Immunomodulation in Septic Shock: Hydrocortisone Differentially Regulates Cytokine Responses

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Abstract. Cortisol is known to be an immunomodulatory hormone that exerts suppressive and permissive effects on the immune response. Little is known regarding the evolution of the cytokine response in human septic shock in the presence of hypercortisolemia induced by infusion of stress doses of hydrocortisone. Twenty-four consecutive patients with high-output circulatory failure (cardiac index, >4 liters/min per m²) who met the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee criteria for septic shock were enrolled in a prospective, double-blind study. The severity of illness at the time of enrollment was graded using the Acute Physiology and Chronic Health Evaluation II system, and the evolution of sepsis-induced organ dysfunction syndrome was assessed using Sepsis-Related Organ Failure Assessment scores. After randomization, hypercortisolemia was induced in 12 patients by infusion of 100 mg of hydrocortisone, followed by continuous infusion of 0.18 mg/kg per h. Levels of the circulating cytokines tumor necrosis factor α (TNF), interleukin 6 (IL-6), IL-8, and IL-10 were serially measured at prospectively defined time points during the first 5 d after randomization. The infusion of hydrocortisone was associated with significant reductions in serum IL-6 and IL-8 levels and with earlier resolution of the sepsis-induced organ dysfunction syndrome. IL-6 levels started to differ between the groups on day 5. The TNF and IL-10 responses were not altered by hydrocortisone infusion. Hydrocortisone infusion in septic shock differentially regulated the cytokine responses. IL-6 and IL-8 levels decreased significantly and IL-6 levels differed between the groups, whereas TNF and IL-10 levels were not affected by hydrocortisone. Stress doses of hydrocortisone may be a valuable immunomodulatory therapy for septic shock.

The endogenous glucocorticoid hydrocortisone plays a pivotal role in the modulation of the immune response to infection. Hydrocortisone interacts with the immune system at several levels, exerting both suppressive and permissive effects. The stress response to infection leads to hypercortisolemia, which counteracts the systemic inflammatory response and thus protects the host from its own overactivated defense reactions. Hypercortisolemia also permits certain immune reactions, which enhance the defense reactions (1).

In healthy volunteers, a stress dose of hydrocortisone (100 mg or 3 μg/kg per min) reduces the clinical response to endotoxin. It also attenuates the production of circulating proinflammatory cytokines (2,3). In patients with sepsis, low-dose hydrocortisone infusions attenuate the systemic inflammatory response, as judged by clinical signs and inflammatory markers (4). Recently, two double-blind, single-center studies demonstrated that stress doses of hydrocortisone reversed septic shock, as defined by cessation of vasopressor therapy (5,6). The earlier weaning from vasopressor therapy in septic shock was associated with a trend toward improvements in organ dysfunction and mortality rates.

The data from these recent trials of low-dose hydrocortisone treatment renewed interest in the use of corticosteroids for the treatment of sepsis. Corticosteroids prevent or suppress inflammation, and they act in a dose-dependent manner. When administered at pharmacologic dosages, corticosteroids alter cytokine responses, leukocyte kinetics, phagocytic cell function, and cell-mediated immunity (7). Little is known regarding the evolution of immune processes when glucocorticoids are administered at moderate doses for longer periods of time to patients with septic shock. The objective of this study, which has been part of a double-blind trial, was to investigate the effects of stress doses of hydrocortisone on the balance of pro- and anti-inflammatory cytokines in hyperdynamic septic shock.

Materials and Methods

Patients

In July 1993, the study protocol was approved by the institutional review board of the Ludwigs-Maximilians-University of Munich. Relatives of the patients were informed regarding the medical problems and the nature and purpose of the study and served as surrogates to determine the judgment of the unconscious patients with respect to participation in the study. The study was conducted in the multidisciplinary intensive care unit of a university hospital.

Patients with septic shock were prospectively enrolled if they met the criteria for septic shock proposed by the members of the American Society of Nephrology.
College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee, i.e., documented infection or positive blood culture results; at least two symptoms of the systemic inflammatory response syndrome, such as fever (body temperature of $>38^\circ C$) or hypothermia (body temperature of $<36^\circ C$), tachycardia ($>90$ beats/min), tachypnea ($>20$ breaths/min), and abnormal white blood cell counts ($>12,000$ or $<4000$ cells/$\mu l$); evidence of organ dysfunction or hypoperfusion abnormalities; and the use of vasopressor support (dopamine dose of $>6 \mu g/kg$ per min or norepinephrine added to dopamine treatment) despite adequate fluid resuscitation (8).

Furthermore, only patients with high-output circulatory failure, defined as a cardiac index of $>4.0$ liters/min per $m^2$ ($>3.5$ liters/min per $m^2$ for patients $>55$ yr of age), were studied. The primary study endpoint was the time to shock reversal, as defined by cessation of vasopressor support. Secondary study endpoints were hemodynamic evolution, multiple organ dysfunction syndrome, and systemic inflammatory response evolution. The severity of illness at the time of enrollment was determined using the Acute Physiology and Chronic Health Evaluation II (APACHE II) scoring system (9). Suspected infection at the time of enrollment had to be proven by microbiologic examination (Table 1). The patient treatment has been described elsewhere (5).

**Intervention**

The patients were prospectively randomized to receive infusions of either stress doses of hydrocortisone or placebo. Hydrocortisone administration was started with a loading dose of $100$ mg, administered intravenously in $30$ min, followed by continuous infusion of $0.18$ mg/kg per h. After the reversal of septic shock (defined as dopamine doses of $<6 \mu g/kg$ per min or cessation of norepinephrine infusion), the dose of hydrocortisone was reduced to $0.08$ mg/kg per h. This dose was kept constant for at least $6$ d. As soon as the underlying infection had been treated successfully, the hydrocortisone infusion was tapered off (in daily $24$-mg steps). Physiologic saline solution was used as placebo. The study drugs were prepared by research assistants at our institution, who were not involved in the study or in the clinical care of the patients. Fifty-milliliter syringes containing $100$ mg of hydrocortisone-21-hemisuccinate (Upjohn, Heppenheim, Germany), diluted in physiologic saline solution, or placebo were prepared daily and stored at $4^\circ C$.

**Measurements**

The evolution of sepsis-induced organ dysfunctions was assessed using Sepsis-Related Organ Failure Assessment (SOFA) scores (10). The relative changes in SOFA scores were calculated in comparison with the baseline score for each patient. The effects of the intervention on markers of the systemic inflammatory response were determined by drawing blood samples at the time of enrollment (baseline values) and, after randomization, at predefined intervals for at least $5$ d after enrollment. Samples (serum and plasma) were coded by number and stored at $-80^\circ C$ until assayed (double assay). The proinflammatory cytokines tumor necrosis factor (TNF) [sandwich enzyme-linked immunosorbent assay (ELISA); Behring Diagnostics, Liederbach, Germany] and interleukin-6 (IL-6) and IL-8 (sandwich ELISA; R&D/DPC Biermann, Germany) and the anti-inflammatory cytokine IL-10 (sandwich ELISA; Bender Med Systems, Vienna, Austria) were assayed. The normal ranges are given in the figures.

**Statistical Analyses**

All data were tested for distribution. We used a repeated-measures ANOVA on ranks because SOFA scores and cytokine levels were not normally distributed. Dunn’s test was used to compare data with baseline values. Comparisons between groups were made using the $t$ test for normally distributed data and the Mann-Whitney $U$ test for non-normally distributed data. $P$ values of $<0.05$ were considered to indicate statistical significance. Statistical analyses were performed using the computer package Sigmastat, version 2.0 (SPSS, Inc., Chicago, IL).

**Results**

**Baseline Findings**

Baseline data did not differ between the two groups, in terms of biometric data, illness severity, and degree of organ dysfunctions (Table 1). No differences in the type or anatomic site of infection were evident (Table 2).

**Changes in Clinical Signs and Organ Dysfunction**

The median values of the daily SOFA scores are depicted in Table 3. There were significant decreases in the SOFA scores for the hydrocortisone-treated patients on days 3, 4, and 5. Two patients in the hydrocortisone-treated group and five patients in the placebo-treated group died as a result of sepsis-induced multiorgan failure ($P = 0.37$).

**Table 2. Underlying infections and type of isolates**

<table>
<thead>
<tr>
<th>Underlying infections</th>
<th>Hydrocortisone $(n = 12)$</th>
<th>Placebo $(n = 12)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosocomial pneumonia</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Community-acquired pneumonia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal infection</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Urogenital tract infection</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Wound infection</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gram-positive infection</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Gram-negative infection</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Polymicrobial infection</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Fungal infection</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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With # Table 1. Comparison of patient characteristics at the time of randomization$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hydrocortisone $(n = 12)$</th>
<th>Control $(n = 12)$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F) $^b$</td>
<td>5/7</td>
<td>6/6</td>
<td>0.41</td>
</tr>
<tr>
<td>Age (yr) $^b$</td>
<td>$45 \pm 4$</td>
<td>$51 \pm 4$</td>
<td>0.59</td>
</tr>
<tr>
<td>APACHE II score $^b$</td>
<td>$25 \pm 1$</td>
<td>$27 \pm 1$</td>
<td>0.67</td>
</tr>
<tr>
<td>APACHE III score $^b$</td>
<td>$92 \pm 6$</td>
<td>$60 \pm 5$</td>
<td>0.63</td>
</tr>
<tr>
<td>SAPS II score $^b$</td>
<td>$57 \pm 4$</td>
<td>$60 \pm 5$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score.  
$^b$ Values are expressed as mean $\pm$ SEM ($t$ test).
Changes in Plasma Cytokine Concentrations and Balance

The plasma concentrations of TNF varied within the normal range for both groups and did not differ between the groups with time (Figure 1). Baseline IL-6, IL-8, and IL-10 levels were considerably elevated for both groups, compared with values for healthy individuals (Table 4). After hydrocortisone infusion, plasma concentrations of IL-6 (Figure 2) and IL-8 (Figure 3) decreased significantly. Plasma concentrations of IL-6, IL-8, and IL-10 for the placebo-treated group did not decrease significantly with time (Figure 4). IL-6 levels at day 5 were significantly lower for the hydrocortisone-treated group, compared with the placebo-treated group.

Table 3. Evolution of SOFA scores during the study period

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>11^b</td>
<td>11^b</td>
<td>9.5^b</td>
</tr>
<tr>
<td>Placebo</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
</tbody>
</table>

^a SOFA, Sepsis-Related Organ Failure Assessment. Values are expressed as median values. The SOFA scores started to differ from baseline values on day 3 for the hydrocortisone-treated group.

^b P < 0.05.

Table 4. Cytokine concentrations before intervention

<table>
<thead>
<tr>
<th></th>
<th>Hydrocortisone (n = 12)</th>
<th>Placebo (n = 12)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF (pg/ml)</td>
<td>4.5 (3/10.5)</td>
<td>6 (3/9)</td>
<td>0.69</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1844 (239/7034)</td>
<td>825 (42/3453)</td>
<td>0.29</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>345 (30/5934)</td>
<td>15 (2/292)</td>
<td>0.16</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>57 (32/167)</td>
<td>64 (10/125)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

^a TNF, tumor necrosis factor; IL, interleukin. Values are expressed as median values (quartiles) (Mann-Whitney U test).

Discussion

A complex network of pro- and anti-inflammatory compounds regulates host humoral and cellular defense mechanisms. A relative deficit of anti-inflammatory compounds leads
to excessive amplification of the inflammatory cascade, with uncontrolled activation of the immune system. Typical clinical sequelae are sepsis-induced organ dysfunctions, such as mental disorders, disseminated intravascular coagulation, acute lung injury, renal failure, acute hepatic dysfunction, and cardiovascular dysfunction (10). The physiologic function of hypercortisolemia associated with stress is to modulate the normal defense mechanisms, thus protecting the host from overactivation of inflammatory reactions (1). Cortisol interacts with the immune system at several levels, exerting suppressive and permissive effects. In a dose-dependent manner, corticosteroids inhibit proinflammatory cytokine synthesis and cellular immunity (11,12).

The major finding of this study is that hydrocortisone administered in stress doses selectively reduced circulating IL-6 and IL-8 levels but not TNF and IL-10 levels in patients with hyperdynamic septic shock. Because IL-6 levels are consistently correlated with the severity of septic shock, the decrease in IL-6 levels may also accelerate the observed improvements in sepsis-induced organ dysfunction, as indicated by the associated decrease in SOFA scores (5,13). These results agree with data reported in the literature. In healthy volunteers, stress doses of hydrocortisone reduced the clinical response to endotoxin and attenuated the appearance of circulating proinflammatory cytokines (2,3). In contrast, hypercortisolemia did not alter the synthesis of anti-inflammatory cytokines such as IL-4 and -10 (14,15). Hypercortisolemia even increased plasma IL-10 concentrations in human endotoxia, which might add to the anti-inflammatory actions of hydrocortisone (15).

Cytokines are peptides that primarily regulate the interaction and communication of cells of the immune system. In case of infection, cytokines initiate the systemic inflammatory response by interacting with specific receptors on inflammatory target cells. In the absence of infection, the injection of TNF has been shown to induce symptoms similar to those of septic shock (16). At the time of enrollment into this study, all patients had circulating TNF levels within the normal range. This phenomenon has been observed in several studies. TNF has a short plasma half-life (<10 min). However, its biologic sequelae can be observed for several days. It is known that only some patients with septic shock have relevant TNF levels (17). In survivors of severe sepsis, circulating concentrations of TNF decreased rapidly within the first 24 h after admission to the intensive care unit (18). In this study, the times of onset of septic shock before enrollment were 20.7 and 26.5 h for the hydrocortisone- and placebo-treated groups, respectively (5).

High levels of IL-6 at the time of enrollment indicated hyperactivated systemic inflammatory responses in the study patients. IL-6 is known to be a favorable marker for the severity of sepsis (13,19). IL-6 induces fever and the synthesis of acute-phase proteins (20). It is a relevant priming factor for the activation of coagulation (21). The observed decrease in IL-6 concentrations to almost normal values denotes a significant reduction in inflammatory activity. As a result of the decrease in IL-6 levels, we also observed a decrease in the levels of the acute-phase protein C-reactive protein and a decrease in the levels of D-dimers, i.e., markers of activated coagulation (data not shown). In a previous study, we demonstrated that hydrocortisone infusion attenuated the systemic inflammatory response, as assessed using inflammatory mediators such as soluble phospholipase A2 and C-reactive protein (4).

IL-8 and chemokines induce the directional migration of leukocytes during normal and inflammatory processes (22). In patients at risk for sepsis, IL-8 is critically involved in the development of acute lung injury (23). In this respect, it is of interest that stress doses of hydrocortisone were shown to reduce the duration of mechanical ventilation, indicating earlier resolution of acute lung injuries (5).

In conclusion, hypercortisolemia induced by the infusion of stress doses of hydrocortisone differentially regulates the cytokine response. In a randomized, double-blind study, we observed significant reductions in IL-6 and IL-8 concentrations but not TNF and IL-10 concentrations in patients with septic shock. As a result of the attenuation of the systemic inflammatory response, we observed earlier resolution of the sepsis-induced organ dysfunction syndrome. Therefore, intervention with stress doses of hydrocortisone seems to be a beneficial immunomodulatory approach in the treatment of septic shock.

Acknowledgments
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References


