Effects of recombinant human growth hormone on rat septic shock with intraabdominal infection by E. coli

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INTRODUCTION

Septic shock is a common and severe disease, the incidence and mortality of septic shock are still very high now-a-days. Growth hormone is an important anabolic hormone. Experimental study showed that recombinant human growth hormone (rhGH) enhanced protein synthesis(1), promoted tissue recovery, improved host defenses(2-4), decreased stress and maintained intestinal mucosa barrier(5-6). The present study was to investigate the therapeutic effects of rhGH on rat septic shock with intraabdominal infection by E. coli and its possible mechanism.

MATERIALS AND METHODS

Animal models and groups

76 female Sprague-Dawley rats weighing between 200 and 240 g were obtained from the Animal Center of Sichuan University. The rats were divided randomly into 3 groups: control group (group C, n=16) without any special treatment; septic shock group (group S, n=30) injected intraperitoneally with a bolus of E.coli (1 x 10^9 cfu·L^-1, 15 ml·kg^-1, ip); treated group (group T, n=30) injected intraperitoneally with a bolus of E.coli (1 x 10^9 cfu·L^-1, 15 ml·kg^-1, ip), treated group (group T, n=30) received bolus injection of E.coli, and then followed by rhGH injection (2.25 U·kg^-1·d^-1, im). Group S and group T were further divided into 1d and 3d subgroups, respectively (n=15 each).

RESULTS: (1) On 1st and 3rd day, MAP in group S decreased markedly, and MAP on 1st day lowered more than that of 3rd day (P<0.01), while MAP in group T just decreased slightly. The survival rate within 1 week was much higher in group T (84.6 %) than in group S (46.2 %) (P<0.01). (2) On 1st day, plasma TNF-α and endotoxin elevated significantly in group S and group T (P<0.05), and endotoxin in group S had more increase (P<0.01). On 3rd day, TNF-α in group S returned to the level of group C (P>0.05), while TNF-α in group T went down below the level of group C (P<0.01). On 3rd day, endotoxin in group S declined, but was still higher than that of group C (P<0.01), endotoxin in group T returned to the level of group C (P=0.05). (3) On 1st day, neutrophil ratio in total leukocyte count in both group S and group T increased significantly (P<0.05 vs group C).

CONCLUSION: rhGH showed beneficial effects on rat septic shock. The possible mechanisms may involve the attenuation of bacteria/endotoxin translocation and decreased systemic endotoxin level; inhibition of the production and release of TNF-α; improved circulatory function; improved systemic host defenses and maintenance of intestinal mucosa barrier.

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Abstract

AIM: To investigate the therapeutic effects of recombinant human growth hormone (rhGH) on rat septic shock with intraabdominal infection by E. coli and its possible mechanism.

METHODS: 76 SD rats were divided into 3 groups randomly: control group (group C, n=16) without any special treatment; septic shock group (group S, n=30) received bolus injection of E.coli (1 x 10^9 cfu·L^-1, 15 ml·kg^-1, ip), treated group (group T, n=30) received bolus injection of E.coli, and then followed by rhGH injection (2.25 U·kg^-1·d^-1, im). Group S and group T were further divided into 1d and 3d subgroups, respectively (n=15 each). Mean arterial pressure (MAP), levels of plasma TNF-α and endotoxin and leukocyte count were determined on 1st day and 3rd day after E.coli injection. Another 39 SD rats were divided into groups C, S and T (n=13 each) just for observing survival rate within 1 week.

RESULTS: (1) On 1st and 3rd day, MAP in group S decreased markedly, and MAP on 1st day lowered more than that of 3rd day (P<0.01), while MAP in group T just decreased slightly. The survival rate within 1 week was much higher in group T (84.6 %) than in group S (46.2 %) (P<0.01). (2) On 1st day, plasma TNF-α and endotoxin elevated significantly in group S and group T (P<0.05), and endotoxin in group S had more increase (P<0.01). On 3rd day, TNF-α in group S returned to the level of group C (P>0.05), while TNF-α in group T went down below the level of group C (P<0.01). On 3rd day, endotoxin in group S declined, but was still higher than that of group C (P<0.01), endotoxin in group T returned to the level of group C (P=0.05). (3) On 1st day, neutrophil ratio in total leukocyte count in both group S and group T increased significantly (P<0.05 vs group C).

CONCLUSION: rhGH showed beneficial effects on rat septic shock. The possible mechanisms may involve the attenuation of bacteria/endotoxin translocation and decreased systemic endotoxin level; inhibition of the production and release of TNF-α; improved circulatory function; improved systemic host defenses and maintenance of intestinal mucosa barrier.
Leukocyte count  0.5 ml blood anticoagulated by heparin was collected through a venous cannula which was inserted into an external jugular vein. Leukocyte count was measured by using CELL-DYN 1600.

**Plasma endotoxin determination**  Levels of plasma endotoxin was determined by LAL test, according to the manual of the kit.

**Measurement of plasma TNFα**  Concentrations of plasma TNFα was measured using RIA, according to the manual of the kit.

**Statistical analysis**  All data except survival rate were expressed as mean ± standard deviation (±s). Data were analyzed by *t*-test or variance analysis using SPSS 10.0 software, and *P*<0.05 was considered as the significant level of difference.

**RESULTS**

**Mean arterial pressure (MAP) and survival rate**  On 1st day and 3rd day, MAP in group S decreased obviously, and MAP on 1st day showed a 46 % decrease (P<0.01, vs group C). MAP in group T was just about 10 % reduction (P<0.05, vs group C). These results suggested that rhGH could attenuate the hypotension induced by septic infection. 7 Rats in group S were dead within 1 week, the survival rate was 46.2 % (6/13). 2 Rats in group T were dead within 1 week, the survival rate was 84.6 % (11/13). All rats in group C survived. The survival rate was much higher in group T than in group S (P<0.01, See Table 1). These findings indicated that rhGH could improve the outcome of septic shock significantly.

**Table 1**  Effects of rhGH on mean arterial pressure (MAP) (±s) and survival rate within 1 week in rat septic shock

| Group | MAP (mmHg) | Survival rate within 1 week (%) |
|-------|------------|---------------------------------|
| C     | 124.6±13.9 | 100.0                           |
| S     | 67.4±22.6  | 98.9±23.2                       |
| T     | 114.4±15.9 | 109.9±10.2                      |

- *P*<0.05 vs group C; *P*<0.01 vs group S in 1 d; *P*<0.01 vs group S.

**Leukocyte count**  On 1st day, the numbers of leukocyte in both group S and group T showed no significant difference from that of group C (P>0.05), while neutrophil ratio in total leukocytes was higher in both group S and group T than in group C, and more in group T (P<0.05). On 3rd day, both the numbers of leukocyte and neutrophil ratio in total leukocytes had no significant changes among the three groups (P>0.05. See Table 2).

**Table 2**  Effect of rhGH on leukocyte count in rat septic shock (±s)

| Time | Leukocyte (×10⁹ L⁻¹) | Neutrophil (%) |
|------|------------------------|----------------|
|      | Group C | Group S | Group T | Group C | Group S | Group T |
| 1 d  | 8.42±2.44 | 9.61±3.58 | 28.75±8.85 | 26.77±11.80 | 7.73±4.57 | 16.14±6.0 |
| 3 d  | 6.75±2.18 | 6.07±2.45 | 13.74±7.06 | 14.96±5.35 | 7.57±3.24 | 16.35±7.06 |

- *P*<0.05 vs group C; *P*<0.01 vs group S in 1 d in group S.

**Plasma endotoxin and TNFα**  On 1st day, plasma TNFα and endotoxin increased significantly in group S and group T (P<0.05), and endotoxin in group S had more increase (P<0.01). On 3rd day, TNFα in group S returned to the level of group C (P>0.05), while TNFα in group T went down below the level of group C (P<0.01). Endotoxin in group S decreased, but was still higher than that of group C (P<0.01). Endotoxin in group T returned to the value of group C (P>0.05, See Table 3). These results suggested that rhGH could extinguish plasma endotoxin and inhibit the production and release of TNFα.

**Table 3**  Effects of rhGH on the concentrations of plasma endotoxin and TNFα in rat septic shock

| Time | Endotoxin (U·L⁻¹) | TNFα (µg·L⁻¹) |
|------|-------------------|---------------|
|      | Group C | Group S | Group T | Group C | Group S | Group T |
| 1 d  | 256±52    | 150±39  | 111±53  | 3.59±0.69 | 3.66±1.33 | 158±25  |
|      |          |          |         | 2.88±0.74 |         |         |
| 3 d  | 189±52    | 108±42  | 22.3±1.09 | 1.54±0.36d |         |         |

- *P*<0.01 vs group C; *P*<0.05 vs group S in 1 d; *P*<0.01 vs group S in 3 d.

**DISCUSSION**  When acute peritoneal bacterial infection happens, there are two lines of host defense against peritoneal bacterial invasion. The first line of host defense is peritoneal resident cells, which consist mainly of macrophages. Peritoneal macrophages begin the process of phagocytosis and killing of bacteria immediately after bacterial inoculation[1]. Inoue T et al demonstrated that administration of rhGH augmented the numbers of peritoneal macrophages significantly in gram-negative sepsis model. The second line of host defense is an acute inflammatory response involving the influx of neutrophils. Neutrophils are attracted to the site of infection by chemotactic factors, then phagocytize, kill and eliminate bacteria[7]. In our study, neutrophil ratio in total leukocyte count in both group S and group T increased significantly than that in group C on 1st day, and more in group T. Our results indicated that rhGH could significantly increase neutrophil ratio in total leukocytes. Taken together, it could be inferred that rhGH could enhance the two lines of host defense, accelerate the clearance of bacteria from the peritoneal cavity, minimize the spread of bacteria to blood, and then diminish plasma endotoxin and proinflammatory cytokines levels.

Endotoxin, the main toxic component of gram-negative bacteria, is the leading cause of sepsis or septic shock[15-19]. Our experiment showed that plasma endotoxin levels both in group S and group T elevated obviously after *E.coli* challenge. The main reason of higher endotoxin level in plasma was related to the proliferation of *E.coli* in blood and peritoneal cavity. In addition, it was also associated with the impairment of intestinal mucosa barrier, which may cause and accelerate bacteria/endotoxin translocation[11].

An important function of intestinal mucosa barrier is to prevent translocation of bacteria/toxins from gut lumen into circulation[12-23]. Glutamine (Gln), the preferred fuel for gut[24], is a required nutrient for maintaining the normal structure and function of intestinal mucosa barrier[25,26]. The ability of intestinal mucosa uptaking and utilizing Gln directly influences the function of intestinal mucosa barrier. In septic shock, ischemia, hypoxia and proinflammatory cytokines may result in impairment of intestinal mucosa barrier, meanwhile, Gln
intake by intestinal mucosa and the activity of glutaminase, which catalyzes the hydrolysis of Gln to glutamate and ammonia, could also be injured, so result in marked reduction of the utilization of Gln by gut. An injured Gln metabolism may be another contributor in the breakdown of the intestinal mucosa barrier. The impairment of intestinal mucosa barrier facilitating the entering of bacteria/endotoxin into portal venous and lymphatic systems was defined as bacteria/endotoxin translocation[27-29]. Moreover, higher systemic endotoxin level could significantly compromise the integrity of intestinal mucosa barrier, and further enhanced the translocation of bacteria/endotoxin[30].

On 1st day, plasma endotoxin increased significantly in group S than in group T. On 3rd day, endotoxin in group S remained at higher level, while endotoxin in group T returned to control level. These results suggested that rhGH could decrease plasma endotoxin level, which might be due to: (1) rhGH binding growth hormone receptors localized extensively in intestinal mucosa could increase the activity of glutaminase, promote intestinal mucosa to uptake and utilize Gln, ameliorate the impairment of intestinal mucosa barrier. (2) rhGH could also protect intestinal mucosa barrier by enhancing the paracrine and autocrine mechanism of IGF-1 and upregulate the expression of IGF-1 mRNA in intestine[30]. Thereby, rhGH administration showed beneficial effects in maintaining the integrity of intestinal mucosal barrier[32,33], attenuating bacteria/endotoxin translocation[27,34,35] and decreasing plasma endotoxin level[36]. In addition, Prieto et al[37] also demonstrated that rhGH could promote the release and chemotaxis of neutrophils, minimize the spread of bacteria and attenuate bacteria/endotoxin translocation, and then reduce plasma endotoxin level.

Endotoxin could trigger a series of inflammatory processes, leading to release of many other proinflammatory cytokines[38], TNF, a central mediator of the complex network of proinflammatory cytokines[39], plays a critical role in the pathogenesis of gram-negative-induced sepsis. Our results showed plasma TNFα increased significantly on 1st day after E. coli injection. The mechanism of higher plasma TNF may be due to[35]; (1) TNF released from macrophages in gut wall and peritoneal cavity in response to endotoxin stimulation, which may be one of the reasons of early higher TNF level in portal vein and systemic circulation. (2) Endotoxin entering liver via portal venous, stimulated Kupffer cells to produce more TNFα and then further elevated plasma TNFα level[36-38]. On 3rd day, TNFα in group S returned to the level of group C, while TNFα in group T reduced even lower than that in group C, suggesting that rhGH could inhibit the production and release of TNFα. The mechanism may involve: rhGH maintained intestinal mucosa barrier, diminished bacteria/endotoxin translocation and downregulated the production of TNFα.

After E. coli injection, MAP in rats of group S decreased obviously, MAP declined even more obviously on 1st day than on 3rd day; while MAP in group T just decreased slightly on 1st day and 3rd day. The data revealed that rhGH could attenuate the decline of blood pressure in septic shock. The mechanism of rhGH improving circulatory function may be related to inhibition of production and release of proinflammatory cytokines and decrease of systemic endotoxin level.

The survival rate within 1 week was much higher in group T (84.6 %) than in group S (46.2 %), which indicated that rhGH could increase survival rate and improve outcome in septic shock.

In summary, the above results showed that rhGH treatment had desirable beneficial effects on rat septic shock, which may involve the following mechanism that rhGH administration could improve host defenses[24]; maintain intestinal mucosa barrier[15,16]; diminish bacteria/endotoxin translocation[17-19]; decrease systemic endotoxin level[18]; inhibit the production and release of TNFα and improve circulatory function.

REFERENCES
1 Kolstad O, Jenssen TG, Ingebretsen OC, Vinners E, Revhaug A. Combination of recombinant human growth hormone and glutamine-enriched total parenteral nutrition to surgical patients: effects on circulating amino acids. Clin N utr 2001; 20: 503-510
2 Van den Berge G. Novel insights into the neuroendocrinology of critical illness. Eur J Endocrinol 2000; 143: 1-13
3 Heemskerk VH, Daemen MA, Buurman WA. Insulin-like-growth factor-1 (IGF-1) and growth hormone (GH) in immunity and inflammation. Cytokine Growth Factor Rev 1999; 10: 5-14
4 Hattori N, Saito T, Yagyu T, Jiang BH, Kitagawa K, Inagaki C, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. J Clin Endocrinol Metab 2003; 86: 4284-4291
5 Miyonos PG, Motsaouka PT, Papandopiu EV, Vagianos C, Kalfarentzos F, Alexandrides TK. Growth hormone and insulin-like growth factor I protect intestinal cells from radiation induced apoptosis. M ol Cel Endocrinol 2000; 160: 115-122
6 Zhou X, Li N, Li JS. Growth hormone stimulates remnant small bowel epithelial cell proliferation. World J Gastroenterol 2000; 6: 909-913
7 Prieto I, Gomez de Segura IA, Garcia Grande A, Guerra A, Pozo F, Garcia P, de Miguel E. Growth hormone reduces bacterial translocation in radiation enteritis in the rat. Rev Esp Enferm Dig 1998; 90: 353-360
8 Meng AH, LingYL, Zhang XP, Zhao XY, Zhang JL. CCK-8 inhibits expression of TNF-α in the spleen of endotoxic shock rats and signal transduction mechanism of p38 MAPK. World J Gastroenterol 2002; 8: 139-143
9 LingYL, Meng AH, Zhao XY, Shan BE, Zhang JL, Zhang XP. Effect of choleysteokin on cytokines during endotoxic shock in rats. World J Gastroenterol 2001; 7: 667-671
10 Fan K. Regulatory effects of lipopolysaccharide in murine macrophage proliferation. World J Gastroenterol 1998; 4: 137-139
11 Forsythe RM, Xu DZ, Lu Q, Deltch EA. Lipopolysaccharide-induced enterocytederived nitric oxide induces intestinal monolayer permeability in an autocrine fashion. Shock 2002;17:180-184
12 Ersin S, Tuncureyk P, Eeesolak M, Alkanat M, Bucole K, Yilmaz M, Telefoncu A, Kose T. The prophylactic and therapeutic effects of glutamine- and arginine-enriched diets on radiation-induced enteritis in rats. J Surg Res 2000; 89: 121-125
13 Sun XQ, Fu XB, Zhang R, Lu Y, Deng Q, Jiang XG, Sheng ZY. Relationship between plasma D(-)-lactate and intestinal damage after severe injuries in rats. World J Gastroenterol 2001; 7: 555-558
14 Dong HL. Intestinal permeability test and its clinical significance. ShiJie Huaren Xiaohua Zazhi 2000; 8: 562-563
15 Luo H, Wang LF, Imoto T, Hijji Y. Inhibitory effect and mechanism of acarbose combined with gymnemic acid on maltoose absorption in rat intestine. World J Gastroenterol 2001; 7: 9-15
16 Qin RY, Zou SQ, Wu ZD, Qiu FZ. Influence of splanchic vascular infusion on the content of endotoxins in plasma and the translocation of intestinal bacteria in rats with acute hemorrhage necrosis pancreatitis. World J Gastroenterol 2000; 6: 577-580
17 Hess DJ, Henry-Stanley MJ, Erickson EA, Wells CL. Effect of tumor necrosis factor alpha, interferon gamma, and interleukin-4 on bacteria-enterocyte interactions. J Surg Res 2002; 104: 88-94
18 Sileri P, Morini S, Sica GS, Sciena S, Rastellini C, Gaspari AL, Benedetti E, Cicalese L. Bacterial translocation and intestinal morphological findings in jaundiced rats. Dig Dis Sci 2002; 47: 929-934
19 Mosenthal AC, Xu D, Deltch EA. Elemental and intravenous total parenteral nutrition diet-induced gut barrier failure is intestinal site specific and can be prevented by feeding nonfermentable fiber. Crit Care Med 2002; 30: 396-402
20 Kouris GJ, Liu Q, Rossi H, Djuuricin G, Gattuso P, Nathan C, Weinstein RA, Prinz RA. The effect of glucagon-like peptide 2
on intestinal permeability and bacterial translocation in acute necrotizing pancreatitis. Am J Surg 2001; 181: 571-575

Parks RW, Stuart Cameron CH, Gannon CD, Pope C, Diamond T, Rowlands BJ. Changes in gastrointestinal morphology associated with obstructive jaundice. J Pathol 2000; 192: 526-532

Erbil Y, Berber E, Ozarmagan S, Seven R, Eminoglu L, Calis A, Olgac V, Gurler N. The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. Hepatogastroenterology 1999; 46: 2791-2795

Gork AS, Usui N, Ceribi E, Drongowski RA, Epstein MD, Coran AG, Harmon CM. The effect of mucin on bacterial translocation in I-407 fetal and Caco-2 adult enterocyte cultured cell lines. Pediatr Surg Int 1999; 15: 155-159

Gu Y, Wu ZH. The anabolic effects of recombinant human growth hormone and glutamine on parenterally fed, short bowel rats. World J Gastroenterol 2002; 8: 752-757

Neu J, DeMarco V, Li N. Glutamine: clinical applications and mechanisms of action. Curr Opin Clin Nutr Metab Care 2002; 5: 69-75

Li YS, Li JS, Jiang JW, Liu FN, Li N, Qin WS, Zhu H. Glycyl-glutamine-enriched long-term total parenteral nutrition attenuates bacterial translocation following small bowel transplantation in the pig. J Surg Res 1999; 82: 106-111

Li JY, Lu Y, Hu S, Sun D, Yao YM. Preventive effect of glutamine on intestinal barrier dysfunction induced by sepsis and trauma. World J Gastroenterol 2002; 8: 168-171

Antequera R, Britana A, Cirac A, Brito A, Romera MA, Zapata R. Disruption of the intestinal barrier and bacterial translocation in an experimental model of intestinal obstruction. Acta Cient Venez 2000; 51: 18-26

Yu P, Martin CM. Increased gut permeability and bacterial translocation in Pseudomonas pneumonia-induced sepsis. Crit Care Med 2000; 28: 2573-2577

Dickinson E, Tuncer R, Nadler E, Boyle P, Alber S, Watkins S, Ford H. NOX, a novel nitric oxide scavenger, reduces bacterial translocation in rats after endotoxin challenge. Am J Physiol 1999; 277: G1281-G1287

Wang X, Wang B, Wu J, Wang G. Beneficial effects of growth hormone on bacterial translocation during the course of acute necrotizing pancreatitis in rats. Pancreas 2001; 21: 149-156

Scopa CD, Koureleas S, Tsamandas A C, 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

Zhou X, Li YX, Li N, Li JS. Effect of bowel rehabilitative therapy on structural adaptation of remnant small intestine: animal experiment. World J Gastroenterol 2001; 7: 66-73

Wang P, Li N, Li JS, Li WQ. The role of endotoxin, TNF-alpha, and IL-6 in inducing the state of growth hormone insensitivity. World J Gastroenterol 2002; 8: 531-536

Luo ZY. Shock. 1st ed. Tianjin: Tianjin Science and Technology Press 2001: 432-440

Gong JP, Wu CX, Liu CA, Li SW, Shi YJ, Yang K, Li Y, Li XH. Intestinal damage mediated by Kupffer cells in rats with endotoxemia. World J Gastroenterol 2002; 8: 923-927

Su GL. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 2002; 283: G256-G265

Su GL, Goyert SM, Fan MH, Aminlari A, Gong KQ, Klein RD, Myc A, Alarcon WH, Steintraesser L, Remick DG, Wang SC. Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. Am J Physiol Gastrointest Liver Physiol 2002; 283: G640-G645

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