Effects of Hydrocortisone on Regulating Inflammation, Hemodynamic Stability, and Preventing Shock in Severe Sepsis Patients

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Background: Severe sepsis is among the most common causes of death in Emergency Departments, with more than 30% mortality. Hydrocortisone is used in severe sepsis patients who were not responsive to fluid resuscitation and vasopressor therapy. However, the effect of hydrocortisone on regulating inflammation, hemodynamic stability, and preventing shock is still unclear in Chinese patients.

Material/Methods: In this prospective observational study, we included 105 severe sepsis patients. We measured the level of serum inflammatory cytokines, hemodynamic variables, and phagocytic ability of innate immune cells during the treatment. We analyzed the relationship between these variables and the hydrocortisone treatment.

Results: We treated 43 (41.0%) patients with hydrocortisone, while the other 62 (59.0%) patients were not, based on their response to fluid resuscitation and vasopressor therapy. The hydrocortisone group had a mean simplified acute physiology score (SAPS) II score of 41.8 with standard deviation (SD) of 7.1, while the non-hydrocortisone group had a mean SAPS II score of 36.7 with SD of 7.3. The mean sequential organ failure assessment (SOFA) scores of these 2 groups were 10.6 and 9.2, respectively. We found an obvious decrease of serum pro-inflammatory cytokines, including interleukin-1β (IL-1β), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-6, after hydrocortisone treatment. However, these changes were not observed in the non-hydrocortisone group. What’s more, amelioration of hemodynamic variables was observed after hydrocortisone treatment. No significant association between hydrocortisone treatment and innate immune cell phagocytic function was observed.

Conclusions: Based on these results, we believe that hydrocortisone treatment has potential anti-inflammatory, hemodynamic reversal, and stability effects on severe sepsis patients. These key benefits may help patients by preventing septic shock.

MeSH Keywords: Hydrocortisone • Immunity, Innate • Inflammation • Sepsis • Shock

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Background

Severe sepsis is among the most common causes of death in Emergency Departments, with a more than 30% mortality rate [1,2]. Severe sepsis is defined as sepsis with sepsis-induced organ dysfunction or tissue hypoperfusion [3]. In recent decades, the incidence of sepsis has continued to increase [3]. It was estimated that one-quarter of sepsis patients finally develop to severe sepsis [3]. Both Gram-positive and Gram-negative bacteria, as well as fungi and viruses, can be the pathogens of sepsis [3–5].

Severe sepsis is thought to be the result of progressive systemic inflammation and procoagulant response caused by infections [6]. The inflammation mediators and procoagulant thrombin act reciprocally, leading to diffuse endothelial injury and multi-organ dysfunction [7]. Volume resuscitation and antimicrobial therapy are the 2 main interventions with high priority for severe sepsis patients [8]. Resuscitation achieving physiologic hemodynamic goals in the first 6 h is associated with significantly reduced mortality [9]. Screening and control of infection source and early administration of effective antibiotics are highly recommended for severe sepsis patients [8]. Other therapies, such as fluid therapy, vasopressors, and inotropic therapy, are currently widely used, aiming to restore and stabilize perfusion [8]. With advances in health care, surveillance, and improved therapy of infections, the mortality of severe sepsis has been significantly reduced in recent decades [10]. Nevertheless, severe sepsis is still among the most common causes of death in the Emergency Department [11]. Clinically, a large proportion of severe sepsis patients are not sensitive to the volume resuscitation. Therefore, more effective interventions that benefit severe sepsis patients are still urgently needed.

Hydrocortisone, a steroid hormone, is usually used as an anti-inflammation medicine in patients with chronic obstructive pulmonary disease, cancers, autoimmune diseases, and adenocortical insufficiency. In septic shock, hydrocortisone is commonly used for patients who were not responsive to fluid resuscitation and vasopressor therapy. However, the effect of hydrocortisone in preventing the development of septic shock from severe sepsis is still controversial [8]. In this study, we evaluated the effects of hydrocortisone treatment on regulating inflammatory response and peripheral circulation, the major causes of septic shock, in severe sepsis patients.

Material and Methods

Study population

We performed a prospective cohort study to evaluate the effect of hydrocortisone treatment on preventing the development of septic shock from severe sepsis. Specifically, we evaluated the inflammatory and peripheral circulation parameters. The study protocol was approved by the Clinical Research Committee of the Second Affiliated Hospital of Dalian Medical University. Adult patients who were treated in the Emergency Department of the Second Affiliated Hospital of Dalian Medical University and diagnosed with sepsis syndrome or severe sepsis during April 2014 to March 2016 were assessed for inclusion in this study. The assessment was finished within 2 h after their arrival at the Emergency Department. The inclusion criteria were: 1) age <75 years; 2) fulfillment of 2 or more systemic inflammatory response syndrome criteria [12]; 3) sepsis-induced hypotension (a systolic blood ≤90 mm Hg), or a blood lactate concentration ≥4 mmol/L; 4) written informed consent from the legal personal representative of the patient. The exclusion criteria were: 1) age >75 years; 2) coincidence of other acute cardiac, pulmonary, renal diseases or hematologic diseases; 3) coincidence of tumors; 4) immunodeficiency; 5) pregnancy. The physicians of the clinical department were blind to the aim and protocol of this study. The laboratory researchers were blind to the treatment schedule of these patients when collecting experimental data. Medical records were collected for review after patients left the hospital. The hydrocortisone treatment was given when adequate fluid resuscitation and vasopressor did not reverse the sepsis-induced hypoperfusion [8]. Baseline treatment of severe sepsis included fluid therapy, vasopressors, antimicrobial therapy, infection source control, infection prevention, and other supportive therapies. There was no significant difference in baseline treatment between the patients who received hydrocortisone and those who did not. Hydrocortisone (200 mg/day) treatment started within 12 h after arriving at the Emergency Department. The average duration of hydrocortisone treatment was 4.3 days.

Measurements

Baseline characteristics of each patient were measured and recorded within 6 h after their admission to the Emergency Department. The baseline data were extracted from the patients’ medical record, including age, sex, simplified acute physiology score (SAPS II), sequential organ failure assessment (SOFA), heart rate, systolic blood pressure, temperature, respiratory rate, white blood cell count, blood lactate, blood culture positivity, antibiotics administrated in the initial 6 hours, comorbidities, and main/suspected source of infection. Central venous oxygen saturation (SvO2), mean arterial pressure (MAP), and central venous pressure (CVP) were continuously monitored. Systemic vascular resistance (SVR) was calculated based on a standard formula. Whole blood was collected at 0 h, 6 h, 24 h, 48 h, 72 h, 96 h, 120 h, and 144 h after admission. Serum inflammation regulatory cytokines were measured.
Cytokine assay

We measured the level of serum inflammatory cytokines (IL-1β, IFN-γ, TNF-α, IL-6, and IL-10) during the treatment. Four milliliters of venous blood samples were collected from the antecubital vein of the patients by using non-heparinized tubes (Becton Dickinson, USA). The blood samples were kept at room temperature and were undisturbed for 20 min, followed by centrifuging in a refrigerated centrifuge. The serum samples were stored at −80°C until testing. The cytokines levels were assessed using enzyme-linked immunosorbent assay kits (R&D Systems, USA).

Monocyte and granulocyte isolation

Heparinized anti-coagulated peripheral blood was collected from the severe sepsis patients. Granulocytes were isolated following the method described previously [13]. In brief, the whole blood cells were separated by lymphocyte separation medium (density of 1.077 g/ml, Histopaque®-1077, Sigma-Aldrich, USA) and Histopaque®-1119 (density of 1.119 g/ml, Histopaque®-1119, Sigma-Aldrich, USA), followed by centrifuging at 300×g for 5 min, and 800×g for 20 min. Granulocytes were collected from the lower layer, followed by washing with PBS and then were resuspend by RPMI 1640 medium (Sigma-Aldrich, USA) with 10% heat-inactivated fetal bovine serum, and 50 mM 2-mercaptoethanol. Then, the granulocytes were further purified by discontinuous Percoll (Amersham Biosciences, Sweden) centrifugation. Monocytes were collected from the upper layer (Histopaque®-1077 layer) and further purified by CD14 antibody-conjugated microbeads (Miltenyi Biotec, Germany). The cells were cultured with RPMI 1640 medium. The recovery of cells was checked by trypan blue staining.

Phagocytosis assay

Phagocytosis assay was performed by following the method previously described [14]. Briefly, isolated granulocytes (2×10⁶/ml) were incubated with green fluorescent protein (GFP)-labeled E. coli (1×10⁹/ml) in RPMI 1640 medium containing 10% FBS at 37°C for 20 min. Then, phagocytosis was analyzed by flow cytometry by measuring the percentage of GFP-positive granulocytes. The samples incubated at 0°C were used as negative control. A similar method was used to measure the phagocytosis of monocytes.

Statistical analysis

GraphPad Prism software (USA) and SPSS 24.0 software were used to perform the statistical analyses. To evaluate the difference in means of quantitative variables, the t test was performed. To evaluate the dependence of frequency distributions between category variables, the chi-square test or Fisher’s exact test was performed. A two-tailed P value of less than 0.05 was considered as statistically significant.

Result

Characteristics of enrolled patients

As shown in Figure 1, this prospective cohort study enrolled a total number of 105 severe sepsis patients from the Emergency Department. The baseline characteristics of these patients were collected when they arrived at the Emergency Department and are summarized in Table 1. These patients were assigned to the hydrocortisone group if they received hydrocortisone treatment at the Emergency Department, while those who did not receive hydrocortisone treatment were assigned to the non-hydrocortisone-treatment group. Hydrocortisone treatment was given when adequate fluid resuscitation and vasopressor treatment were unable to reverse the sepsis-induced hypoperfusion [8].

The mean age of patients in the hydrocortisone-treated group was 59.5 years, while that of the non-hydrocortisone-treated group was 55.6 years (Table 1). The hydrocortisone-treated group had a mean SAPS II score of 41.8 with standard deviation (SD) of 7.1, while the non-hydrocortisone-treated group had a mean SAPS II score of 36.7 with SD of 7.3 (Table 1). The mean SOFA scores of these 2 groups were 10.6 and 9.2, respectively (Table 1). The hydrocortisone-treated group also showed more severity than the non-hydrocortisone-treated group in some other characteristics (Table 1), such as heart rate (P value=0.042), white blood cell count (P value=0.029), and blood lactate concentration (P value=0.006). Among the patients who received hydrocortisone treatment, 24 (55.8%) showed obvious improvement in hemodynamic parameters within 48 h after hydrocortisone treatment, while 11 (25.6%) patients showed septic shock symptoms.
Hydrocortisone treatment reduced pro-inflammatory cytokines

We measured the serum levels of multiple inflammation regulatory cytokines at time of admission and analyzed their changes during the treatment. Interestingly, we found that various pro-inflammatory cytokines, such as IL-1β, IFN-γ, TNF-α, and IL-6, were decreased in the hydrocortisone-treated patients much faster than in the patients who did not receive hydrocortisone treatment (Figure 2A–2D). We also found that the anti-inflammation cytokine IL-10 was also enhanced in the hydrocortisone-treated patients faster than in the non-hydrocortisone-treated group (Figure 2E).

Hydrocortisone treatment promoted hemodynamic stability

As shown in Figure 3, hemodynamic parameters were plotted at different time points after admission to the Emergency Department. The mean arterial pressure (MAP), central venous pressure (CVP), systemic vascular resistance (SVR), and...
central venous oxygen saturation (ScvO₂) were enhanced after adequate fluid resuscitation and vasopressor administration in the non-hydrocortisone-treated group (from 0 h to 6 h). However, these hemodynamic parameters were not ameliorated sufficiently to meet the goal of initial resuscitation by adequate fluid resuscitation and vasopressor administration in the hydrocortisone-treated group (from 0 h to 6 h). Upon hydrocortisone administration (at around 6 h), these hemodynamic parameters started to improve (Figure 3).

Hydrocortisone treatment did not affect the phagocytosis of immune cells

Since the innate immune cells are the major cell type that eliminates pathogens in sepsis, we evaluated the influence of hydrocortisone administration on phagocytosis of innate immune cells. As shown in Figure 4, we measured the phagocytic ability of monocytes and granulocytes from the severe sepsis patients before and after they received the hydrocortisone...
We found there was no significant difference in phagocytic ability of monocytes and granulocytes before and after the hydrocortisone treatment. These data indicate that the hydrocortisone treatment does not affect the phagocytic function of innate immune cells.

**Discussion**

Although the mortality rates of infectious diseases have been significantly decreased by antibiotics and supportive care, they are still threats to health, especially in immunocompromised patients [15–17]. Severe sepsis places a large burden on Emergency Departments due to its high short-term mortality, which may reach 50% and its high use of health care resources [1,18]. Systemic inflammation and hypoperfusion are the main features of the clinical course of severe sepsis and are the causes of sepsis shock. Corticosteroids have long been used as an adjuvant in severe sepsis [19,20] due to their anti-inflammatory properties. Nevertheless, the effects of corticosteroids on septic patients are still controversial [21,22]. Some previous studies showed that short-time administration of high doses of corticosteroids had no significant effects on the outcome of septic patients [22,23]. On the contrary, other studies have shown that prolonged administration of low-dose hydrocortisone improved shock reversal [24,25]. More importantly, there have been no relevant studies in the Chinese population. Thus, more well-designed studies focusing on the effects of hydrocortisone treatment in septic patients are still greatly needed.

In the present study, we measured serum inflammatory cytokines during treatments to explore the potential mechanisms by which hydrocortisone benefits severe sepsis patients. Interestingly, we found obviously attenuated pro-inflammatory cytokines in the hydrocortisone-treated patients vs. the

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**Figure 3.** Alterations of hemodynamic variables during the treatment of severe sepsis patients. Alterations of MAP (A), CVP (B), SVR (C), and ScvO₂ (D) of the severe sepsis patients who received hydrocortisone treatment (light blue lines or dots) vs. those who did not (deep blue lines and dots) were measured and plotted during the therapy. The mean start time of hydrocortisone treatment was less than 12 h after admission to the Emergency Department. An obvious increase in these hemodynamic variables were observed after hydrocortisone treatment. Values showed here are means and standard deviations. (ScvO₂ – central venous oxygen saturation; MAP – mean artery pressure; CVP – central venous pressure; SVR – systemic vascular resistance; NS – no significance; * P value less than 0.05; ** P value less than 0.01; *** P value less than 0.001; **** P value less than 0.0001).
non-hydrocortisone-treated patients, including IL-1β, IFN-γ, TNF-α, and IL-6. These pro-inflammatory cytokines have been shown to activate neutrophils and stimulate the coagulation system, thus acting synergistically in inducing shock [26]. At baseline, the hydrocortisone-treated patients had an elevated level of pro-inflammatory cytokine expression. After they received hydrocortisone treatment, these serum concentrations of cytokines clearly fell. However, the levels of cytokines in non-hydrocortisone-treated patients remained relatively stable or decreased gradually during treatment. Previous studies have shown that pro-inflammatory cytokines, such as IL-6, TNF-α, and IFN-γ, are markers of disease severity and are associated with poor outcomes [27–31]. Therefore, our results suggest that hydrocortisone reduces the pro-inflammatory condition in severe sepsis patients, thus potentially improving outcome.

Our results also indicated that increased MAP, CVP, SVR, and ScvO₂ were associated with hydrocortisone treatment, evidenced by the synchronous increase of hemodynamic parameters with hydrocortisone treatment. The hemodynamic reversal is critical for preventing septic shock and the survival of severe sepsis patients [32,33]. Our results were consistent with a previous study by Keh et al. [34]. Innate immune response is the major anti-pathogen mechanisms in most septic infections. Monocytes and granulocytes eliminate bacterial pathogens via phagocytic effects. In the present study, we also explored the effect of hydrocortisone on phagocytosis of monocytes and granulocytes. Importantly, there was no significant difference in monocyte phagocytosis and granulocyte phagocytosis before vs. after the hydrocortisone treatment. These data indicate that the hydrocortisone treatment does not impair the anti-bacterial function of innate immune cells and is in line with results of previous studies [34,35]. Treatment of

Figure 4. Phagocytic ability of monocytes and granulocytes from severe sepsis patients with or without hydrocortisone treatment. Phagocytosis of monocytes and granulocytes collected from severe patients at 6 h and 96 h after admission at the Emergency Department was measured. (A, B) There was no significant difference in monocyte phagocytosis rates between 6 h and 96 h in the severe patients receiving hydrocortisone treatment vs. those who did not. (C, D) No significant difference in granulocyte phagocytosis rate was observed between 6 h and 96 h in severe patients who received hydrocortisone treatment vs. those who did not. The data were normalized to the result at 6 h after admission. Values showed here are means and standard deviations (NS – no statistical significance).
severe sepsis can be very complicated, and outcome can be influenced by many factors, such as the time of finding the source of infection and administration of effective antibiotics [8]. Our observational study included a moderate number of patients, which might not have been sufficient to control all the confounding factors between the hydrocortisone-treated patients and non-hydrocortisone-treated patients. Thus, large-scale observational studies and clinical trials are still needed to validate the value of using hydrocortisone treatment in certain severe sepsis patients.

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Conclusions

In conclusion, our prospective cohort study indicates that hydrocortisone treatment has potential anti-inflammatory and hemodynamic reversal and stability effects in severe sepsis patients. These key ameliorations may benefit the patients by preventing septic shock.

Conflict of interest

None.