70.52 ± 5.13b   | 74.89 ± 5.16b    |
| Albumin (g/L)                  | 35.74 ± 1.75    | 39.44 ± 1.18b   | 42.39 ± 1.68b    |
| Prealbumin (mg/L)              | 180.88 ± 48.28  | 231.38 ± 52.31  | 286.38 ± 65.61b  |
| Transferrin (g/L)              | 2.41 ± 0.14     | 2.49 ± 0.12     | 2.61 ± 0.12a     |
| Fibronectin (mg/L)             | 172.5 ± 21.4    | 256.3 ± 16.5b   | 275.8 ± 16.9b    |

All values are expressed as mean ± SD. *$P < 0.05$, **$P < 0.01$ 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 assayed 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.

ACKNOWLEDGMENTS

We thank the staff of the Research Institute of General Surgery of Jinling hospital for helpful assistance. We also thank the staff of the Department of Pathology of Jinling hospital for technical assistance.

REFERENCES

1 Gu Y, Wu ZH, Xie JX, Jin DY, Zhuo HC. Effects of growth hormone (rhGH) and glutamine supplemented parenteral nutrition on intestinal adaptation in short bowel rats. Clin Nutr 2001; 20: 159-166

2 Byrne TA, Morrissey TB, Nattakom TV, Ziegler TR, Guerrero JA, Rosell J, Ruiz-Requena E. 1992;

3 Zhang X, Li J, Li N. Growth hormone improves graft mucosal structure and recipient protein metabolism in rat small bowel transplantation. Chin Med J (Engl) 2002; 115: 732-735

4 Shulman DI, Hu CS, Duckett G, Lavallee-Grey M. Effects of short-term growth hormone therapy in rats undergoing 75% small intestinal resection. J Pediatr Gastroenterol Nutr 1992; 14: 3-11

5 Wheeler EE, Challacombe DN. The trophic action of growth hormone, insulin-like growth factor-I, and insulin on human duodenal mucosa cultured in vitro. Gut 1997; 40: 57-60

6 Challacombe DN, Wheeler EE. Trophic action of epidermal growth factor on human duodenal mucosa cultured in vitro. Gut 1991; 32: 991-993

7 Chen JY, Liang DM, Gan P, Zhang Y, Lin J. In vitro effects of recombinant human growth hormone on growth of human gastric cancer cell line BGC823 cells. World J Gastroenterol 2004; 10: 1132-1136

8 Wang GF, Ren JA, Jiang J, Fan CG, Wang XB, Li JS. Catheter-related infection in gastrointestinal fistula patients. World J Gastroenterol 2004; 10: 3345-1348

9 Wang XH, Ren JA, Li JS. Sequential changes of body composition in patients with enterocutaneous fistula during the 10 days after admission. World J Gastroenterol 2002; 8: 1149-1152

10 Fan CG, Ren JA, Wang XB, Li JS. Refeeding syndrome in patients with gastrointestinal fistula. Nutrition 2004; 20: 346-350

11 Ren JA, Mao Y, Wang GF, Wang XB, Fan CG, Wang ZM, Li JS. Enteral refeeding syndrome after long-term total parenteral nutrition. Chin Med J (Engl) 2006; 119: 1856-1860

12 Jung SE, Youn YK, Lim YS, Song HG, Rhee JE, Suh GJ. Combined administration of glutamine and growth hormone synergistically reduces bacterial translocation in sepsis. J Korean Med Sci 2003; 18: 17-22

13 Scopa CD, Kourelas S, Tsamandas AC, Spiliopoulou I, Alexandrides T, Filos KS, Vagianos CE. Beneficial effects of growth hormone and insulin-like growth factor I on intestinal bacterial translocation, endotoxemia, and apoptosis in experimentally jaundiced rats. J Am Coll Surg 2000; 190: 423-431

14 Sigalet DL, Martin GR. Hormonal therapy for short bowel syndrome. J Pediatr Surg 2000; 35: 360-363; discussion 364

15 Zhang W, Frankel WL, Adamson WT, Roth JA, Mantell MP, Bain A, Ziegler TR, Smith RJ, Rombeau JL. Insulin-like growth factor-I improves mucosal structure and function in transplanted rat small intestine. Transplantation 1995; 59: 755-761

16 Zhou X, Li YX, Li N, Li JS. Glutamine enhances the gutrophic effect of growth hormone in rat after massive small bowel resection. J Surg Res 2001; 99: 47-52

17 Vara-Thorbeck R, Guerrero JA, Rosell J, Ruiz-Queuena E, Capitan JM. Exogenous growth hormone: effects on the catabolic response to surgically produced acute stress and on postoperative immune function. World J Surg 1993; 17: 530-537; discussion 537-538

18 Seguy D, Vahedi K, Kapel N, Souberbielle JC, Messing B. Low-dose growth hormone in adult home parenteral nutrition-dependent short bowel syndrome patients: a positive study. Gastroenterology 2003; 124: 293-302

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 mucosal 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.
25 Byrne TA, Cox S, Karimbakas M, Veglia LM, Bennett HM, Lautz DB, Robinson MK, Wilmore DW. Bowel rehabilitation: an alternative to long-term parenteral nutrition and intestinal transplantation for some patients with short bowel syndrome. Transplant Proc 2002; 34: 887-890

26 Szkudlarek J, Jeppesen PB, Mortensen PB. Effect of high dose growth hormone with glutamine and no change in diet on intestinal absorption in short bowel patients: a randomised, double blind, crossover, placebo controlled study. Gut 2000; 47: 199-205

27 Vanderhoof JA, Kollman KA, Griffin S, Adrian TE. Growth hormone and glutamine do not stimulate intestinal adaptation following massive small bowel resection in the rat. J Pediatr Gastroenterol Nutr 1997; 25: 327-331

28 Park JH, Vanderhoof JA. Growth hormone did not enhance mucosal hyperplasia after small-bowel resection. Scand J Gastroenterol 1996; 31: 349-354

29 Jorgensen PH, Oxlund H. Growth hormone increases the biomechanical strength and collagen deposition rate during the early phase of skin wound healing. Wound Repair Regen 1996; 4: 40-47

30 Oxlund H, Christensen H, Seyer-Hansen M, Andreassen TT. Collagen deposition and mechanical strength of colon anastomoses and skin incisional wounds of rats. J Surg Res 1996; 66: 25-30

S- Editor Cheng JX   L- Editor Logan S   E- Editor Lin YP