EFFECT OF HYDROCORTISONE IN A LOW DOSAGE ON APOPTOSIS OF T LYMPHOCYTE SUBSET IN TREATMENT OF SEPSIS

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ABSTRACT

Objective: *To investigate the effect of hydrocortisone in a low dosage on T lymphocyte subset in peripheral blood in treatment of sepsis.*

Methods: *A total of 84 sepsis patients were divided randomly into the observation group (treatment with low-dosage hydrocortisone, n=42) and treatment control group (n=42), and 42 healthy subjects were selected as healthy control group. Flow cytometer and enzyme-linked immunosorbent assay (ELISA) kit were used to detect the levels of T lymphocyte subset, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and calcitonin to observe the effects of hydrocortisone in a low dosage on these indicators in treatment of sepsis.*

Results: *In sepsis patients, the level of CD^{4+} was significantly decreased in comparison with the healthy control group ($p<0.01$), while difference of CD^{8+} level between sepsis patients and healthy subjects had no statistical significance ($p>0.05$). After 72 h of treatment with low-dosage hydrocortisone, the level of CD^{4+} was increased, and the ratio of CD^{4+}/CD^{8+} approached nearly 1, suggesting that the efficacy of hydrocortisone is superior to that in treatment control group. The levels of inflammatory factors in sepsis patients were significantly higher than those in healthy control group, which, however, was significantly ameliorated after 72 h of treatment with hydrocortisone in a low dosage, and the efficacy was superior to that in the treatment control group.*

Conclusion: *Low-dosage hydrocortisone can ameliorate the prognosis of sepsis patients, which may act through regulating the levels of T lymphocyte subset and inflammatory factors.*

Keywords: *Hydrocortisone, sepsis, T lymphocyte subset, inflammatory factors, calcitonin.*

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Introduction

Sepsis refers to the systemic acute inflammatory response caused by factors like infection, which may further trigger the clinical syndrome affecting the organs and tissues[(1)] with manifestations including fever, diffuse coagulation or acute decrease in blood pressure. With a high mortality rate, sepsis has become one of the major causes of deaths of patients in intensive care unit (ICU)[(2)]. Due to the high morbidity rate, high mortality rate and high treatment expense, sepsis has brought heavy burden to the society and economy. Thus, Society of Critical Care Medicine (SC-CM), European society of intensive medicine (ESICM) and International Sepsis Forum (ISF) have already initiated the Surviving Sepsis Campaign (SSC), aiming to decrease the mortality rate of sepsis by 25% within 5 years[(3)]. Programmed cell death, a major mechanism for auto-regulation of the body, is critical to maintaining the homeostasis and adapting to the environment[(4)]. In this study, we observed the effect of hydrocortisone in a low dose on T lymphocyte subset in treatment of sepsis, so as to investigate the mechanisms of hydrocortisone to eliminate the excessively activated lymphocytes and alleviate the inflammation through apoptosis, and the detailed information of this study is reported as follows.

Materials and methods

Inclusion and exclusion criteria of cases

Sepsis diagnosis was made in accordance with the New Criteria for Diagnosis of Sepsis in International Definition Conference of Sepsis in 2001[(5)].

Exclusion criteria:

• Patients who had taken immunosuppression agent within 6 months that might affect the results of this study;

• Patients with diseases in immune system;

• Patients aged below 18 years old;

• Patients with missed data or those who were not eligible to this study.

General data

Data of 84 patients who were admitted to the ICUs in this hospital between July 2014 and June 2017 were collected, and before study, 84 cards (random digit and therapeutic procedure) generated randomly by SAS software were deployed into two groups, i.e. the hydrocortisone group (observation group, n=42) and treatment control group (n=42). When the eligible patients were enrolled into the study, treatment would be initiated based on the card. At the same time, 42 healthy people who came to this hospital for physical examination were enrolled as healthy control group. Differences in comparisons of gender, age and Acute Physiology and Chronic Health Evaluation II (APACHE II) score had no statistical significance ($p>0.05$), suggesting that the data were comparable (Table 1).

Group	n	Gender (male/female)	Age (years old)	APACHE II (point)
Healthy control group	42	23/19	44.1±3.2	18.7±3.8
Treatment control group	42	18/24	42.3±6.2	19.6±3.3
Observation group	42	22/20	43.7±4.2	19.3±3.6
p		>0.05	>0.05	>0.05

Table. 1: Comparisons of gender, age and APACHE II scores between two groups (n, $\bar{x}\pm s$).

Treatment methods

Treatment control group:

Patients underwent regular fluid resuscitation, anti-infection therapy and other symptomatic treatment strategy according to the disease condition.

Observation group:

Based on the strategy for treatment control group, patients would take hydrocortisone acetate orally (20 mg/tablet; one tablet for once or twice per day) which would be withdrawn when disease condition was stabilized.

Observation indicators and detection

Apoptosis of T lymphocyte subset

In this part, we assayed the levels of CD^{4+}, CD^{8+} and CD^{4+}/CD^{8+} ratio using BD FACSCalibur flow cytometer with FITC-labeled CD^{4+} monoclonal antibody and PE-labeled CD^{8+} monoclonal antibody (BD). Measurements of the indicators above were performed before and 24, 48 and 72 h after treatment, while subjects in healthy control group only underwent one time of determination for baseline level.

Detection of inflammatory factors and other factors

Factors to be detected in this part included tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and calcitonin (CT) through enzyme-liked immunosorbent assay (ELISA), and Measurements of the indicators above were performed before and 24, 48 and 72 h after treatment, while subjects in healthy control group only underwent one time of determination for baseline level.

Statistical methods

SPSS 17.0 software was applied for data analysis. Analysis of variance was carried out for comparison of continuous variables among three groups, least significant difference (LSD) method for pairwise comparison, t test for comparison of continuous variables between two groups and chi-square test for comparison of enumeration data.

Results

Treatment outcome

In observation group, there were 8 death cases and 34 survival cases with a mortality rate of 19%; in the treatment control group, there were 18 death cases and 17 survival cases with a mortality rate of 43%; comparison between two groups showed that difference had statistical significance ($p=0.047$; Table 2).

Group	n	Death cases (n)	Survival cases (n)	Mortality rate (%)
Treatment control group	42	8	34	19
Observation group	42	18	24	43
p				0.047

Table. 2: Comparison of the treatment outcomes between two groups.

Apoptosis of T lymphocyte subset

Before treatment, in sepsis patients, the level of CD^{4+} T lymphocyte was significantly lower than that in the healthy control group, and the difference had statistical significance ($p<0.01$). Within 48 h after treatment, a persistent decrease was identified in

level of CD^{4+} lymphocyte of sepsis patients, while decrease was suspended within 72 h after treatment or even a slight increase, suggestive of an effective amelioration; although the level was slightly higher than that before treatment, the difference had no statistical significance ($p>0.05$). In observation group, at 48 and 72 h after treatment, the level of CD^{4+} T lymphocyte was significantly higher than that in the treatment control group ($p<0.05$). Similar variations were also observed in the level of CD^{8+} T lymphocyte ($p<0.05$ or 0.01). CD^{4+}/CD^{8+} ratio, reflecting the immune functions, revealed that there was no statistically significant difference in comparison between the observation group and the treatment control group within 48 h after treatment ($p>0.05$), but the ratio was increased nearly to 1 in the observation group at 72 h after treatment, and the difference in comparison with the treatment control group showed statistical significance ($p<0.01$; Table 3).

Time point	Group	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Before treatment	Healthy control group	35.12±7.08	20.87±4.90	1.71±0.07
	Treatment control group	27.86±6.69$^{\times}$	22.17±5.19	1.28±0.51$^{\times}$
	Observation group	26.41±6.42$^{\times}$	23.83±6.07	1.26±0.40$^{\times}$
24h after treatment	Treatment control group	25.80±3.63	21.85±5.66	1.25±0.29
	Observation group	26.88±5.86	21.74±5.34	1.34±0.54
48h after treatment	Treatment control group	23.35±5.55	18.92±4.12	1.28±0.39
	Observation group	26.52±5.84$^{\#}$	22.07±3.49$^{\#\#}$	1.23±0.31
72h after treatment	Treatment control group	26.02±5.29	17.03±4.26	1.65±0.61
	Observation group	29.43±4.83$^{\#}$	27.65±5.10$^{\#\#}$	1.10±0.22$^{\#\#}$

Table. 3: Comparison of apoptosis of T lymphocyte subset in peripheral blood among three groups.
$^{\times}p<0.01$ vs. healthy control group; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ vs. treatment control group at the same times point.

Detection of inflammatory factors and other factors

Massive release of TNF-α was detected in sepsis patients, and comparison of TNF-α level in blood between the sepsis patients and healthy subjects showed that the difference had statistical significance ($p<0.01$). Within 48 h after treatment, a persistent increase was identified in level of TNF-α of sepsis patients, and was suspended within 72 h after treatment or even a slight decrease, suggestive of an effective amelioration; although the level was slightly lower than that before treatment, the difference had no statistical significance ($p>0.05$). In the observation group, at 72 h after treatment, the level of TNF-α was significantly lower than that in the treatment control group ($p<0.05$). After treatment, persistent fluctuation was observed in levels of IL-1 and CT, but started to decrease at 72 h after treatment in the observation group, and the difference in comparison with the treatment control group showed statistical significance ($p<0.05$; Table 4).

Time point	Group	TNF-α (pg/mL)	IL-1 (pg/mL)	CT (μg/L)
Before treatment	Healthy control group	14.74±8.45	12.04±4.52	0.28±0.15
	Treatment control group	31.87±10.01$^{\times}$	29.29±7.75$^{\times}$	3.32±1.13$^{\times}$
	Observation group	31.63±5.59$^{\times}$	31.97±8.79$^{\times}$	3.58±1.26$^{\times}$
24h after treatment	Treatment control group	36.73±10.01$^{\times}$	28.31±10.89	3.72±1.22
	Observation group	37.75±12.05$^{\times}$	30.98±15.50	3.74±1.45
48h after treatment	Treatment control group	42.11±15.20$^{\times}$	32.05±11.92	3.52±1.16
	Observation group	37.71±11.29$^{\times}$	34.24±9.56	3.53±1.46
72h after treatment	Treatment control group	35.92±9.57$^{\times}$	32.73±11.30	2.88±1.06
	Observation group	28.85±8.30$^{\times\#\#}$	26.67±12.01$^{\#}$	2.27±0.84$^{\#}$

Table. 4: Comparisons of inflammatory factors and CT in serum among three groups.
$^{\times}p<0.01$ vs. healthy control group; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ vs. treatment control group at the same times point.

Discussion

Sepsis, as the most common clinical symptoms in ICU, leads to an extremely high mortality rate, and induces systemic immune responses under infection or severe trauma. Although antibiotics can pertain the bacteremia effectively through killing the bacteria, the massive release of endotoxin from the killed bacteria will dramatically increase the endotoxin, finally inducing the excessive activation of immune responses, and generating the damage to tissues and cells, thereby aggravating the disease condition[6-7].

Currently, research has focused on the damage to tissues, cell apoptosis and protective effect of drug on cells of sepsis, but there remain few studies designed for discovering the pathogenesis of immune regulation in sepsis. Thus, from the perspective of immune regulation, we investigated the apoptosis of T lymphocyte subset and inflammatory factors.

Clinically, there remains controversy on administration of glucocorticoid[8], and a large dose of corticosteroid hormone (30 mg/kg methylprednisolone) could not increase the survival rate of sepsis patients, but may increase the possibility of second infection, which may contribute to the exacerbation in disease condition.

However, corticosteroid hormone in a "physiological" dose may be beneficial to some patients with critical sepsis, like those concomitant with persistent shock who require vasopressor or prolonged mechanical ventilation[9]. A recent multicenter clinical study showed that daily application of hydrocortisone in a dose of 50 mg for 4 times can effectively decrease the mortality rate of sepsis patients with shock. For example, Annane D et al[10] reported that sepsis patients with shock and adrenocortical insufficiency who are less responding to the ACTH, a low dose of hormone may decrease the mortality rate with a reduction in dose of vasoactive drugs. Keh et al[11] found that the disorder in hemodynamics in sepsis patients with shock can be corrected by administration of hormone at a low dose, which can also serve as a regulator for immune functions with a stronger anti-inflammatory effect than the immunosuppressive effect.

Studies[12] have shown that T lymphocyte mainly includes CD^{4+} T lymphocytes and CD^{8+} T lymphocytes. CD^{4+} T lymphocytes serve as helpers or factors inducing immune responses, while CD^{8+} T lymphocytes are principally cytotoxic t lymphocytes. During the induction of cellular immune and humoral immune, CD^{4+} T lymphocytes are critical, and the quantity and quality of CD^{4+} T lymphocytes are closely associated with the prognosis of sepsis patients. CD^{4+}/CD^{8+} ratio can reflect the immunosuppression. In this study, we applied hydrocortisone in a low dose for treatment of sepsis, and the results of effect on T lymphocyte subset in peripheral blood showed: a) Before treatment, in sepsis patients, the level of CD^{4+} T lymphocyte was significantly lower than that in the healthy control group, and within 48 h after treatment, a persistent decrease was identified in level of CD^{4+} lymphocyte of sepsis patients, while decrease was suspended within 72 h after treatment or even a slight increase; in treatment with hydrocortisone at a low dose, the decrease of CD^{4+} T lymphocyte was slowed down at 48 h after treatment, and the increase was much faster than the treatment control group at 72 h after treatment. Decrease in CD^{4+} T lymphocyte in an early stage of sepsis may be caused by the anomaly in immune mechanism and secondary excessive elimination by cell apoptosis, thereby resulting in a massive apoptosis of CD^{4+} T lymphocyte. Treatment with hydrocortisone at a low dose may curb the excessive immune responses to alleviate the apoptosis of CD^{4+} T lymphocyte, which is conducive to ameliorating the prognosis of sepsis patients. b) During the onset of sepsis, a fluctuated decrease was identified in level of CD^{8+} T lymphocytes in treatment control group, which was reversed to a fluctuated increase during patients in treatment with hydrocortisone at a low dose, but to elucidate the mechanism, further studies are required. c) There was a fluctuated decline in CD^{4+}/CD^{8+} ratio, and nearly to 1 at 72 h after treatment, manifesting the symptoms of immunosuppression, which might be correlated with the treatment with low-dose hydrocortisone.

It was also reported by some studies[13] that under the stimulation of various inflammatory factors like TNF-α and ILs, NF-κB will be massively released, activated and combine with the corresponding intracellular promotor of genes regulating the genetic expression to initiate the transcription and expression of genes of TNF-α and ILs which will further aggravate the release of NF-κB, thereby forming a positive feedback loop. Meanwhile, NF-κB can induce the infiltration of inflammatory cells to exacerbate the edema and necrosis of tissues, which keeps intensifying the damage to tissues and cells. The results of this study revealed that during the onset of sepsis, inflammatory factors and CT sustained at a high level, and fluctuations were observed in treatment; however, the levels of inflammatory factors and CT could be curbed significantly at 72 h after treatment with a low dose of methylprednisolone, which is critical to regulation of excessive immune reactions, and conducive to ameliorating the prognosis of patients.

In conclusion, hydrocortisone at a low dose can improve the prognosis of sepsis, which might be realized through the regulation of levels of T lymphocyte subset and inflammatory factors, and the treatment should focus more on the T lymphocyte subset.

References

1) Bennett N T, Sehultz G S. Growth factors and wound heal-ing: PartII. Role in normal and chronic wound healing (J). Am JSurg, 1993, 166(1) : 74-81.
2) Zehler T R, Pierce G F, Herndon D N. Growth factorsand wound healing: basic science and potential clinical applicati-om (M). New York: Springer, 1997: 206-228.
3) Manglik S, FloresE, Lubarsky L, eta.l Glucocorticoid insufficiency in patients who present to the hospital with sever sepsis: A prospective clinical[J]. Crit Care Med, 2003, 31: 1668 -1675.
4) Riad M, Mogos M, Thangthurai D, et a.l Steroid[J]. CurrOpinCritCare, 2002, 8: 281-284.
5) Bornstein SR, Briegel J. A new role for glu-cocorticoids in septic shock[J]. Am JRespir CareMed, 2003, 167: 485-486.

6) Liu ZH, Yu YQ, Jiang Y, et a.l Growthhormone in crease circulating neutrophil acti-vation and provokes lungmicrovascular injury in septic peritonitis rats[J]. J Surg Res, 2002, 105: 195-199.
7) Das UN. Insulin and inflammation: further evidence and discussion [J]. Nutrition, 2002, 18: 526-527.
8) Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis[J]. N Engl JMed, 2003, 348: 138-150.
9) Van den Berghe, Wouters P, Weekers F, et a.l Intensive in insulin therapy in critically ill patients[J]. N Engl J Med, 2001, 345: 1359-1367.
10) JeschkeMG, Einspanier R, Klein D, et a.l Insulin attenuates the systemic inflammatory re-sponse to thermal trauma [J]. Mol Med, 2002, 8: 443-450.
11) Annane D, Sebille V, CharpentierC, et a.l Effectof treatmentwith low doses ofhydrocorti- sone and fludrocortisone onmortality in patients with septic shock[J]. JAMA, 2002, 288: 862-871.
12) Keh D, Boehnke T, Weber-Cartens S, eta.l Immunologic and hemodynamic effects of " Low - dose" hydrocortisone in septic shock: a double -blind, randomized, placebo-controlled, crossover study[J]. AmJ RespirCritCareMed, 2003, 167: 512-520.
13) Gao F, Gao E, Yue TL, eta.l Nitric oxide mediates the anti -apoptotic effect of insulin in myocardial is chemia -reperfusion: the roles of PI3 -kinase, Akt, and endothelial nitric oxide synthase phosphorylation [J]. Circulation, 2002, 105 (12): 1497-1502.

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