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BRIEF REPORT

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Stress doses of hydrocortisone in septic shock: beneficial effects on opsonization-dependent neutrophil functions

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Abstract *Objective:* To assess the effects of stress doses of hydrocortisone (HC) on clinical parameters and neutrophil functions in patients with septic shock. *Design:* Prospective, double-blind, randomized, placebo-controlled study. *Setting:* Intensive care units of a university hospital. *Patients and participants:* 30 adult patients with septic shock. *Interventions:* Patients were allocated to receive either HC (intravenous bolus of 100 mg preceding a continuous infusion 10 mg/h, $n = 15$) or placebo ($n = 15$), respectively. The effects of HC were assessed at baseline and after 24 h. *Measurements and results:* As compared with placebo-treated patients, administration of HC significantly decreased norepinephrine requirements (from 1.5 to 0.8 mg/h; $p < 0.001$), interleukin-6 serum concentrations (from 388.8 to 88.8 pg/ml; $p < 0.02$), and the spontaneous release of hydrogen peroxide (H_2O_2) by neutrophils (–33.0%; $p < 0.05$). Additionally, HC treatment preserved the autologous plasma-induced amplification of phagocytosis of zymosan particles [factor of opsonin-induced amplification of phagocytosis of unopsonized particles: 1.80 for placebo vs. 1.75 for HC at baseline (not significant between groups) and 0.50 for placebo vs. 1.75 for HC after 24 h of treatment ($p < 0.05$)]. These effects were paralleled by respective changes in the phagocytosis-associated H_2O_2 production. *Conclusions:* In patients with septic shock stress doses of HC exert beneficial effects in terms of improvements in hemodynamics, decrease in pro-inflammatory mediators, and oxidative stress without the compromise of opsonization-dependent phagocytic neutrophil functions; thus, HC treatment does not aggravate non-specific immunosuppression but instead improves innate immunity in the early stage of septic shock.

Keywords Sepsis · Hydrocortisone · Innate immunity · Neutrophil · Opsonization

Introduction

Whereas the use of high-dose corticosteroids in sepsis is known to cause immunosuppression [1], administration of stress doses of hydrocortisone (HC) has been shown to improve survival without an increase in superinfections [2]. While hemodynamic improvements are likely due to both an attenuation of the systemic cytokine response and catecholamine-permissive actions [3], effects of stress doses of HC on the non-specific part of the immune system have not been fully explored in sepsis to date.

The cellular effector arm of the non-specific immunity is represented mostly by neutrophils. These cells constitute the first line of defense in the protection of the host against invading microorganisms, and they

are also needed for removal of cellular debris during reparative processes; thus, neutrophils are armed with highly efficient bactericidal mechanisms which, however, may also cause collateral tissue damage. While under physiological conditions both microbicidal and tissue toxic effector functions of neutrophils are well balanced, neutrophils might become dysregulated during sepsis [4]. In fact, we recently demonstrated in septic patients an enhanced release of tissue-damaging oxygen radicals from circulating neutrophils, while bactericidal effector functions decreased.

In this study we set out to characterize the effects of stress doses of HC on (a) clinical parameters, (b) potentially tissue-toxic spontaneous production of hydrogen peroxide (H_2O_2, and (c) plasma opsonin-independent and plasma opsonin-dependent phagocytic functions of blood neutrophils.

Materials and methods

Patients

Patients admitted to the intensive care unit (ICU) were prospectively enrolled in this double-blind, randomized, placebo-controlled trial if they met the criteria for septic shock [5]. Thirty patients were randomized in a blinded fashion by an ICU physician not involved in the study to receive either HC (intravenous bolus of 100 mg followed by a continuous infusion of 10 mg/h) or placebo, respectively. Severity of illness at baseline and after 24 h was assessed by the Acute Physiology and Chronic Health Evaluation (APACHE) scoring system II and III, multiorgan dysfunction score (MODS), and Sepsis-related Organ Failure Assessment (SOFA). Clinical parameters and blood values were determined at baseline and 24 h after admission. Patients with pregnancy, immunosuppression, hemorrhage, transplantation, or burns were excluded. Treatment of septic shock according to ICU standard regimes was not influenced by the study protocol, which was approved by the local ethics committee.

Blood values

Blood samples were taken from arterial catheters with a standardized time interval until analyses (7–10 min). Differential hemogram and leukocyte counts, platelets, C-reactive protein (CRP), procalcitonin (PCT), serum bilirubin, creatinine, lactate, and arterial blood gas analyses were performed by the Department of Clinical Chemistry, University Hospital, Munich. Interleukin-6 (IL-6) was obtained by Milenia Quickline-IL-6 test (Milenia Biotec GmbH, Bad Nauheim, Germany) and analyzed with the PicoScan system (Milenia Biotec GmbH, Bad Nauheim, Germany) (range: 50–10,000 pg/ml;intra-/interassay coefficients of variation: 12.1 and 15.5%, respectively).

Determination of functional capabilities of neutrophils

The capabilities of neutrophils to (a) spontaneously produce H_2O_2, (b) phagocytose unopsonized or autologous plasma-opsonized yeast particles (zymosan), and (c) produce H_2O_2 upon challenge with zymosan were determined by flow cytometry as described elsewhere [4, 6].

Statistical analysis

Statistical analyses were performed with SPSS-13.0 software (SPSS, Chicago, Ill.). Data were normally distributed (Kolmogorov–Smirnov test). Mean values between groups were compared by independent Student's *t*-test. Within the same group, data were compared by paired *t*-test. Data were considered to be significantly different at $p < 0.05$.

Results

Baseline data

There were no significant differences between both groups for baseline characteristics, microbiological causes of septic shock, and calculated scores before start of treatment. The PCT and serum IL-6 were similar in both groups. The CRP was higher in the placebo-treated subjects as compared with the HC group (Table 1). Extent of functional organ impairment and frequency of failing organs showed no differences between the two groups (Table 2).

Clinical parameters

As compared with baseline conditions and placebo treatment, application of HC was followed by a significant decrease in norepinephrine requirements [from 1.5 to 0.8 mg/h (–46.6%); within group: $p < 0.001$, between groups: $p < 0.05$). Regarding other parameters of organ functions and clinical scores, no differences were determined between the two groups.

Interleukin-6 plasma levels

In contrast to placebo, HC administration lowered plasma IL-6 significantly by 77.2% (from 388.8 to 88.8 pg/ml; $p < 0.02$) resulting in significantly different IL-6 plasma levels after 24 h between HC- and placebo-treated patients ($p < 0.05$; Fig. 1).

Table 1 Baseline data of septic shock patients (mean ± SD). *APACHE*, Acute Physiology and Chronic Health Evaluation scoring system; *MODS*, Multi-organ Dysfunction Score; *SOFA*, Sepsis-related Organ Failure Assessment; *CRP*, C-reactive protein; *PCT*, procalcitonin

	Placebo group ($n = 15$)	Hydrocortisone group ($n = 15$)
Age (years)	63.5 ± 13.6	61.0 ± 21.8
Gender (female/male)	3/12	7/8
Time from onset of sepsis (h)	23.3 ± 8.8	29.5 ± 19.2
Temperature (°C)	38.0 ± 0.8	38.1 ± 1.0
Microbiological cause of septic shock		
Bacteria strains		
Gram positive	6 (40.0%)	6 (40.0%)
Gram negative	3 (20.0%)	2 (13.3%)
Gram positive and Gram negative	2 (13.3%)	2 (13.3%)
Candida albicans	1 (6.7%)	3 (20.0%)
No microbes detected	3 (20.0%)	2 (13.3%)
Clinical scoring-system values		
Apache II	26.8 ± 4.9	25.5 ± 4.7
Apache III	57.1 ± 18.8	53.3 ± 12.8
MODS	8.9 ± 2.9	8.8 ± 2.7
SOFA	13.0 ± 1.9	12.3 ± 2.9
Inflammation markers		
CRP (mg/dl)	28.1 ± 9.7	19.3 ± 9.6
PCT (ng/ml)	5.4 ± 7.0	8.3 ± 8.7

Table 2 Organ functions of septic shock patients (mean ± SD). p_aO_2, mean arterial oxygen partial pressure; FiO_2, inspiratory oxygen fraction

	Placebo group ($n = 15$)	Hydrocortisone group ($n = 15$)
Organ function parameters		
p_aO_2/FiO_2 ratio	241.0 ± 66.9	243.6 ± 82.8
Norepinephrine (mg/h)	1.6 ± 1.4	1.5 ± 1.0
Serum bilirubin (mg/dl)	1.6 ± 1.4	1.9 ± 1.9
Serum creatinine (mg/dl)	2.0 ± 1.5	1.3 ± 0.5
Platelets (G/l)	239 ± 121	220 ± 161
Lactate (mmol/l)	2.2 ± 2.0	2.0 ± 1.0
Incidence of organ failure		
Lung ($p_aO_2/FiO_2 < 300$)	12 of 15	11 of 15
Cardiovascular system (norepinephrine requirement > 0.5 mg/h)	13 of 15	13 of 15
Liver (bilirubin ≥ 1 mg/dl)	6 of 15	7 of 15
Kidney (creatinine ≥ 1.1 mg/dl)	13 of 15	9 of 15
Renal replacement therapy	2 of 15	1 of 15
Platelets (< 150 G/l)	3 of 15	4 of 15
Σ	49	45
No. of organ failures per patient	3.3	3.0
Lactate (> 0.63 mmol/l)	15 of 15	15 of 15

Neutrophil functions

The HC therapy decreased spontaneous H_2O_2 production to a significant extent, whereas placebo-treated subjects displayed higher values after 24 h (Fig. 2). At baseline and after 24 h, both groups showed no significant differences for phagocytosis of unopsonized zymosan particles and the phagocytosis-associated H_2O_2 production (Figs. 3, 4).

Rate of phagocytosis and the associated H_2O_2 production could be further enhanced by neutrophil stimulation with opsonized zymosan. While the extent of the enhancement of phagocytic activities by opsonization with autologous plasma was not different between patient groups at baseline, it became significantly different between groups 24 h after start of treatment. Accordingly, neutrophils from placebo-treated patients almost completely lost their capability to elevate phagocytosis of particles and the associated H_2O_2 production when stimulated by opsonized zymosan (both $p > 0.05$). In contrast, neutrophils of HC-treated patients were still able to respond with a significantly enhanced rate of phagocytosis ($p < 0.05$) and associated H_2O_2 production ($p < 0.05$) following activation with opsonized zymosan (Figs. 3, 4).

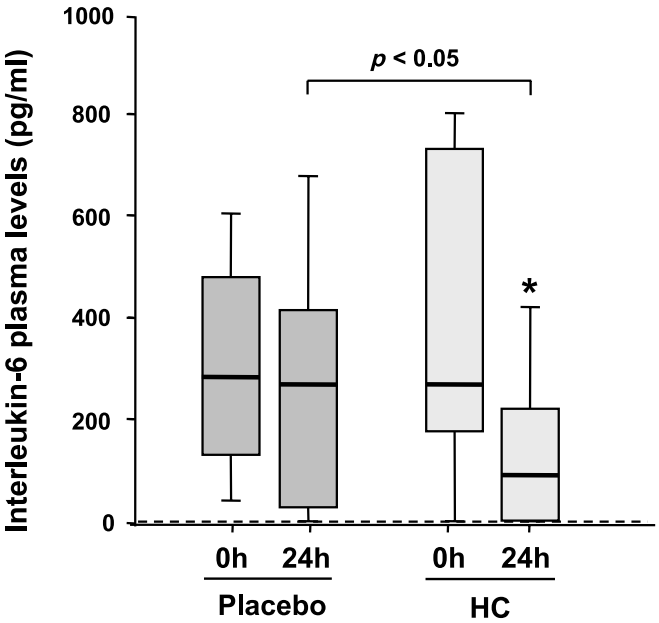


Fig. 1 Plasma interleukin-6 (IL-6) levels in septic shock patients before and after 24-h treatment with placebo or hydrocortisone (HC, 100 mg i.v. bolus followed by 10 mg/h i.v.). *Dashed line* close to the *x*-axis gives the upper limit value of IL-6 for healthy volunteers. $^{*}p < 0.02$ vs. value before HC treatment (0 h)

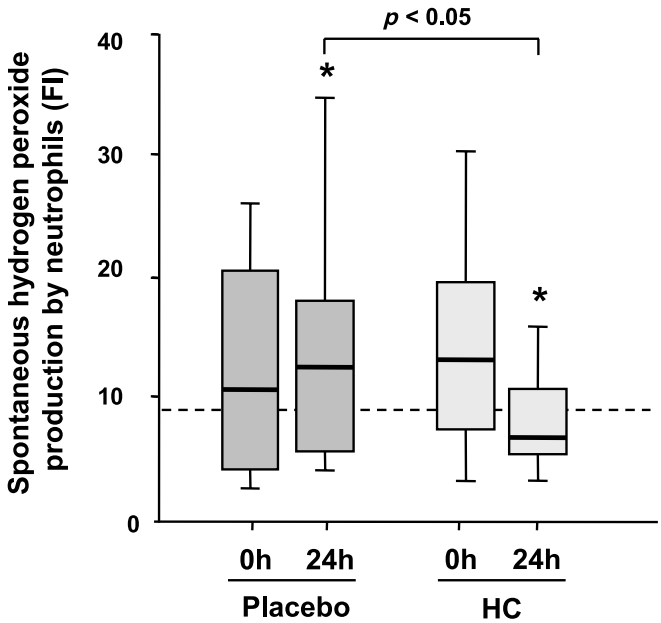
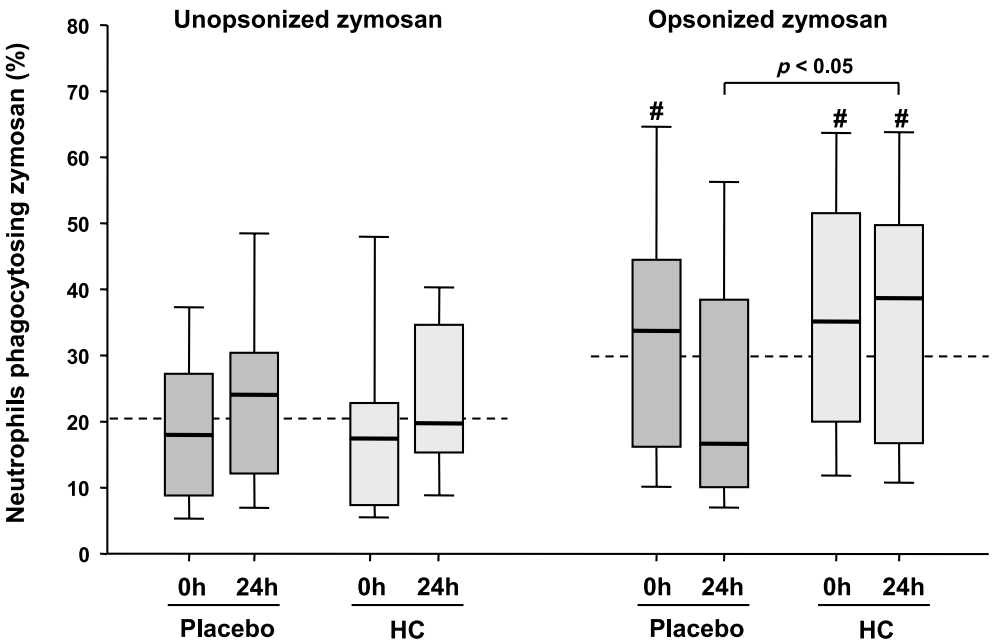


Fig. 2 Spontaneous in-vitro hydrogen peroxide (H_2O_2) production by neutrophils from septic shock patients before and after 24-h treatment with placebo or hydrocortisone (*HC*, 100 mg i.v. bolus followed by 10 mg/h i.v.). *Dashed line* gives the median value of spontaneous H_2O_2 production for healthy volunteers. *FI* fluorescence intensity. $^{*}p < 0.05$ vs. values before start of placebo or HC treatment (0 h)

Fig. 3 Percentage of neutrophils from septic shock patients before and after 24-h treatment with placebo or hydrocortisone (*HC*, 100 mg bolus i.v. followed by 10 mg/h i.v.) which phagocytose unopsonized and opsonized zymosan particles, respectively. *Dashed line* gives median value for neutrophils of healthy volunteers. $\#p < 0.05$ vs. values obtained with unopsonized zymosan for the respective group and time point



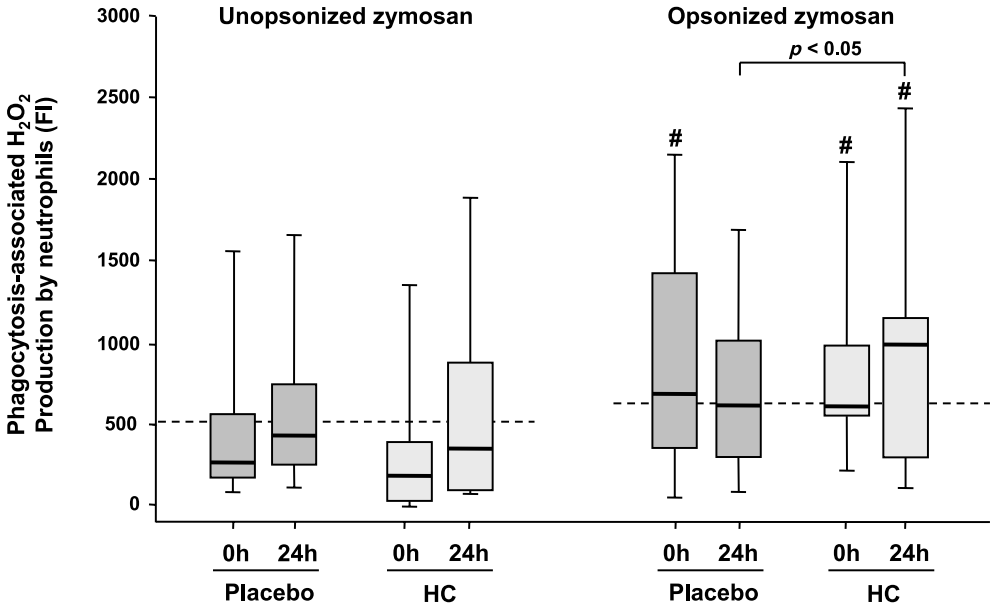


Fig. 4 Phagocytosis-associated hydrogen peroxide (H_2O_2) production ex vivo with unopsonized and opsonized zymosan particles stimulated neutrophils from septic shock patients before and after 24-h treatment with placebo or hydrocortisone (*HC*, 100 mg i.v. bolus followed by 10 mg/h i.v.). *Dashed line* gives the respective median value for neutrophils of healthy volunteers. *FI*, fluorescence intensity. $\#p < 0.05$ vs. values obtained with unopsonized zymosan for the respective group and time point

Discussion

In this study, continuous i.v. administration of stress doses of HC for 24 h resulted in (a) a significant decrease in norepinephrine requirements, (b) lowered IL-6 plasma levels, (c) suppression of spontaneous H_2O_2 production by neutrophils, and (d) maintenance of opsonization-dependent phagocytosis and phagocytosis-associated production of H_2O_2 by neutrophils.

Use of stress doses of corticosteroids improved survival rates in animal models of septic shock by stabilization of capillary permeability [7], enhancement of cardiac output [8], stimulation of albumin synthesis [9], and attenuation of hepatic lysosomal enzymes [10].

Accordingly, in our study, administration of stress doses of HC to septic shock patients resulted in a significant decrease in norepinephrine dosage requirements as described elsewhere [2, 11]. Besides various currently discussed mechanisms, including induction of phenyl-*N*-methyltransferase, an enzyme for epinephrine synthesis, prolongation of catecholamine actions by reuptake inhibition in neuromuscular junctions, increase in catecholamine sensitivity by enhancement of β-adrenergic binding affinity, blockage of vasodilatory prostaglandin synthesis [3], and hemodynamic stabilization might also result from the dampening of inflammation thereby counteracting sepsis-induced compromises of the micro- and macrocirculation.

Indeed, HC treatment decreased IL-6 levels (Fig. 1) supporting the hypothesis that the drug exerts its beneficial "permissive" effects via inhibition of the synthesis, release and/or efficacy of pro-inflammatory cytokines [12, 13] most likely due to nuclear factor κB-dependent pathway suppression [14].

As overactivated neutrophils are also important in the pathogenesis of sepsis, it is interesting to note that we observed a significantly decreased spontaneous H_2O_2 production of neutrophils in HC-treated patients. Since Levine et al. was not able to detect any inhibition of H_2O_2 production by incubation of neutrophils with physiological concentrations of corticosteroids in vitro [15], inhibition of H_2O_2 production in HC-treated patients is likely due to indirect effects, e.g. attenuation of cytokines.

Stress doses of HC preserved phagocytic neutrophil functions. As shown in Fig. 3, HC treatment for 24 h had no effect on the phagocytosis and the associated H_2O_2 production elicited by unopsonized zymosan. This finding is in agreement with those of other authors [12, 16]. Zymosan particles consist of β-glucan-rich membrane fragments of *Saccharomyces cerevisiae* and bind specifically to two sites on phagocytic cells: (a) the lectin-binding site of CD11b of the complement receptor type-III [17]; and (b) dectin-1 [18]; thus, the results of our study strongly suggest that both signaling pathways are not deteriorated by HC administration. As compared with the effects of unopsonized zymosan particles, percentages of phagocytosing cells and intensity of phagocytosis-associated H_2O_2 production increased significantly upon challenge of neutrophils with opsonized particles, indicating enhancement of phagocytic functions by plasma opsonins to the same extent in both patient groups at baseline; however, the opsonization-dependent enhancement of phagocytic functions was completely lost 24 h after placebo treatment but well preserved in the HC group; hence, HC treatment appears to maintain potentially microbicidal phagocyte functions which are dependent on plasma opsonins. Such an effect might be due to a less severe loss in humoral factors such as

complement-dependent opsonins or the stimulation of the production of humoral factors that are able to facilitate ingestion by neutrophils. Irrespective of these speculations, our findings of significantly better preserved plasma opsonin-dependent phagocyte functions in HC-treated patients might be of microbicidal relevance, as the most frequent reason for defective neutrophil functions is diminished serum opsonic capacity [19].

Our study has a major limitation because we investigated only patients in the early phases of septic shock. Since pattern of immune response may change in septic shock, there is a need to characterize the effect of stress doses of HC on innate immunity also in the later stages of sepsis. Another limitation is the higher CRP value in the placebo group at baseline; however, PCT and IL-6, which sensitively detect infectious and non-infectious causes for a systemic inflammation, were not significantly different between groups at baseline.

Conclusion

In conclusion, administration of stress doses of HC protects from overproduction of potentially tissue toxic H_2O_2 but preserves plasma opsonin-dependent phagocytic functions of neutrophils; thus, stress doses of HC do not appear to aggravate non-specific immunosuppression but instead improves innate immunity in the early stage of septic shock.

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