

Immunologic and Hemodynamic Effects of “Low-Dose” Hydrocortisone in Septic Shock

A Double-Blind, Randomized, Placebo-controlled, Crossover Study

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Within the last few years, increasing evidence of relative adrenal insufficiency in septic shock evoked a reassessment of hydrocortisone therapy. To evaluate the effects of hydrocortisone on the balance between proinflammatory and antiinflammation, 40 patients with septic shock were randomized in a double-blind crossover study to receive either the first 100 mg of hydrocortisone as a loading dose and 10 mg per hour until Day 3 ($n = 20$) or placebo ($n = 20$), followed by the opposite medication until Day 6. Hydrocortisone infusion induced an increase of mean arterial pressure, systemic vascular resistance, and a decline of heart rate, cardiac index, and norepinephrine requirement. A reduction of plasma nitrite/nitrate indicated inhibition of nitric oxide formation and correlated with a reduction of vasopressor support. The inflammatory response (interleukin-6 and interleukin-8), endothelial (soluble E-selectin) and neutrophil activation (expression of CD11b, CD64), and antiinflammatory response (soluble tumor necrosis factor receptors I and II and interleukin-10) were attenuated. In peripheral blood monocytes, human leukocyte antigen-DR expression was only slightly depressed, whereas *in vitro* phagocytosis and the monocyte-activating cytokine interleukin-12 increased. Hydrocortisone withdrawal induced hemodynamic and immunologic rebound effects. In conclusion, hydrocortisone therapy restored hemodynamic stability and differentially modulated the immunologic response to stress in a way of antiinflammation rather than immunosuppression.

Keywords: sepsis; glucocorticoids; immune system

During sepsis, the systemic inflammatory response comprises reciprocal communication between the neuroendocrine and the peripheral immune system (1). Proinflammatory mediators recruit the hypothalamic-pituitary-adrenal axis to counterregulate inflammation through the synthesis of the stress hormone cortisol. The fundamental roles of glucocorticoids in stress response to infection and increasing knowledge of the antiinflammatory and immunosuppressive pharmacodynamic profile have been the rationale for its use in sepsis trials for decades. Timing, dosage, and duration of glucocorticoid administration were adapted to different disease pathophysiologic models and probably had a major impact on outcome (2). Several randomized controlled trials unequivocally re-

vealed that short-time (1 to 2 days) administration of high doses of glucocorticoids (up to 40 g of hydrocortisone equivalent per day) in early septic shock was without effect on outcome or was even harmful—most probably because of immunosuppression and increased incidence of secondary infections (3, 4). Only one study showed an initial improvement of survival and shock reversal with high-dose methylprednisolone, but with ongoing disease, the differences were no longer significant (5).

In contrast to these former approaches, recent randomized controlled trials indicate that prolonged (5 days or more) administration of “low” doses (compared with the doses above) of hydrocortisone (240–300 mg per day) in early or late septic shock improves shock reversal (6, 7) and outcome (8, 9). These results are in agreement with the concept of impaired adrenocortical reserve in septic shock (2, 10–13). However, the diagnosis and predictive value of relative adrenal insufficiency in septic shock is still a matter of current discussions (14). Although it was reported that shock reversal and outcome were independent from adrenal reserve (8), other reports indicate that the degree of adrenal dysfunction correlates with outcome (15) and that hydrocortisone therapy reduces mortality only in patients with impaired adrenal reserve (9).

Overall, the encouraging results of the recent low-dose hydrocortisone trials evoked a reassessment of the role of glucocorticoids in septic shock. It is well established that glucocorticoids modulate the stress response to sepsis dose dependently through permissive (e.g., enhancing the cardiovascular response to vasopressors) and suppressive (e.g., inhibition of cytokine synthesis) effects (16). However, there is little known about the immunologic effects of continuously infused low doses of hydrocortisone in septic shock. In particular, protection from overshooting inflammatory response has to be weighted against the risk of aggravated immunosuppression, which gained increasing importance in understanding of sepsis pathophysiology and multiple organ failure (17). Because low doses of glucocorticoids are increasingly used as an adjunctive therapy to stabilize blood pressure in septic shock patients, knowledge of immune reactions is of clinical importance to elucidate possible risks accompanied with this therapeutic approach. Therefore, we investigated the effects of a 3-day hydrocortisone treatment on hemodynamic and immunologic parameters, comparing the response with the application and the withdrawal of hydrocortisone in a two-period crossover design.

METHODS

The study protocol was approved by the institutional ethics committee. Patients who met these criteria were found to be eligible for enrollment: (1) written informed consent from the next of kin; (2) the presence of septic shock (18), including (a) proven or strongly suspected infection,

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(b) three or more of these conditions: mechanical ventilation, heart rate of more than 90 beats per minute, temperature of more than 38°C or less than 36°C, a white blood cell count of more than 12,000 cells/ μ l or less than 4,000 cells/ μ l, or more than 10% immature cells, and (c) sepsis-induced hypotension (systolic blood pressure of less than 90 mm Hg or a reduction of more than 40 mm Hg from baseline in the absence of other causes of hypotension); and (3) patients requiring norepinephrine to maintain a mean arterial pressure of more than 70 mm Hg despite adequate fluid resuscitation. An age of less than 18 years, glucocorticoid medication within the last 3 months, immunosuppressive therapy, hematologic diseases, pregnancy, and a moribund state were exclusion criteria.

Eligible patients were randomized by the pharmacist into two groups: one group (hydrocortisone group 1 [HC-1]) first received 100 mg of a hydrocortisone loading dose over 30 minutes, followed by 10 mg/hour until Day 3 and isotonic saline from Days 4 to 6; the other group (HC-2) first received the placebo and then hydrocortisone. Treatment of septic shock patients according to standard regimes of the intensive care unit was not affected by the study protocol (*see* the online supplement for additional information). Norepinephrine therapy was tapered individually with ongoing hemodynamic stabilization, keeping the mean arterial pressure more than 70 mm Hg. Patients continued to receive standard treatment after Day 6, and in case of ongoing hemodynamic instability, hydrocortisone was administered according to the physician's decision.

Measurements

The average of triple cardiac output measurements (thermodilution technique) was recorded together with norepinephrine requirement at the time of hemodynamic investigation. Mean arterial pressure heart rate, central venous pressure, pulmonary capillary wedge pressure, and mean pulmonary arterial pressure were recorded from online monitoring devices; systemic and pulmonary vascular resistance and cardiac index were calculated with standard formulae.

Cortisol was measured with radioimmunoassay. Interleukin (IL)-4, IL-6, IL-8, IL-10, IL-12 p70, and interferon- γ , soluble E-selectin, and soluble tumor necrosis factor receptors I and II were measured with ELISA, and nitrite/nitrate (NOx) was measured with Griess reaction. Leukocyte differentiation, expression of human leukocyte antigen (HLA)-DR on monocytes and of CD11b and CD64 on granulocytes, and *in vitro* phagocytosis and respiratory burst of monocytes and granulocytes were measured with flow cytometry (*see* the online supplement for additional information). Blood from 10 healthy individuals was used for control subjects. For assessment of the Simplified Acute Physiology Score II (19) and the Sepsis-related Organ Failure Assessment (20), daily recorded data were analyzed.

Statistical Analysis

Statistical procedures are based on recommendations for analysis of crossover trials (21) and included analyses of hydrocortisone-dependent and -independent effects (*see* Figure 1). Within-group changes of variables for the hydrocortisone early (Days 0–3), hydrocortisone late (Days 3–6), placebo early (Days 0–3), and placebo late (Days 3–6) periods were analyzed with Friedman repeated-measures analysis of variance on ranks, when indicated. Nominal data proportions were compared with Fisher's exact test. Spearman rank correlation coefficient was calculated to quantify correlations. The false discovery rate procedure (22) revealed a corrected p value of less than 0.02 to be significant for multiple comparisons.

RESULTS

Fifty-nine patients with septic shock were consecutively examined for eligibility between March 1997 and September 2000. Forty patients were enrolled in the study, and 19 patients were not included (13 with steroid history, 1 with hematologic disease, 2 without consent, 1 with extracorporeal membrane oxygenation, and 2 moribund patients). Patients were included within 48 hours after the onset of septic shock or as soon as possible after referral from tertiary hospitals.

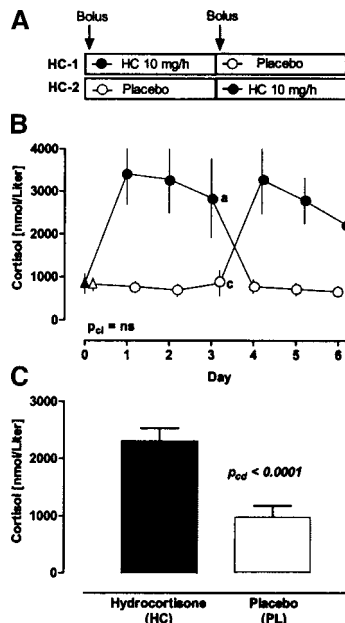


Figure 1. (A–C) Study design and plasma cortisol concentration. (A) Crossover study design. Patients received either 100 mg of HC or placebo as a bolus immediately after obtaining baseline values (*closed triangle, open triangle in B*) on Day 0 and Day 3 after an infusion of HC (*closed circle*) or placebo (*open circle*). (B) HC infusion induced a significant hypercortisolemia, which completely reversed after HC withdrawal within 24 hours. Values are means and 95% confidence interval. The possibility of a carryover period or other treatment effect was assessed by comparing the means of the differences at the end of each period between groups HC-1 and HC-2 (*a minus d* versus *b minus c*) with Mann-

Whitney U signed rank sum test, with a cortisol-independent p value (p_c) below 0.02 indicating significance. (C) The treatment effect of HC (individual crossover difference between HC and placebo) was analyzed by comparing the means at the end of each period (*a* versus *d* and *b* versus *c*) with Wilcoxon-matched paired signed rank test, with a "cortisol-dependent" p value (P_{cd}) of less than 0.02 indicating significance (*bar chart, mean + SEM*). To convert values for cortisol to μ g/dl, divide by 27.5.

Baseline Characteristics

No significant differences between HC-1 and HC-2 were evident at the time of inclusion for age, sex, Simplified Acute Physiology Score II, Sepsis-related Organ Failure Assessment, time between the onset of septic shock and inclusion, the diagnosis of underlying diseases, the source of infection, and bacteriologic results (Table 1). None of the patients died within the observational period of 6 days. Intensive care unit and hospital mortality were 30% in both groups.

HC Infusion Increased Plasma Cortisol Concentration in All Patients

Baseline plasma cortisol was not significantly different between groups, for HC-1 844 (mean) nmol/l (95% confidence interval, 614, 1,074) and for HC-2 822 nmol/l (670, 974). Two patients in the HC-2 group had very low cortisol baseline values; in one of these, cortisol increased spontaneously from 218 to 351 nmol/l until hydrocortisone treatment; in the other patient, values ranged between 209 and 222 nmol/l until hydrocortisone infusion. Both patients finally survived.

Hydrocortisone treatment increased plasma cortisol levels in all patients of both the HC-1 group and the HC-2 group by about factor 5 (1.6- to 15-fold). Peak levels were reached within 1 day after the start of hydrocortisone infusion (means of 3,500 and 3,200 nmol/l in HC-1 and HC-2, respectively). The individually different increase in plasma cortisol during hydrocortisone treatment was not related to the distinct groups, baseline cortisol, or other patient characteristics. Hydrocortisone withdrawal in HC-1 induced a complete reversal of hypercortisolemia to baseline level within 1 day (Figure 1).

TABLE 1. PATIENT CHARACTERISTICS ON INCLUSION

	HC-1 (n = 20)	HC-2 (n = 20)	p
Age, years (mean, 95% CI)	54 (46, 63)	50 (42, 58)	0.37
Male sex	13	13	
SAPS II	42 (35, 49)	42 (36, 48)	0.96
SOFA	9.7 (8.5, 10.9)	10.4 (9.1, 11.7)	0.42
Nonsurvivors (ICU mortality)	6	6	
Time between onset of septic shock and inclusion, hours			
< 24	4	4	
24-48	7	10	0.52
48-120	6	4	0.71
> 120	3	2	
Underlying diseases (patients may have more than one disease)			
Trauma	6	5	
Pneumonia	14	16	
ARDS	8	10	
Gastrointestinal disease	10	8	
Other	1	1	
Main source of infection			
Pulmonary	12	13	
Gastrointestinal	8	6	
Wound	—	1	
Microbiology			
Gram positive	3	5	0.69
Gram negative	10	5	0.19
Mixed	3	3	
Fungal	1	—	
Not identified	3	7	0.27

Definition of abbreviations: ARDS = acute respiratory distress syndrome; ICU = intensive care unit; SAPS II = simplified acute physiology score; SOFA = sepsis-related organ failure assessment.

Hydrocortisone Improved Hemodynamic Variables and Reduced Plasma NOx

The increase in plasma cortisol was accompanied by a significant systemic hemodynamic stabilization, evidenced by an increase of mean arterial pressure and systemic vascular resistance and a concomitant reduction of heart rate and cardiac index. These hemodynamic effects were significantly more pronounced in patients who received hydrocortisone, although norepinephrine requirement in these patients declined. Withdrawal of hydrocortisone on Day 3 had opposite effects on hemodynamic parameters in HC-1. Mean pulmonary arterial pressure, pulmonary vascular resistance, pulmonary capillary wedge pressure, and central venous pressure were not affected by hydrocortisone treatment (Table 2). On Day 3, in HC-1 and HC-2, 14 of 20 and 6 of 20 patients, respectively, were without norepinephrine (p = 0.025);

on Day 6, in HC-1 and HC-2, 12 of 20 and 17 of 20 patients, respectively, were weaned from norepinephrine (p = 0.15). In HC-1, 6 of 20 (30%) patients required norepinephrine again after glucocorticoid withdrawal.

Plasma NOx concentrations were reduced during both hydrocortisone treatment periods and remained attenuated after hydrocortisone cessation in HC-1, whereas placebo had no effect on NOx in HC-2. Because NOx remained low in HC-1 after drug withdrawal, a carryover effect was considered, and an additional within-period analysis of variance (Friedman) was performed, yielding significance only during hydrocortisone treatment (Figure 2). At the end of a hydrocortisone-treatment period, patients not requiring norepinephrine had significant lower plasma NOx than patients who required any dose of norepinephrine (25 [27, 42] μmol/l versus 64 [39, 90]; p = 0.015). Plasma NOx concentra-

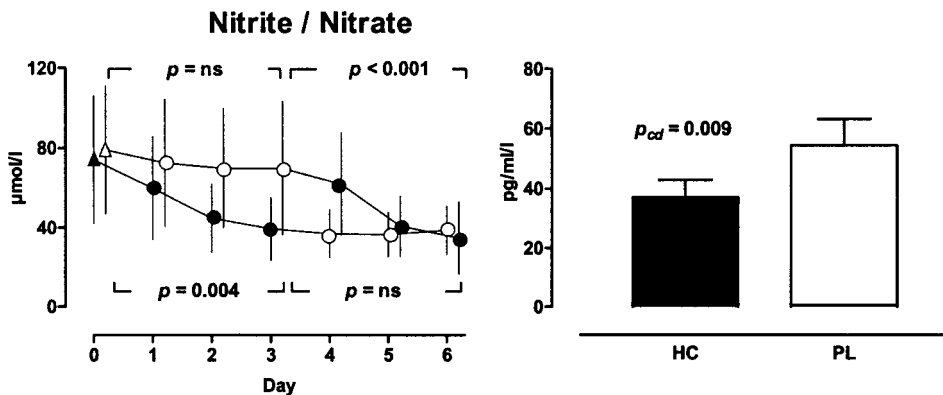


Figure 2. NOx plasma concentration. HC administration, but not placebo, reduced the NOx plasma concentration, indicating inhibition of nitric oxide formation. Note that NOx remained unchanged after cessation of HC infusion. On Day 3, NOx was significantly different between HC-1 and HC-2 (p < 0.02, Mann-Whitney-U test). (Line chart) p values of different treatment periods represent results of variance analysis (Friedman test). (Bar chart) Compared with placebo, a 3-day exposure to hydrocortisone reduced NOx to approximately 32% (p = 0.009). Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

TABLE 2. HEMODYNAMIC PARAMETERS

Parameter	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	P _{cd}	P _{ci}
MAP, mm Hg									
HC-1	77 (72, 83)	87 (82, 91)	92 (86, 96)	91 (86, 95)	83 (79, 87)	80 (75, 85)	81 (77, 84)	0.0003	0.24
HC-2	81 (76, 87)	81 (77, 85)	80 (77, 84)	82 (79, 84)	87 (84, 92)	88 (84, 93)	89 (83, 95)		
HR, beats/min									
HC-1	111 (106, 116)	100 (93, 107)	93 (86, 100)	91 (86, 96)	92 (85, 98)	102 (93, 112)	105 (96, 114)	< 0.0001	0.22
HC-2	108 (99, 117)	111 (104, 118)	105 (99, 111)	105 (98, 112)	92 (85, 100)	86 (79, 94)	85 (79, 92)		
SVR, dyn × sec × cm ⁻⁵									
HC-1	647 (514, 780)	743 (621, 864)	823 (696, 967)	884 (692, 1076)	824 (689, 957)	687 (553, 821)	634 (529, 739)	< 0.0001	0.072
HC-2	638 (575, 701)	638 (575, 701)	830 (696, 964)	648 (582, 715)	728 (637, 820)	776 (679, 827)	810 (671, 949)		
CI, L/min/m ²									
HC-1	4.2 (4.0, 5.0)	4.5 (4.0, 5.0)	4.3 (3.9, 4.7)	4.1 (3.6, 4.6)	3.9 (3.5, 4.3)	4.4 (4.0, 4.9)	4.9 (4.2, 5.5)	0.0068	0.96
HC-2	4.7 (4.2, 5.1)	4.7 (4.2, 5.2)	4.5 (4.1, 4.9)	4.6 (4.3, 4.9)	4.4 (4.1, 4.7)	5.4 (4.0, 4.8)	4.3 (3.8, 4.7)		
PCWP, mm Hg									
HC-1	14 (12, 15)	13 (12, 15)	14 (12, 15)	14 (13, 15)	13 (12, 15)	13 (12, 15)	13 (11, 15)	0.18	0.06
HC-2	14 (12, 15)	13 (11, 14)	11 (10, 13)	13 (12, 14)	14 (13, 15)	13 (12, 14)	13 (12, 15)		
PVR, dyn × sec × cm ⁻⁵									
HC-1	155 (125, 185)	176 (146, 207)	171 (145, 198)	160 (123, 198)	158 (119, 197)	170 (127, 213)	156 (124, 189)	0.50	0.01
HC-2	178 (148, 209)	174 (140, 208)	188 (163, 213)	178 (148, 207)	175 (145, 205)	159 (124, 193)	156 (124, 188)		
CVP, mm Hg									
HC-1	12 (11, 13)	13 (11, 14)	12 (11, 14)	12 (11, 14)	11 (10, 12)	12 (10, 13)	12 (10, 13)	0.60	0.07
HC-2	12 (10, 14)	12 (11, 13)	12 (11, 13)	12 (11, 14)	13 (12, 14)	11 (10, 12)	11 (10, 13)		
MPAP, mm Hg									
HC-1	30 (28, 32)	31 (28, 33)	30 (28, 33)	29 (26, 31)	27 (24, 29)	29 (27, 32)	29 (27, 32)	0.20	0.07
HC-2	33 (29, 36)	31 (28, 34)	30 (28, 33)	32 (30, 34)	32 (29, 34)	28 (26, 31)	28 (26, 31)		
Norepinephrine, µg/kg/min									
HC-1	0.21 (0.10, 0.32)	0.24 (0.0, 0.49)	0.08 (0.0, 0.16)	0.02 (0.0, 0.04)	0.06 (0.0, 0.11)	0.06 (0.01, 0.10)	0.06 (0.01, 0.10)	< 0.0001	0.06
HC-2	0.25 (0.11, 0.39)	0.25 (0.1, 0.42)	0.23 (0.09, 0.37)	0.18 (0.05, 0.30)	0.17 (0.0, 0.35)	0.06 (0.0, 0.15)	0.03 (0.0, 0.07)		

Definition of abbreviations: CI = cardiac index; CVP = central venous pressure; HC-1/2 = hydrocortisone treatment day 1–3/4–6; HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; P_{cd} = cortisol dependent p value; P_{ci} = cortisol independent p value; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

Values are means and 95% confidence interval; “cortisol dependent” p values (P_{cd}) < 0.02 indicate effects of HC administration. “Cortisol independent” p values (P_{ci}) < 0.02 indicate HC-independent effects.

Mean confidence interval (95% confidence interval). For further details, see METHODS section.

tions correlated with the norepinephrine requirement ($r = 0.34$ [0.25, 0.44], $p < 0.001$). This effect was more pronounced during hydrocortisone infusion ($r = 0.54$ [0.39, 0.66], $p < 0.0001$) than during the placebo period or at baseline ($r = 0.17$ [0.01, 0.32], $p = 0.027$). Correlations between NOx and any other hemodynamic variable were not significant.

Hydrocortisone Downregulated Systemic Inflammatory Immune Response but Did Not Inhibit the Th1-related Response

Hydrocortisone infusion differentially modulated the release of inflammatory mediators and expression of cell adhesion molecules. IL-6 and the chemoattractant cytokine IL-8 were both significantly reduced during hydrocortisone treatment (Figure 3). Almost 24 hours after hydrocortisone initiation, concentrations

of both cytokines were significantly lower in HC-1 than in HC-2 ($p < 0.01$). The cell surface expression of CD11b and of CD64 on peripheral granulocytes (Figure 4) was downregulated, similar to the amount of soluble adhesion molecule soluble E-selectin (Figure 5), indicating attenuation of granulocyte–endothelial interactions. In contrast to these inhibitory effects, concentrations of IL-12 p70 and interferon- γ were not suppressed but were rather increased. This was more pronounced for IL-12 than for interferon- γ . The measured concentrations of IL-12 p70 and interferon- γ were within or above the range of values obtained from healthy individuals, suggesting a normal Th1 response (Figure 6). IL-4 concentrations, as a marker of Th2 response, remained unchanged during hydrocortisone infusion. Hydrocortisone withdrawal induced a significant reincrease of IL-6 and IL-8, soluble E-selectin, CD11b, and CD64 (Friedman test).

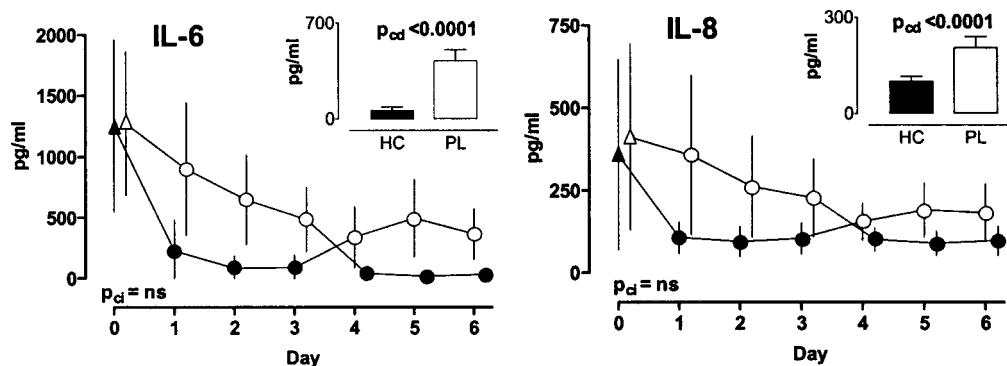


Figure 3. IL-6 and IL-8 plasma concentration. HC infusion reduced IL-6 and IL-8 plasma concentrations significantly, which was reversed to a level comparable to that of control subjects in HC-1 when HC therapy was stopped on Day 3. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

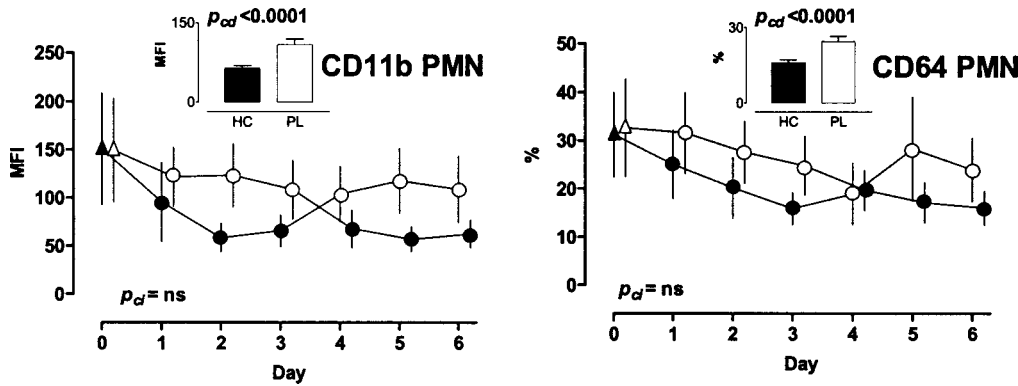


Figure 4. CD11b and CD64 expression on circulating neutrophils. The geometric mean fluorescence intensity (MFI) of CD11b and CD64 expression as a percentage of circulating granulocytes was significantly depressed during hydrocortisone infusion. The inhibitory effect was reversible after hydrocortisone withdrawal. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

Hydrocortisone Inhibited the Antiinflammatory Immune Response

Hydrocortisone infusion modulated release of mediators and expression of receptors, which are associated with an antiinflammatory immune response. IL-10 and soluble tumor necrosis factor receptors I and II were significantly reduced. Again, hydrocortisone withdrawal had opposite effects and induced a significant increase of IL-10 and tumor necrosis factor receptors ($p < 0.01$, Friedman test) (Figure 7).

Hydrocortisone Did Not Induce Severe Monocyte/Granulocyte Dysfunction

The HLA-DR expression on monocytes is essential for their antigen-presenting activity and is a sensitive marker of monocyte function. Baseline HLA-DR antigen expression, quantified as a percentage of positive monocytes, was significantly depressed compared with values of healthy individuals (more than 85%) but did not reach the level of “immunoparalysis” (less than 30%). Most importantly, the levels were not further reduced within 3 days of hydrocortisone treatment (65 [58, 72] versus 63% [57, 69], 95% CI). However, the more sensitive HLA-DR antigen density analysis expressed as geometric mean fluorescence intensity revealed a marginal loss of receptor density within the first 2 days of treatment. On the other hand, mean fluorescence exceeded control values after HC withdrawal in HC-1 group, thus resulting in an overall significant difference between the HC and placebo phases (Figure 8).

Monocyte phagocytosis *ex vivo* was enhanced during hydrocortisone treatment, whereas granulocyte phagocytosis was slightly depressed (2%). Granulocyte respiratory burst was not affected by hydrocortisone treatment (*see* Table E1 in the online supplement).

Hydrocortisone Showed Marginal Effects on Leukocyte Counts

A 3-day exposure to hydrocortisone induced a significant increase of circulating monocytes (750 [590, 911]/ μl versus 548 [406, 691]/ μl ; $p = 0.008$) and a significant decrease of circulating lymphocytes (663 [553, 773]/ μl versus 830 [702, 958]/ μl ; $p = 0.006$) and had no effect on the numbers of circulating granulocytes (10,043 [8,300, 11,780]/ μl versus 11,090 [9,186, 12,995]/ μl ; $p = 0.28$).

DISCUSSION

The purpose of this study was to investigate the effects of a low-dose continuous hydrocortisone infusion on hemodynamic and immunologic variables in patients with septic shock and to determine whether this therapeutic approach bears the risk of severe immunosuppression. Our data demonstrated that low-dose hydrocortisone treatment (1) improved hemodynamic parameters, (2) inhibited systemic inflammation and prevented overwhelming compensatory antiinflammatory response, and (3) maintained Th1-related immune responsiveness.

The presented data are in accordance with other results of hydrocortisone-induced hemodynamic improvement and reduction of vasopressor support in septic shock (6–9, 23–26). Hydrocortisone treatment induced significant changes of the systemic circulation without affecting pulmonary vascular resistance or cardiac filling pressures. The increase of mean arterial pressure was accompanied by a significant reduction of norepinephrine requirement, an increase of systemic vascular resistance, and a reduction of cardiac index and heart rate, implying effects predominantly on vascular tone of resistance vessels. Most patients could be weaned from vasopressor therapy within 2–3 days. However, a significant but less impressive reduction of norepinephrine was also observed with placebo, indicating an improvement of the clinical course independent from hydrocortisone therapy. Overall, shock reversal in hydrocortisone-treated patients was faster than in the placebo group, as reported by others (6, 8). Although HC therapy was shown to reverse septic shock independently from adrenal functional reserve (8), other trials indicate that patients with impaired response to corticotropin are more susceptible to cortisol replacement (26, 27). In this study, abrupt drug withdrawal reinduced norepinephrine dependency in 30% of patients previously weaned from vasopressors. Because we did not perform adrenal function tests, it remains speculative whether shock after hydrocortisone withdrawal correlated with adrenal reserve.

In septic shock, overwhelming production of nitric oxide mediates peripheral vasodilation, catecholamine resistance, tissue hypoxia, and cardiomyopathy (28–30). In our patients, increased

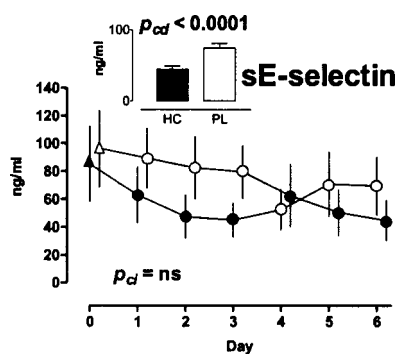


Figure 5. Plasma levels of soluble adhesion molecules E-selectin. Hydrocortisone therapy significantly reduced shedding of E-selectin, indicating an attenuation of endothelial/leukocyte interactions. HC cessation had opposite effects. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

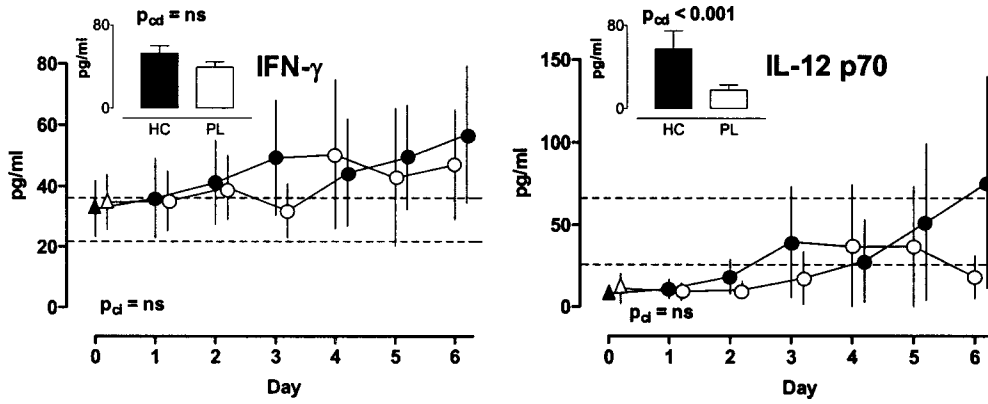


Figure 6. Proinflammatory mediators interferon- γ (IFN- γ) and IL-12 p70. HC administration did not suppress IFN- γ and IL-12 p70, and plasma concentrations rather increased during application of HC. The dashed lines indicate a 95% confidence interval of the mean of control values obtained from healthy individuals. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

plasma NOx formation as the main breakdown product of cytokine-induced nitric oxide metabolism was found to correlate with shock severity, organ failure, and outcome (31–34). However, experimental sepsis models and a recent phase III study with L-arginine analogue N^G-monomethyl-L-arginine strongly indicate that excessive and nonspecific blocking of nitric oxide synthases (NOSs) is fatal, stressing the importance of cytoprotective and regulative properties of maintained basal nitric oxide production (35–37). Therefore, selective inhibition of inducible NOS might be more effective and targets the “pathologic” nitric oxide while leaving the “physiologic” nitric oxide unaffected (35). In contrast to nonspecific competitive inhibitors of nitric oxide synthesis (L-arginine analogues, isothioureas), glucocorticoids inhibit inducible NOS but not the constitutively expressed endothelial NOS (38, 39). Inhibition of nitric oxide formation by glucocorticoids was demonstrated *in vitro* at different levels: transcription, translation, substrate, or enzyme cofactor availability and calpain-induced inducible NOS degradation (40–43). The presented data give evidence that inhibition of nitric oxide formation contributed to hemodynamic improvement, al-

though we were unable to find direct correlations between plasma NOx concentrations and hemodynamic variables. This discrepancy might be explained by the study design determining a stable blood pressure by titrating norepinephrine dosage. Indeed, plasma NOx correlated significantly only with norepinephrine requirement, an effect that was even more pronounced during hydrocortisone treatment, indicating that inhibition of nitric oxide formation increased vascular tone. Furthermore, hydrocortisone treatment seemed to maintain basal nitric oxide synthesis, as NOx concentrations were reduced from 70–80 to 30–40 μ M, which is in agreement with reported levels in nonseptic control subjects in the order of 30–40 and 60–100 μ M in patients with severe septic shock (35). However, the discrepancy between sustained NOx suppression and hemodynamic deterioration in some patients after hydrocortisone cessation emphasizes nitric oxide-independent rebound mechanisms. In fact, the physiologic role of glucocorticoids in regulation of systemic blood pressure includes numerous mechanisms of action: signal transduction, prostaglandin metabolism, sodium and calcium transport, and modulation of adrenoreceptors, angiotensin re-

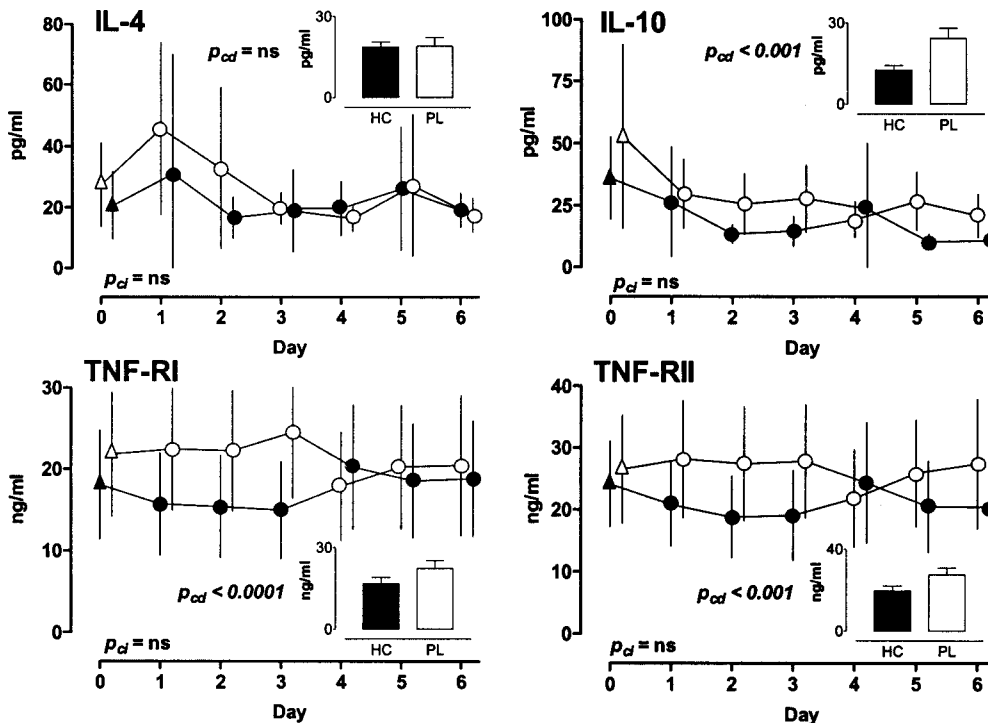


Figure 7. Effects of hydrocortisone on antiinflammatory mediators. HC significantly attenuated release of IL-10 and reduced concentrations of both soluble tumor necrosis factor (TNF) receptors I and II, whereas HC had no effect on IL-4. The inhibitory effects of HC were reversed with drug cessation. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

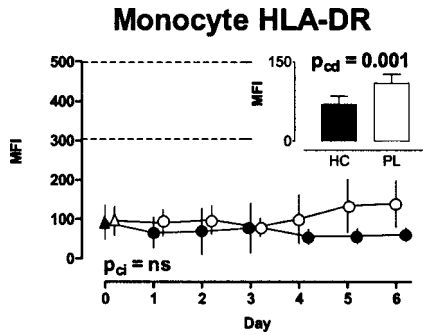


Figure 8. HLA-DR expression on monocytes. Monocyte HLA-DR geometric mean fluorescence intensity (MFI) was depressed. However, the significant difference was mainly due to increased HLA-DR expression after cessation of therapy. The dashed lines indicate means and 95% confidence interval of control values obtained from healthy individuals. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

ence interval of control values obtained from healthy individuals. Triangles = baseline values; closed circles = hydrocortisone; open circles = placebo. For further explanation see Figure 1.

ceptors, endothelin receptors, and mineralocorticoid receptors (44, 45). Therefore, the impact of hydrocortisone on vascular tone in septic shock needs further investigation. Taken together, inhibition of inducible NOS by a short-time treatment with hydrocortisone contributed to hemodynamic stabilization, and this approach might be as effective, less expensive, and perhaps safer than the use of competitive NOS inhibitors.

Because glucocorticoids affect the immune system at different levels, possibly influencing the balance between inflammation and antiinflammation, we measured several parameters representing different immune responses.

Overall, the inflammatory response was attenuated by hydrocortisone. The amount of inflammatory markers such as IL-6 and IL-8, which were found to correlate with disease severity and worse outcome (46–50), and soluble E-selectin, reflecting endothelial activation (51–53), was significantly suppressed. Furthermore, circulating neutrophils expressed less markers of activation (complement receptor 3 [CD11b/CD18] and high-affinity Fc γ -receptor I [CD64]) (54, 55) without a reduction of cell-bound L-selectin (data not shown), which can be induced by glucocorticoids impairing cell recruitment at the site of inflammation (56–58). Importantly, *in vitro* granulocyte function (respiratory burst and phagocytosis) remained intact, indicating that low-dose hydrocortisone did not suppress innate defense mechanisms. Our results are in agreement with recent reports of hydrocortisone-induced attenuated systemic inflammatory response, evidenced by reduced phospholipase A₂, neutrophil elastase, C-reactive protein, and IL-6 plasma concentrations (25, 59).

Overall, hydrocortisone did not induce immunosuppression. Several markers such as IL-10 and soluble tumor necrosis factor receptors decreased during hydrocortisone therapy. Moreover, the inhibition of IL-6 synthesis attenuated the antiinflammatory response, as this cytokine is increasingly recognized as an antiinflammatory mediator (60). A switch from Th1 to Th2 cells and overproduction of antiinflammatory cytokines promote the risk of infection and may worsen outcome (61–63). There is some evidence that low-dose hydrocortisone did not promote proliferation of antiinflammatory T cell subsets. The amount of Th2-derived cytokines did not change (IL-4) or decrease (IL-10), and interferon- γ , which is released by Th1 and suppressor cells, was not suppressed. Recently, it could be shown that a switch of suppressor cell subsets with increased release of IL-4 and decreased production of IFN- γ is often found in nonsurvivors after burns (64). Furthermore, in concanavalin A-stimulated whole-blood samples, analyzed for release of IL-4 and IL-10, both antiinflammatory cytokines rather decreased during hydrocortisone infusion. Finally, IL-12, a central regulatory cytokine directing Th1 development and monocyte activation, increased

during hydrocortisone treatment. This stands in contrast to dose-dependent *in vitro* inhibition of IL-12 synthesis by glucocorticoids in nonseptic conditions (65–67). Dose-dependent promoting properties of glucocorticoids in regulation of host resistance, receptor regulation, and cytokine synthesis are currently debated (68). Therefore, further studies are needed to define the impact of hydrocortisone on proliferation of T cell subsets and cytokine release in septic shock.

Monocytes are crucially involved in adaptive immune responses. The reduced capability of monocytes to present antigens to T cells due to reduction of surface HLA-DR promotes risk of infection and may worsen outcome (69–72). Recent observational studies indicate that interferon- γ treatment may be advantageous in immunoparalyzed septic shock patients (73). In our study, *in vitro* monocyte phagocytosis was not impaired during hydrocortisone treatment. However, hydrocortisone further depressed HLA-DR expression, which was already low in patients compared with healthy control subjects. The hydrocortisone-induced receptor downregulation was transient and limited, followed by a rebound increase of receptor expression immediately after drug withdrawal. Hence, adverse effects have to be considered in severely immunodepressed patients. In early septic shock when inflammation often predominates, hydrocortisone might be safer than in prolonged septic shock with increasing incidence of immunosuppression.

Most of the observed hemodynamic and immune effects were followed by a rebound effect when hydrocortisone was withdrawn. It is therefore recommended that patients be weaned from hydrocortisone over several days to avoid adverse effects.

It may be considered that constant hydrocortisone doses administered in this study had probably not met individual needs and that it could be advantageous to titrate hydrocortisone therapy more individually. Furthermore, our data are only representative for a 3-day observational period of hydrocortisone infusion, and prolonged treatment might have different effects on the immune system. Nevertheless, most patients could be weaned from vasopressors in early septic shock within a few days, which might allow a reduction of the dose, thus minimizing the risk of possible adverse effects with prolonged treatment.

Subgroup analyses revealed no significant difference of hemodynamic or immunologic parameters after 3 days of hydrocortisone therapy between 11 patients who received hydrocortisone in early septic shock (less than 48 hours) compared with 29 patients who were treated in late septic shock (more than 48 hours).

Whether the distinct effects of hydrocortisone on the immunologic profile, demonstrated in this study, finally result in a better outcome could not be answered because measurements were performed within a 6-day window using a crossover design. Furthermore, a correlation with a relative adrenal insufficiency was not tested. In this context, the impact of low-dose hydrocortisone application in patients with appropriate adrenal function would be of special interest, possibly elucidating effects beyond restoration of cortisol deficiency. Therefore, further randomized clinical trials with an intent-to-treat design have to be performed to answer these questions.

In conclusion, low-dose hydrocortisone treatment in septic shock rapidly induced hemodynamic stabilization, probably by a reduction of nitric oxide formation. Both inflammatory and antiinflammatory responses were attenuated, whereas the innate cell function of granulocytes and monocytes was preserved. Although hydrocortisone did not aggravate immunosuppression, downregulation of HLA-DR receptors on monocytes has to be considered in severely immunodepressed patients. To avoid rebound effects, low-dose hydrocortisone has to be tapered over days.

We postulate that modulation of the complex immune network in a more widely ranging fashion by the use of low doses

of hydrocortisone may be advantageous compared with the inhibition of single mediators, possibly provoking microenvironmental imbalances.

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