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• BASIC RESEARCH •

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

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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* (1×10^{10} 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 TNFα 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 TNFα 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, 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). 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 TNFα; improved circulatory function; improved systemic host defenses and maintenance of intestinal mucosa barrier.

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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* (1×10^{10} cfu· L^{-1}, 15 ml· kg^{-1}); treated group (group T, n=30) received a bolus injection of *E.coli*, and then followed by rhGH intramuscular injection (2.25 U· kg^{-1}· d^{-1}). Group S and group T were further divided into 1d and 3d subgroups, respectively (n=15 each).

Another 39 female Sprague-Dawley rats weighing between 200 and 240 g were divided into groups C, S and T (n=13 each) just for observing the survival rate within 1 week.

Reagents

rhGH (Saizen) was obtained from Serono Co. in Switzerland. Bacterial suspension of *E. coli* was supplied by Dept. of Microbiology in Huaxi School of Basic Medical Sciences and Forensic Medicine. Limulus amebocyte lysate (LAL) kit and radioimmunoassay (RIA) kit of rat TNFα were purchased from Shanghai Laboratory of Medical Science and Institute of Radioimmunology, Chinese PLA General Hospital, respectively.

Methods

Measurement of blood pressure Rats were anesthetized with sodium pentobarital (15 ml· kg^{-1}, ip) on 1st day or 3rd day after receiving *E.coli*. An arterial cannula connected with Four-Channel Physiological Measuring Instrument (RM-6200, Japan) was inserted into a carotic artery for recording mean arterial pressure (MAP).

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 TNFa 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 ($\bar{x}\pm s$). 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) ($\bar{x}\pm s$) and survival rate within 1 week in rat septic shock

Group	MAP (mmHg)		Survival rate within
	1 d	3 d	1 week (%)
C	124.6±13.9		100.0
S	67.4±22.6[b]	98.9±23.2[ad]	46.2
T	114.4±15.9[ad]	109.9±10.2[a]	84.6[f]

[a]$P<0.05$,[b]$P<0.01$, *vs* group C,[d]$P<0.01$, *vs* group S in 1 d; [f]$P<0.01$, *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 ($\bar{x}\pm s$)

Time	Leukocyte ($\times 10^9$/L)			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±8.83[a]	36.77±11.84[bc]
	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

[a]$P<0.05$, [b]$P<0.01$, *vs* group C; [c]$P<0.05$, *vs* 1 d in group S.

*Plasma endotoxin and TNF*a

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[a]	150±39[bc]		3.59±0.69[b]	3.66±1.33[b]
	111±53			2.88±0.74		
3 d		189±52[a]	108±42[d]		2.23±1.09	1.54±0. 36[ad]

[a]$P<0.01$,[b]$P<0.05$, *vs* group C; [c]$P<0.01$,*vs* group S in 1 d; [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[7]. 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[8-10]. 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[31]. 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[31]. In addition, Prieto *et al*[7] 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[34]. TNF, a central mediator of the complex network of proinflammatory cytokines[35], 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 TNF.

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[2-4]; maintain intestinal mucosa barrier[5,6], diminish bacteria/endotoxin translocation[17-19]; decrease systemic endotoxin level[18]; inhibit the production and release of TNFα and improve circulatory function.

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