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Original Article

Early low-dose glucocorticoid therapy effectively suppresses serum pro-inflammatory factors such as IL-6 and inhibits apoptosis of CD4+ cells in septic shock patients

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Abstract: Objective: To investigate the timing of low-dose glucocorticoid therapy in treatment of septic shock, especially the changes in inflammatory factors including interleukin-6 (IL-6) and interleukin-10 (IL-10), and corresponding immune function. Methods: A randomized clinical trial was conducted in sixty patients with septic shock from June 2013 to August 2015. During the early- or late-low-dose glucocorticoid treatment, the serum levels of IL-6 and IL-10 were detected by ELISA, and the apoptosis rate of CD4+ cells and expression of serum human leucocyte antigen DR locus (HLA-DR) in peripheral blood were detected by flow cytometry. Finally, the occurrence of complications after treatment was evaluated. Results: After low-dose glucocorticoid treatment, level of IL-6 in patients was significantly reduced. The level of IL-6 in the early-hormone group was significantly lower than that in the late-hormone group. The apoptosis rate of CD4+ cells was significantly reduced after treatment, and the decrease in the early-hormone group was significantly greater than in late-hormone group. Levels of IL-10 and HLA-DR were not significantly changed between early- and late-hormone treatments. The occurrence of complications among the groups had no significant difference. Conclusions: Early low-dose glucocorticoid can be more effective in suppressing serum pro-inflammatory factors such as IL-6 and apoptosis rate of CD4+ cell in patients with septic shock.

Keywords: Septic shock, inflammatory factors, glucocorticoids, hydrocortisone, immune function

Introduction

Inflammation and immune dysfunction have important roles in the pathogenesis of septic shock [1]. The dynamic equilibrium of pro-and anti-inflammatory mediators determines the process of occurrence and development of sepsis [2]. Glucocorticoids play important roles in systemic reactions during inflammation via suppressing the over-activated immune system and they have been widely used in current clinical practice [3]. Currently, in addition to appropriate fluid resuscitation and vasoactive drugs, glucocorticoids are recommended for application in sepsis patients. It has been demonstrated that low-dose glucocorticoid replacement therapy can be beneficial due to the improvements of hemodynamic stability and infected organs functions during the treatment of septic shock [4-6]. However, the timing of low-dose glucocorticoid therapy in treatment of septic shock still remains controversial. This study is aimed to investigate the effect of early low-dose glucocorticoid on serum inflammatory factor and immune function in patients with septic shock.

Material and methods

Research objects

A randomize clinic trial of low-dose glucocorticoid therapy was conducted in septic shock patients who had been hospitalized in The First Hospital of Hebei Medical University from June 2013 to August 2015. Sixty patients who were insensitive to fluid resuscitation and vasoactive drugs (definition of septic shock based on The Guideline of Diagnosis and Treatment of septic shock) were included and subdivided into three groups randomly (**Figure 1**): the control group (septic shock), the early-hormone

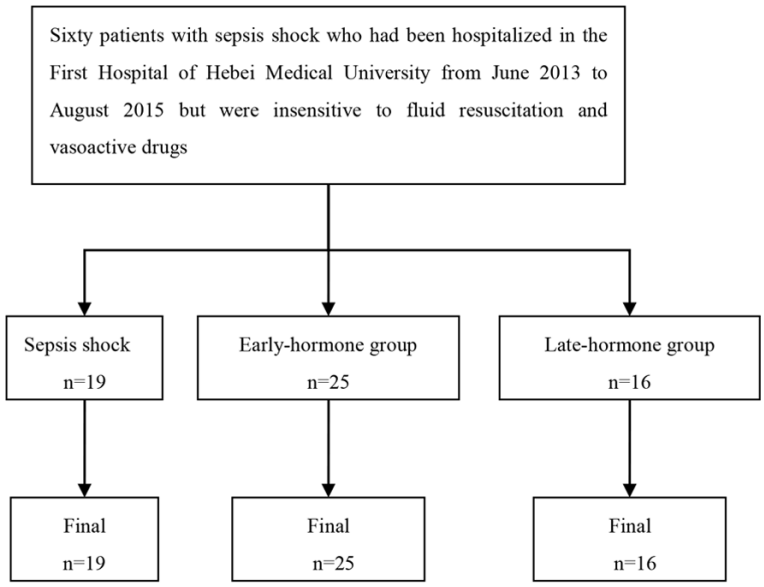

Figure 1. Flow chart for recruitment of septic shock patients.

group and the late-hormone group according to the timing of low-dose glucocorticoid. The control group (n=19): not given hormone treatment; the early-hormone group (n=25): patients were given low dose hydrocortisone within 6 hours after the occurrence of septic shock; the late-hormone group (n=16): patients were given low dose hydrocortisone within 12-24 hours after the occurrence of septic shock. The difference of the three groups in gender, age, basis diseases, and severity degree of the disease had no statistical significance ($P>0.05$), so these three groups were comparable (**Table 1**). Investigation was conducted in accordance with the ethical standards and according to the Declaration of Helsinki and according to the national and international guidelines approved by the Hospital Review Board. Written informed consent was obtained for all patient samples.

Exclusion criteria

Patients with a history of blood disease, immune system disease or immune deficiency syndrome, or those who took immune suppressor continuously, pregnant women, or patients with malignant tumor or risk of sudden cardiac death.

Therapeutic schedule

The control group without treatment: comprehensive treatment procedures including routine anti-infection, fluid resuscitation, nutritional support, and organ protection. The glucocorticoid treatment group: the patients were given low-dose hydrocortisone (50 mg) on the basis of comprehensive treatment including routine anti-infection and nutrition support, intravenous drip, one time every 6 hours, for 7 days.

Collection and detection of blood samples

Ten mL of peripheral venous blood was drawn and collected from normal control group and patients at fasting state at pro-treatment, and at 1 day, 3 days and 7 days post-treatment. Blood samples were centrifuged for 10 minutes at 3000 rpm, stored at -20°C for further examination.

Double-antibody sandwich enzyme linked immunosorbent assay (ELISA) was adopted to detect inflammatory factors including serum interleukin-6 (IL-6) and interleukin-10 (IL-10). Flow cytometry was adopted to detect the expression of serum human leucocyte antigen DR locus (HLA-DR) [7, 8] and the apoptosis rate of peripheral blood CD4+ cells.

Occurrence of complications

Outcomes and occurrence of complications were evaluated as previously described [9]. The occurrence of shock recurrence, arrhythmia, gastrointestinal bleeding, blood glucose ≥ 10 mmol/L and fungal infections during the hospitalization period were recorded for each included patient.

Statistical analysis

The normality test was adopted for all data. Data are shown as mean ± standard deviation ($\bar{x} \pm s$). Non-parametric test for dependent sample was adopted by SPSS17.0 statistical package. The student *t* test and repeated-measures ANOVA were adopted for two group and intra-group comparisons, respectively. $P<0.05$ was considered statistically different.

Table 1. Comparison of patient data

	Control group (n=19)	Early-hormone group (n=25)	Late-hormone group (n=16)	F/x^2	P
Age	61.21±11.29	59.12±11.36	56.94±8.23	0.706	0.498
Sex (male)	11 (57.9%)	13 (52.0%)	7 (43.8%)	0.698	0.705
Diabetes (Yes)	6 (31.6%)	9 (36.0%)	4 (25.0%)	0.546	0.761
Hypertension (Yes)	9 (47.4%)	15 (60.0%)	6 (37.5%)	2.053	0.358
Apache II	32.21±6.41	29.92±6.63	29.25±6.08	1.079	0.347
Sofa Score	17.11±2.11	16.08±2.75	17.44±2.42	2.479	0.091

Table 2. Septic complications after low-dose glucocorticoid therapy

Group	n	Recurrent shock	Arrhythmias	Gastrointestinal bleeding	Blood glucose ≥ 10 mmol/L	Concurrent fungal infection
Control group	19	3 (15.8%)	2 (10.5%)	3 (15.8%)	12 (63.2%)	1 (5.3%)
Early-hormone group	25	1 (4.0%)	4 (16.0%)	2 (8.0%)	16 (64.0%)	3 (12.0%)
Late-hormone group	16	1 (6.3%)	3 (18.8%)	3 (18.8%)	8 (50.0%)	2 (12.5%)
x^2		2.088	0.601	1.271	0.912	0.757
P		0.352	0.741	0.530	0.634	0.685

Note: Recurrent shock is defined as the required of vasopressor agents in maintaining the blood pressure. Once drugs discontinued, blood pressure reduced.

Results

Levels of IL-10 and apoptosis rate of CD4+ cells were increased, but the level of HLA-DR was decreased in patients with septic shock

Serum levels of IL-10 in the septic shock patients were lower than that in the normal subjects, with statistical significance (P<0.05) (**Figure 2A** and **2B**). The apoptosis rate of CD4+ cells in peripheral blood in patients with septic shock was higher than that in the normal subjects (**Figure 3A**). The expression level of mononuclear cells HLA-DR in peripheral blood in patients with septic shock was lower than that in the normal subjects, and the difference was statistically significant (P<0.05) (**Figure 3B**).

Low-dose glucocorticoid treatment decreased the levels of IL-6

After low-dose glucocorticoid treatment of 1, 3 and 7 day, the serum IL-6 levels were significantly decreased compared with the levels in patients before treatment, and the difference had statistical significance (P<0.05) (**Figure 2A**). Serum IL-6 levels in early-hormone group reduced more than that in late-hormone group at 1, 3 and 7 days.

Serum levels of IL-10 in all groups was decreased gradually (P<0.05), but there were no significant differences among groups at the same time point (**Figure 2B**).

Low-dose glucocorticoid treatment decreased the apoptosis rate of CD4+ cell

After 3 and 7 days of treatment, the apoptosis rate of peripheral blood CD4+ cell was significantly decreased comparing with that at pretreatment (**Figure 3A**). The apoptosis rate of peripheral blood CD4+ cells significantly decreased with treatment time (P<0.05). Among groups after treatment, the apoptosis rate of peripheral blood CD4+ cells in both the early-hormone group and the late-hormone group at 3 and 7 days after treatment were significantly lower than that before treatment (P<0.05). The apoptosis rate of peripheral blood CD4+ cells in the early-hormone group were significantly lower than that in the late-hormone group (P<0.05).

Low-dose glucocorticoid treatment enhanced the HLA-DR expression in peripheral blood mononuclear cells

After 1, 3 and 7 days of treatment, the expression level of peripheral blood mononuclear cells HLA-DR were detected, respectively, and results showed that HLA-DR expression increased comparing with that before treatment

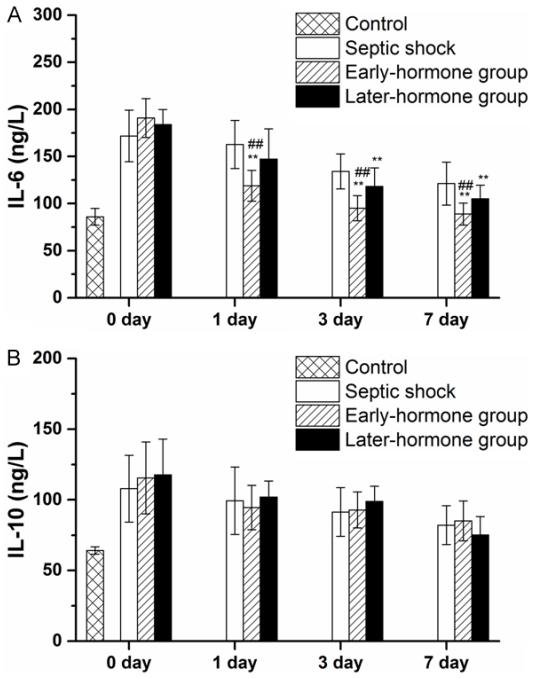

Figure 2. Expression of IL-6 and IL-10 after low-dose glucocorticoid therapy. A. IL-6; B. IL-10. Changes of IL-6 and IL-10 in serum in septic shock patients were also shown. *, $P<0.05$, **, $P<0.01$ vs. septic shock. ##, $P<0.01$ vs. late-hormone group.

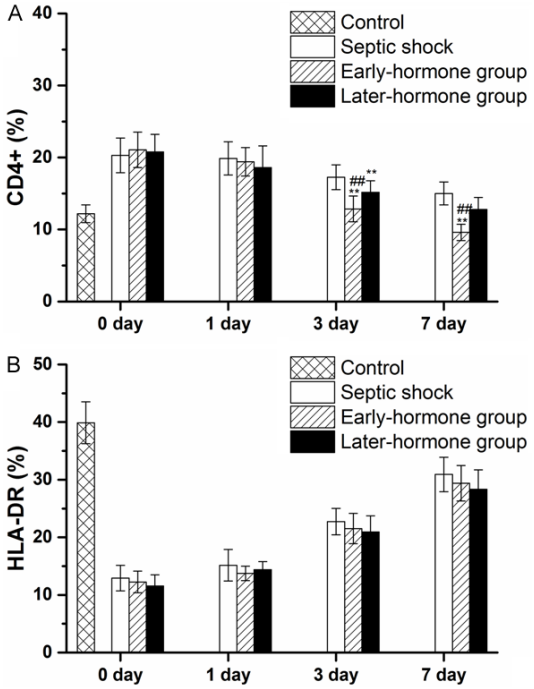

Figure 3. Changes in immune function after low-dose glucocorticoid therapy. A. CD4+; B. HLA-DR. Changes of CD4+ and HLA-DR in serum in septic shock patients were also shown. **, $P<0.01$ vs. septic shock; ##, $P<0.01$ vs. late-hormone group.

(**Figure 3B**). The expression level of HLA-DR increased with treatment time, and the difference had statistical significance ($P<0.05$). There was no statistical difference in the expression level of HLA-DR between early- and late-hormone groups.

No significant difference in complications between early- and later-hormone groups

The early-hormone group and the late-hormone group had not increased the occurrence of complications such as shock recurrence, arrhythmia, gastrointestinal bleeding, blood glucose rise (blood glucose ≥ 10 mmol/L) and fungal infection (**Table 2**).

Discussion

Glucocorticoids have very strong anti-inflammatory action and exhibit a good effect on inhibition of the release of cytokines [10]. Low-dose glucocorticoid was used in treatment of septic shock patients who are insensitive to fluid resuscitation and vasoactive drugs. Several studies focused on the timing of low-dose glucocorticoid therapy [11, 12]. It has been demonstrated that giving glucocorticoids within 4 hours of septic shock can improve the survival rate of animals [13]. The administration of low-dose glucocorticoid within 9 hours after septic shock can improve the survival rate of patients, reduce the use of noradrenaline, and downregulate the serum TNF-α level [14]. Few randomized controlled trials reported that the use of low- and median-dose glucocorticoids in septic shock patients during the first 24 h in Intensive Care Unit improved hemodynamics of patients [15, 16], and during 48 hours can decrease the mortality rate of severe septic shock patients [17]. Hemodynamics and its associated serum biomarkers play important roles in endothelial functions, such as cell migration [18-20]. Here, we found that early- (during the first 6 hours) and late- (during the 12-24 hours) hormone treatment significantly decreased the serum IL-6 level in septic shock at 1, 3 and 7 days after treatment. The levels of IL-6 in the early-hormone group were significantly lower than that in the late-hormone group. The serum IL-10 level had no statistical difference after treatment. Thus, low-dose glucocorticoids can

effectively inhibit the serum IL-6 level but has no influence on serum IL-10.

This research also discussed the influence of early low-dose glucocorticoid on the apoptosis rate of peripheral blood CD4+ cells and immune function. The results showed that at 3 and 7 days after treatment, the serum HLA-DR expression level was significantly increased with the treatment time, and the apoptosis rate of peripheral blood CD4+ cells was significantly decreased with the treatment time.

The apoptosis rate of peripheral blood CD4+ cells decreased in the early-hormone group compared with that in the late-hormone group. This is consistent with the results of other investigations [11, 12]. The apoptosis rates of peripheral blood CD4+ cells are closely related to immune function of the body [21]. Low-dose glucocorticoids have a slight inhibiting effect on the expression of HLA-DR without significant difference. Because of the resistance of glucocorticoids receptor and relative adrenal cortex insufficiency with septic shock, low-dose glucocorticoid does not induce the apoptosis of lymphocytic while inhibited inflammatory reactions. This may be one of the reasons why early low-dose glucocorticoid has a low influence on the expression level of peripheral blood HLA-DR. Nevertheless, early application of low-dose glucocorticoids is better.

We also observed the occurrence of complications during the early low-dose glucocorticoid therapy and the results showed that there was no difference between the early-hormone and the late-hormone groups in the occurrence of complications including shock recurrence, arrhythmia, gastrointestinal bleeding, blood glucose ≥ 10 mmol/L and fungal infection. It was indicated that the clinical application of early low-dose glucocorticoid for treating patients with septic shock is relatively safe.

In summary, early low-dose glucocorticoid can inhibit the level of serum IL-6 and the apoptosis rate of CD4+ cells, but it did not significantly influence the HLA-DR, suggesting that the clinical application effect of early low-dose glucocorticoid in septic shock is better and it also has some immunomodulatory effects on some cytokines.

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Disclosure of conflict of interest

None.

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