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Prednisolone Dose-Dependently Influences Inflammation and Coagulation during Human Endotoxemia¹

Martijn D. de Kruif,^{2*} Lucienne C. Lemaire,[†] Ida A. Giebelen,^{**‡} Marieke A. D. van Zoelen,^{**‡} Jennie M. Pater,^{**‡} Petra S. van den Pangaart,^{**‡} Angelique P. Groot,^{*‡} Alex F. de Vos,^{**‡} Peter J. Elliott,[¶] Joost C. M. Meijers,[§] Marcel Levi,[§] and Tom van der Poll^{**‡}

The effects of steroids on the outcome of sepsis are dose dependent. Low doses appear to be beneficial, but high doses do not improve outcome for reasons that are insufficiently understood. The effects of steroids on systemic inflammation as a function of dose have not previously been studied in humans. To determine the effects of increasing doses of prednisolone on inflammation and coagulation in humans exposed to LPS, 32 healthy males received prednisolone orally at doses of 0, 3, 10, or 30 mg ($n = 8$ per group) at 2 h before i.v. injection of *Escherichia coli* LPS (4 ng/kg). Prednisolone dose-dependently inhibited the LPS-induced release of cytokines (TNF- α and IL-6) and chemokines (IL-8 and MCP-1), while enhancing the release of the anti-inflammatory cytokine IL-10. Prednisolone attenuated neutrophil activation (plasma elastase levels) and endothelial cell activation (von Willebrand factor). Most remarkably, prednisolone did not inhibit LPS-induced coagulation activation, measured by plasma concentrations of thrombin-antithrombin complexes, prothrombin fragment F1+2, and soluble tissue factor. In addition, activation of the fibrinolytic pathway (tissue-type plasminogen activator and plasmin- α_2 -antiplasmin complexes) was dose-dependently enhanced by prednisolone. These data indicate that prednisolone dose-dependently and differentially influences the systemic activation of different host response pathways during human endotoxemia. *The Journal of Immunology*, 2007, 178: 1845–1851.

Inflammation and coagulation can activate each other in a complex way (1, 2). In patients with sepsis, activation of both processes contributes to organ failure (1, 2). As is known from the strategies used in the treatment of sepsis, successful intervention by aiming at the activation of inflammation and coagulation is difficult (3–5). One of the oldest, and most cost-effective, anti-inflammatory treatments used is that of corticosteroid therapy. Multiple studies have been published that investigated the effects of corticosteroids in sepsis (6–17). A recent meta-analysis by Minneci et al. (18) showed that the overall effects of steroids on survival and shock during sepsis were largely dose dependent. Studies published before 1989 administered higher doses of glucocorticosteroids (median total dose 24,000 mg of hydrocortisone equivalent) compared with those published after 1997 (median total dose 1,209 mg of hydrocortisone equivalent) and there was a negative linear relationship between steroid dose and survival. Similarly, another meta-analysis performed by Annane et al. (19) reported a reduced mortality in patients treated with steroids in five recent sepsis trials in which low dose corticosteroids were used, whereas such a beneficial effect was not seen in trials using high-dose steroids. It has been suggested that the harmful effect of the high-dose

steroids may have been caused by a pronounced immunosuppressive effect because the time to resolution of secondary infections and, subsequently, the mortality from these secondary infections, was increased in these patients (18).

The human endotoxemia model, in which healthy subjects are i.v. injected with a standardized LPS preparation, has been widely used to study the mechanisms of activation of inflammatory pathways during a systemic inflammatory response syndrome (20). Three studies using this model investigated the effects of corticosteroids on cytokine release and showed variable results regarding inhibition of TNF- α and IL-6 release (21–23). The release of the anti-inflammatory cytokine IL-10 was enhanced by hydrocortisone in this model (24). The knowledge of the effects of steroids on inflammatory pathways other than the cytokine network in humans in vivo however is highly limited. Moreover, the effects of glucocorticoids on inflammatory pathways as a function of the dose have only been investigated in vitro (25, 26). In light of the renewed interest in the use of steroids in sepsis, we designed a study seeking to establish whether steroids have a dose-dependent effect on the LPS-induced systemic inflammation and coagulation in healthy humans.

Materials and Methods

Subjects and study design

The study was approved by the institutional scientific and ethics committees. Written informed consent was obtained from all subjects. Thirty-two healthy, male volunteers (mean (\pm SE) age 23.9 \pm 0.7 years) were admitted to the Clinical Research Unit of the Academic Medical Center. Medical history, physical examination, hematological and biochemical screening, and electrocardiograms were all normal. Prednisolone (prednisolone sodium phosphate oral solution 5 mg/ml; prepared by institutional pharmacy) was administered orally at a dose of 0, 3, 10, or 30 mg ($n = 8$ /group) 2 h before LPS injection. All participants were challenged at $t = 0$ h with LPS (*Escherichia coli* LPS, lot G; US Pharmacopeia) as a bolus i.v. injection at a dose of 4 ng/kg body weight. Oral temperature, blood pressure, heart rate, and oxygen saturation were measured at half-hour intervals (Dinamap1846 SX; Critikon). Clinical symptoms such as headache, chills, nausea, and

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myalgia were recorded throughout the study period using a graded scale (0, absent; 1, mild; 2, moderate; 3, severe).

Assays

Blood was collected from a cannulated forearm vein at 2.5 h and at 1 h before LPS injection, directly before LPS administration ($t = 0$ h) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 24 h thereafter. For leukocyte and differential counts, blood was drawn into sterile 4.5-ml tubes containing EDTA-K₃ (15%; Vacutainer Systems/BD Biosciences). Heparinized plasma (for measurement of C-reactive protein (CRP)³), EDTA-K₃ anticoagulated plasma (cytokines, chemokines, and elastase) or citrated plasma (assays of coagulation and fibrinolysis) was obtained by immediate centrifugation (4°C, 10 min, 3000 rpm) and stored at -20°C until assayed. CRP was measured by immunoturbidimetric assay (Roche). Cytokine concentrations (TNF- α , IL-1 β , IL-6, IL-8, IL-10, and IL-12p70) were measured using a cytometric bead array immunoassay (BD Biosciences/BD Pharmingen) (27). IL-1R antagonist (IL-1RA) and soluble TNFRs types 1 and 2 (soluble TNFR1 and TNFR2) were measured with Luminex¹⁰⁰ (BioSource International). Elastase was measured by ELISA (28), as were parameters for endothelial cell activation: von Willebrand factor (vWF; DakoCytomation); parameters for coagulation activation: soluble tissue factor (American Diagnostics), prothrombin fragment F1+2, and thrombin-antithrombin complexes (TATc; both obtained from Dade Behring); and parameters for fibrinolytic activation: tissue-type plasminogen activator (tPA; Asserachrom tPA; Diagnostica Stago), plasmin- α_2 -antiplasmin complexes (PAPc; Enzygnost PAP Micro; Dade Behring), and plasminogen activator inhibitor type 1 (PAI-1; Monozyme).

Statistical analysis

Differences between treatment groups were analyzed using a nonparametric mixed model approach (repeated measures ANOVA) (29, 30). The effects reported are treatment effects adjusted for time effects. All analyses were conducted using SPSS version 11.5 and p values <0.05 were considered statistically significant. With a sample size of eight volunteers per group, a similar difference in TNF- α peak values, as observed earlier (21), could be detected with >95% power using a two-sided ($p < 0.05$) t test. Values presented are given as mean \pm SE.

Results

Vital signs, clinical symptoms, and acute phase response

Intravenous injection of LPS elicited a febrile response, peaking after 4 h ($39.1 \pm 0.2^\circ\text{C}$ in the control group). The febrile response was inhibited in all prednisolone groups (peak temperatures: 30 mg of prednisolone, $38.0 \pm 0.2^\circ\text{C}$; 10 mg, $38.0 \pm 0.2^\circ\text{C}$; 3 mg, $38.4 \pm 0.1^\circ\text{C}$). The febrile response was associated with tachycardia and transient flu-like symptoms, including headache, chills, nausea, and myalgia. Overall, the symptoms were attenuated by administration of prednisolone in all groups, indicating that these doses were high enough to evoke a clinical effect (Table I). In addition, the acute phase response induced by LPS was significantly inhibited by 30 and 10 mg of prednisolone. After LPS infusion, CRP plasma levels rose to 64.1 ± 10.7 mg/L in the control group ($t = 24$ h). The rise was inhibited by 30 and 10 mg of prednisolone (levels at $t = 24$ h: 38.1 ± 2.3 mg/L and 36.8 ± 1.5 mg/L, respectively; $p < 0.05$) but not by 3 mg of prednisolone (level at $t = 24$ h: 74.4 ± 8.0 mg/L). Administration of prednisolone was not associated with adverse effects.

Cytokines and chemokines

As shown in Fig. 1, LPS challenge induced a profound rise in concentrations of TNF- α , IL-6, IL-8, and MCP-1, with peak values of 1627 ± 590 pg/ml, 3273 ± 1223 pg/ml, 832 ± 401 pg/ml, and 28.86 ± 2.88 ng/ml, respectively. Prednisolone inhibited these increases in a dose-dependent manner. TNF- α production was inhibited by 30 and 10 mg of prednisolone (peak

Table I. Clinical symptoms and signs^a

Symptoms	Controls ($n = 8$)				3 mg ($n = 8$)				10 mg ($n = 8$)				30 mg ($n = 8$)			
	Total no. ^b	Sev/mod/mild ^c	Peaking time ^d	Total no. ^b	Sev/mod/mild ^c	Peaking time ^d	Total no. ^b	Sev/mod/mild ^c	Peaking time ^d	Total no. ^b	Sev/mod/mild ^c	Peaking time ^d	Total no. ^b	Sev/mod/mild ^c	Peaking time ^d	
Headache	8	5/2/1	1.5	8	4/0/4	1.5	7	2/2/3	1.5	8	1/3/4	1.5	8	1/3/4	1.5	
Chills	8	7/1/0	1.5	7	1/2/4	1.5	7	2/1/4	1.5	8	2/3/3	1.5	8	2/3/3	1.5	
Myalgia	4	2/1/1	2	3	0/0/3	3	2	0/1/1	1	6	0/2/4	3	6	0/2/4	3	
Nausea	6	1/2/3	2	5	1/2/2	3	5	0/2/3	3	1	0/0/1	3	1	0/0/1	3	
Vomiting	2	1/1/0	1.5	2	0/1/1	4	1	0/1/0	3	0	0/0/0	NA	0	0/0/0	NA	
Abdominal pain	2	1/0/1	2	2	0/0/2	2	1	0/0/1	3	0	0/0/0	NA	0	0/0/0	NA	
Backache	0	0/0/0	NA	4	0/0/4	2	3	0/0/3	1	3	0/1/2	1.5	3	0/1/2	1.5	
Signs	Peak value	\pm SEM	Peaking time ^d	Peak value	\pm SEM	Peaking time ^d	Peak value	\pm SEM	Peaking time ^d	Peak value	\pm SEM	Peaking time ^d	Peak value	\pm SEM	Peaking time ^d	
Temperature ($^\circ\text{C}$)	39.1	0.2	4	38.4	0.1	4	38.0	0.2	4	38.0	0.2	4	38.0	0.2	4	
Heart rate (bpm)	100	5	4	100	6	3.5	102	5	4.5	109	4	4	109	4	4	
MABP ^e (mm Hg)	111	6	1.5	97	4	1.5	116	7	1.5	110	7	1.5	110	7	1.5	

^a Clinical symptoms and signs after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h.

^b Total number of subjects suffering from the indicated symptom.

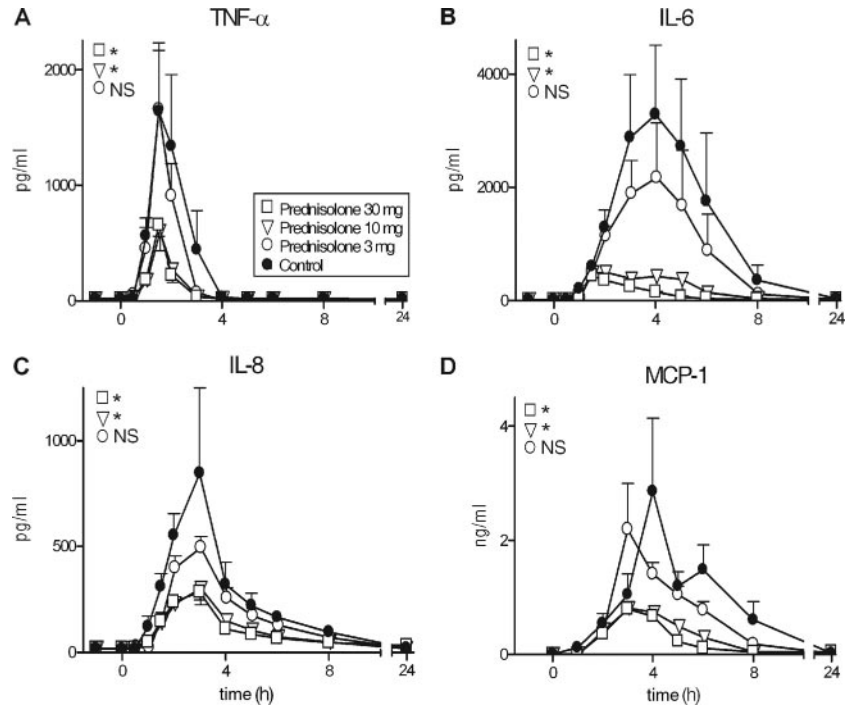
^c A further distinction was made between severe (sev), moderate (mod), and mild (mild) symptoms.

^d Peaking time refers to the maximum number of subjects suffering from the indicated symptom or time of peak value from the indicated clinical sign (hours after LPS).

^e MABP, Mean arterial blood pressure.

³ Abbreviations used in this paper: CRP, C-reactive protein; IL-1RA, IL-1R antagonist; vWF, von Willebrand factor; TAT, thrombin-antithrombin; TATc, TAT complex; tPA, tissue-type plasminogen activator; PAP, plasmin- α_2 -antiplasmin; PAPc, PAP complex; PAI-1, plasminogen activator inhibitor type 1.

FIGURE 1. Cytokines and chemokines. Mean (\pm SE) plasma levels of TNF- α (A), IL-6 (B), IL-8 (C), and MCP-1 (D) after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.



values: 640 ± 98 and 584 ± 166 pg/ml at $t = 1.5$ h, respectively; $p < 0.05$ vs controls), while 3 mg of prednisolone had no effect. IL-6 production was similarly inhibited by 30 and 10 mg of prednisolone (peak values: 430 ± 61 and 485 ± 90 pg/ml at $t = 1.5$ h and $t = 2$ h, respectively; $p < 0.05$ vs controls), and, similarly, 3 mg of prednisolone had no effect. In addition, IL-8 production was inhibited by 30 and 10 mg of prednisolone (peak values: 272 ± 38 and 288 ± 76 pg/ml at $t = 3$ h; $p < 0.001$ and $p < 0.05$ vs controls; respectively), as was MCP-1 production (peak values: 7.9 ± 1.0 and 8.1 ± 0.5 ng/ml at $t = 3$ h, respectively; $p < 0.05$ vs controls). These proteins were not significantly attenuated by 3 mg of prednisolone.

Anti-inflammatory cytokine response

LPS injection also induced an anti-inflammatory cytokine response (Fig. 2). Levels of IL-10 were raised with a peak value of 134 ± 34 pg/ml at $t = 3$ h, and were stimulated by 30 mg of prednisolone (peak value: 442 ± 94 pg/ml at $t = 3$ h; $p < 0.001$ vs controls). Levels of IL-1RA increased to a peak values of 48.6 ± 3.6 ng/ml at $t = 4$ h. Both 30 and 10 mg of prednisolone inhibited this response (peak values: 43.9 ± 2.8 ng/ml at $t = 3$ h and 42.1 ± 2.6 ng/ml at $t = 4$ h, respectively; $p < 0.05$ vs controls). Soluble TNFR1 increased to peak values of 4.02 ± 0.40 ng/ml at $t = 4$ h. Both 30 and 10 mg of prednisolone inhibited this response (peak values: 3.03 ± 0.26 ng/ml

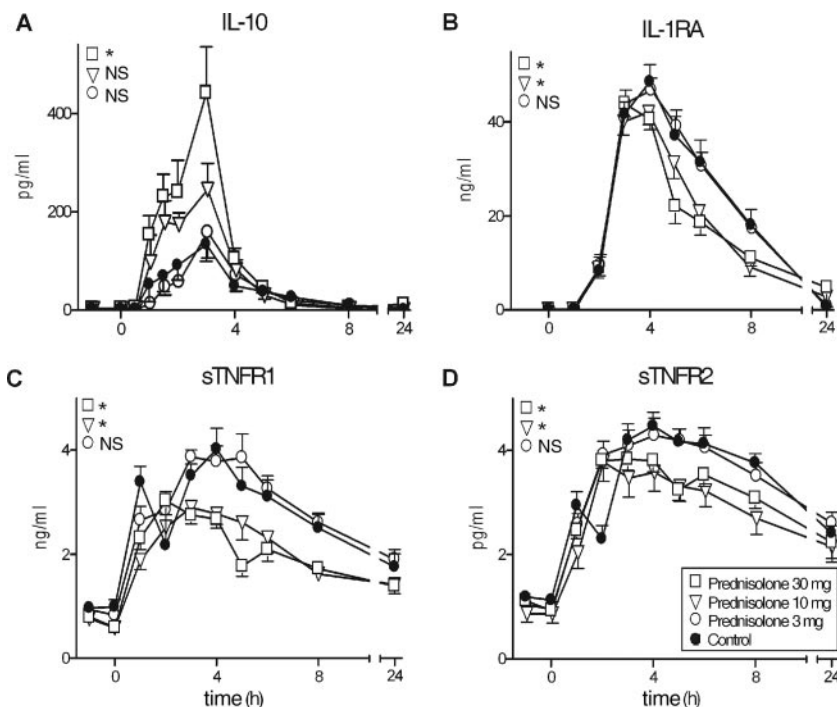


FIGURE 2. Anti-inflammatory cytokine response. Mean (\pm SE) plasma levels of IL-10 (A), IL-1RA (B), soluble TNFR1 (C), and soluble TNFR2 (D) after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.

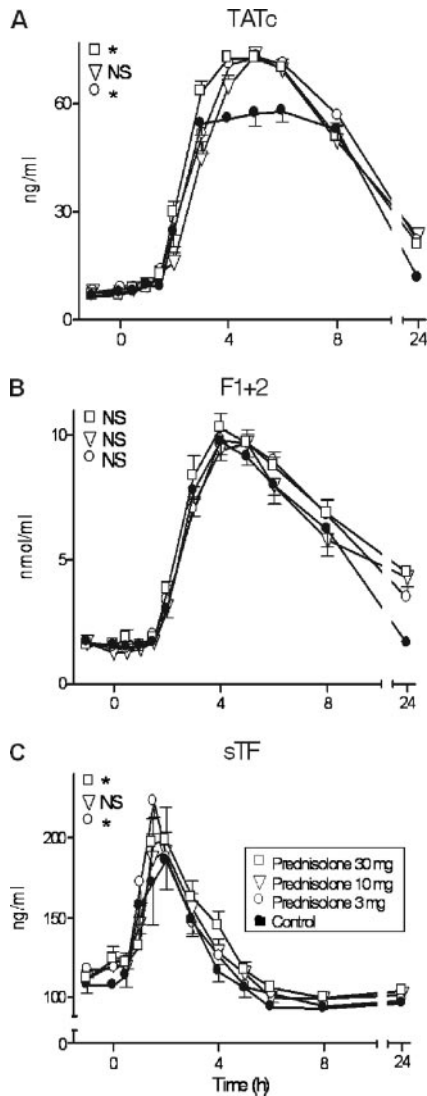


FIGURE 3. Activation of coagulation. Mean (\pm SE) plasma levels of TAT complexes (A), F1+2 (B) and soluble tissue factor (C) after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.

at $t = 2$ h and 2.90 ± 0.15 ng/ml at $t = 3$ h; $p < 0.05$ and $p < 0.001$ vs controls; respectively). Levels of soluble TNFR2 increased to 4.46 ± 0.27 ng/ml at $t = 4$ h. Both 30 and 10 mg of prednisolone inhibited the rise (peak values: 3.83 ± 0.21 ng/ml at $t = 3$ h and 3.78 ± 0.37 ng/ml at $t = 2$ h, respectively; $p < 0.05$ vs controls). Administration of 3 mg of prednisolone had no effect on any of these anti-inflammatory mediators.

Activation of coagulation

LPS injection induced activation of coagulation, as indicated by an increase in the plasma concentrations of TATc with a peak of 57.8 ± 3.1 ng/ml at $t = 6$ h (Fig. 3). Both 30 and 3 mg of prednisolone augmented this response (peak values: 72.5 ± 1.6 and 72.9 ± 1.1 ng/ml at $t = 5$ h, respectively; $p < 0.05$ vs controls), and 10 mg of prednisolone showed a trend to enhance this effect (peak value: 73.6 ± 1.7 ng/ml at $t = 5$ h; $p < 0.10$). Plasma levels of prothrombin fragments F1+2 were elevated with a peak of 9.7 ± 0.5 nM/L at $t = 4$ h. A dose of 30 mg of prednisolone showed a trend to further increase this elevation (peak value: 10.3 ± 0.6 nM/L at $t = 4$ h; $p < 0.10$ vs controls). Levels of

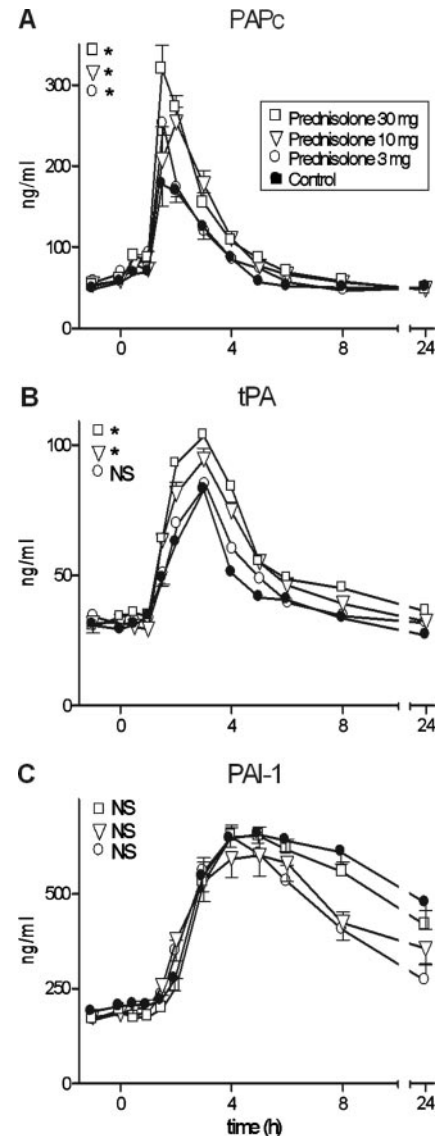


FIGURE 4. Activation and inhibition of fibrinolysis. Mean (\pm SE) plasma levels of PAP complexes (A), t-PA (B), and PAI-1 (C) after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.

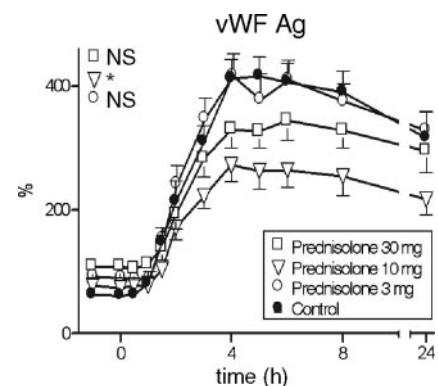
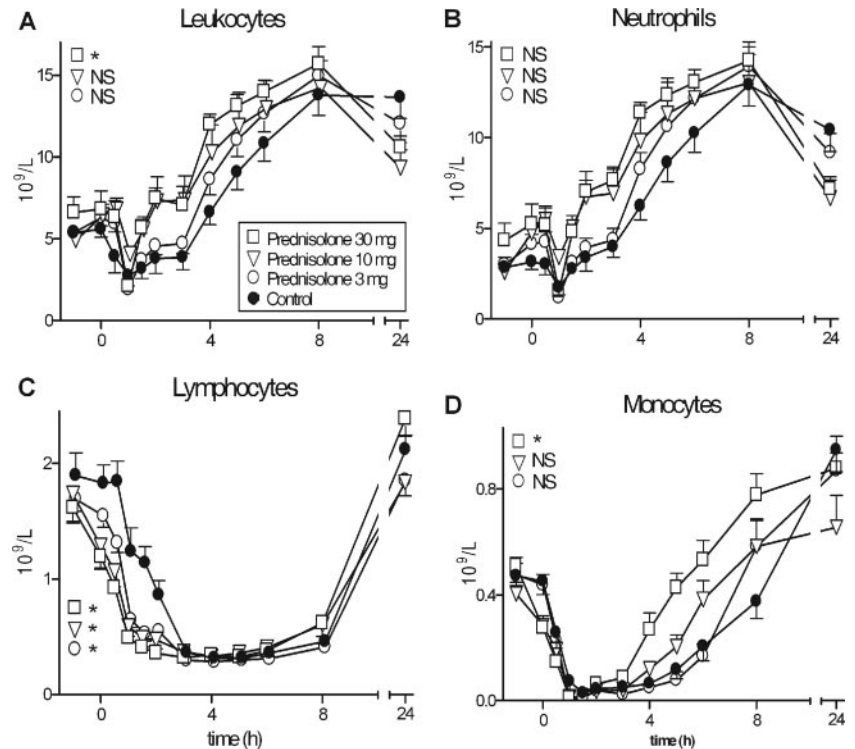


FIGURE 5. Endothelial cell activation. Mean (\pm SE) plasma levels of vWF after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.

FIGURE 6. White blood cell counts. Mean (\pm SE) plasma concentrations of leukocytes (A), neutrophils (B), lymphocytes (C), and monocytes (D) after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.



circulating soluble tissue factor were increased at $t = 2$ h with a peak of 185 ± 18 ng/ml. Both 30 and 3 mg of prednisolone stimulated this response (peak values: 198 ± 22 ng/ml at $t = 2$ h and 223 ± 10 ng/ml at $t = 1.5$ h, respectively; $p < 0.05$ vs controls), while 10 mg of prednisolone showed a trend to enhance this effect (peak value: 191 ± 13 ng/ml at $t = 2$ h; $p < 0.10$).

Activation and inhibition of fibrinolysis

After LPS challenge, activation and inhibition pathways of fibrinolysis were stimulated. Levels of PAPc were increased with a peak of 177 ± 26 ng/ml at $t = 1.5$ h (Fig. 4). All doses of prednisolone stimulated this increase (peak values: 251 ± 14 ng/ml at $t = 1.5$ h after 3 mg of prednisolone, 253 ± 18 ng/ml at $t = 2$ h after 10 mg of prednisolone and 319 ± 30 ng/ml at $t = 1.5$ h after 30 mg of prednisolone; $p < 0.05$). The plasma levels of tPA were increased with a peak of 82.8 ± 0.7 ng/ml at $t = 3$ h. Both 30 and 10 mg of prednisolone augmented this effect (peak values: 103.6 ± 1.4 and 94.5 ± 4.4 ng/ml at $t = 3$ h; $p < 0.001$ and $p < 0.05$ vs controls; respectively), and 3 mg of prednisolone showed a trend to enhance this effect (peak value: 84.6 ± 2.1 ng/ml at $t = 3$ h; $p < 0.10$). Levels of PAI-1 peaked at $t = 5$ h (642 ± 23 ng/ml). Prednisolone had no effect on LPS-induced PAI-1 release.

Endothelial cell activation

LPS injection elicited endothelial cell activation, as indicated by increases in the plasma levels of vWF Ag from $62 \pm 16\%$ at $t = 0$ h to $422 \pm 31\%$ at $t = 4$ h (Fig. 5). A dose of 10 mg of prednisolone limited this increase (peak value: $272 \pm 26\%$; $p < 0.05$), but not 30 or 3 mg of prednisolone.

Leukocyte responses

After LPS challenge, plasma leukocyte counts initially decreased from $5.6 \pm 0.4 \times 10^9/L$ at $t = -2.5$ h to $2.7 \pm 0.6 \times 10^9/L$ at $t = 1$ h, and subsequently increased up to $13.8 \pm 1.2 \times 10^9/L$ at $t = 8$ h (Fig. 6). A dose of 30 mg of prednisolone increased the leukocytosis (peak value: $15.7 \pm 1.1 \times 10^9/L$ at $t = 8$ h; $p < 0.05$

vs controls). These changes were also reflected in absolute plasma neutrophil counts, although the effect of 30 mg of prednisolone did not reach statistical significance (peak value: 30 mg of $14.2 \pm 1.0 \times 10^9/L$ vs controls $12.9 \pm 1.2 \times 10^9/L$ at $t = 8$ h; $p = 0.10$). Absolute plasma lymphocyte counts were decreased from $2.30 \pm 0.16 \times 10^9/L$ to $0.32 \pm 0.04 \times 10^9/L$ at $t = 4$ h and slowly returned to baseline levels at $t = 24$ h. Prednisolone treatment enhanced the decrease in lymphocyte counts in all groups ($p < 0.001$ vs control group). Monocyte counts initially decreased from $0.504 \pm 0.041 \times 10^9/L$ to $0.030 \pm 0.004 \times 10^9/L$ ($t = 1.5$ h), followed by an increase up to $0.947 \pm 0.083 \times 10^9/L$ at $t = 24$ h. The increase was enhanced by 30 mg of prednisolone ($p < 0.001$ vs controls). Neutrophil degranulation was inhibited by 30 mg of prednisolone, as reflected by the inhibition of plasma elastase levels (peak value 170 ± 24 ng/ml at $t = 8$ h vs 215 ± 27 ng/ml at $t = 4$ h for controls, $p < 0.05$) (Fig. 7).

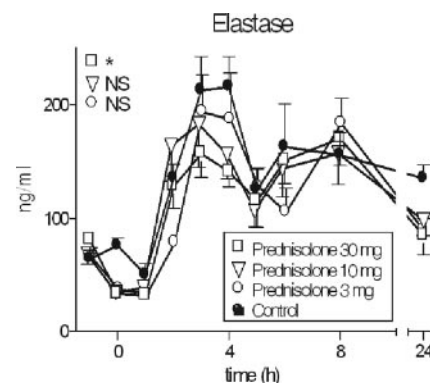


FIGURE 7. Neutrophil function. Mean (\pm SE) plasma levels of elastase, a neutrophil degranulation product, after LPS administration (4 ng/kg i.v., $t = 0$ h), preceded by oral administration of 3, 10, and 30 mg of prednisolone or 0 mg in the control group at $t = -2$ h. *, $p < 0.05$ vs control group.

Discussion

The present study is the first to investigate the effect of increasing doses of corticosteroids on the inflammatory and coagulation responses during human endotoxemia. Our data show that prednisolone dose-dependently inhibited the release of cytokines and chemokines. Furthermore, while prednisolone enhanced the LPS-induced release of the anti-inflammatory cytokine IL-10 in a dose-dependent way, it attenuated the secretion of the cytokine antagonists IL-1RA and soluble TNFR. Prednisolone also inhibited other proinflammatory responses elicited by i.v. LPS, in particular degranulation of neutrophils and activation of the vascular endothelium. Most remarkably, activation of the coagulation and fibrinolytic systems was not inhibited by prednisolone treatment. These data suggest that prednisolone differentially affects systemic host responses to i.v. LPS.

The current findings on the effect of steroids in human volunteers challenged with LPS should be viewed upon in the context of three earlier investigations that examined the effect of i.v. hydrocortisone on LPS-induced cytokine release (21–23). The first two studies (21, 23) used hydrocortisone at a dose of 3 $\mu\text{g}/\text{kg}/\text{min}$ starting 6 h before LPS injection and continuing up to 6 h after LPS injection; hence, these subjects received the equivalent of 18.8 mg of prednisolone before LPS and 18.8 mg of prednisolone after LPS. Remarkably, these two previous studies reported different results on the inhibition of TNF- α and IL-6 by hydrocortisone: whereas Rock et al. (23) found inhibition of LPS-induced IL-6 and no effect on TNF- α release, Barber et al. (21) demonstrated inhibition of LPS-induced TNF- α secretion with no effect on IL-6 levels. The latter study also found enhanced IL-10 release (24) and a reduction in IL-1RA and soluble TNFR (31) in hydrocortisone-treated subjects. Santos et al. (22) administered hydrocortisone as a bolus i.v. dose of 100–200 mg directly before LPS injection and observed a reduction in both TNF- α and IL-6 levels but no effect on IL-1RA release. Our present study extends these earlier data (21–24, 31) by showing a dose-response relationship of prednisolone with inhibition of both TNF- α and IL-6 release and also inhibition of IL-1RA release. Moreover, the studies cited above did not examine other inflammatory responses such as chemokine release and activation of neutrophils, the vascular endothelium, and the coagulation and fibrinolytic systems, which are all host responses implicated in the pathophysiology of the sepsis syndrome.

An important finding of the current study is the modest enhancement of LPS-induced activation of coagulation and the enhancement of the fibrinolytic pathways by prednisolone, as measured by increased levels of TATc, soluble tissue factor, PAPc, and tPA. The effects were most significant at the highest dose used, i.e., 30 mg of prednisolone. It is well-known that inflammatory pathways closely interact with coagulation and fibrinolytic pathways (2, 32). Most often, inhibition of proinflammatory cytokines results in decreased activation of coagulation and fibrinolysis (33, 34). Interestingly, in the present study, prednisolone reduced levels of proinflammatory cytokines whereas it modestly enhanced the procoagulant response. Corticosteroids have been associated with activation of coagulation and fibrinolysis in vitro and in vivo previously. In monocytic cell lines, corticosteroids stimulated coagulation by inducing tissue factor expression (35). The enhanced tissue factor expression resulted from a complex mechanism, where corticosteroid treatment reduced tissue factor gene transcription in endotoxin-stimulated monocytes, but increased the stability of the generated tissue factor mRNA (36). Considering that activation of coagulation is strictly dependent on tissue factor in this model of human endotoxemia (37), it seems likely that pred-

nisolone enhanced coagulation activation through an effect on tissue factor expression. In the present study, levels of soluble tissue factor were indeed affected by corticosteroids, however, it needs to be noted that it is not sure whether this marker represents the biologically active form of tissue factor (38). Observational studies have further provided evidence that steroids may enhance coagulation activation. In humans, the diurnal pattern of endogenous cortisol production synchronized with diurnal coagulation changes (39). In addition, during emotional stress conditions, cortisol release was associated with rises in levels of TATc, factor VIII, factor XII, tPA, fibrinogen, and D-dimer (40). When dexamethasone was administered to healthy volunteers, levels of factor VII, factor VIII, factor XI, fibrinogen, vWF, and P-selectin were increased (41, 42).

There are several restrictions of this study to be mentioned. First, the present study does not completely reflect the clinical situation, because we used a model for Gram-negative sepsis in healthy volunteers. This model lacks an infectious source and is self-limiting. The “treatment” is administered before the systemic inflammatory response syndrome has fully developed, which is necessary to reach clinically relevant concentrations of the drug intervention at the time of LPS challenge considering that LPS has a very short half-life. Furthermore, during sepsis the stimulus is ongoing and episodic, and corticosteroids are often given repetitively over several days. Due to the restrictions of the model, our data cannot be directly extrapolated to clinical sepsis and should be confirmed in patient studies. Nonetheless, the model of human endotoxemia is the best available in healthy humans and has proven useful to obtain a proof of principle of the actions of drugs and/or to study mechanisms that contribute to the activation of inflammatory pathways (20).

With these limitations in mind, we here demonstrate that prednisolone dose-dependently inhibits proinflammatory cytokine release, endothelial cell activation, neutrophil activation, and the acute phase response whereas it modestly enhances, thus does not inhibit, the activation of coagulation and fibrinolysis. Further studies are warranted to determine the effect of steroids on the various components of the systemic inflammatory response in patients with septic shock.

Disclosures

P. J. Elliott was employed by CombinatorX at the time of study design and completion. Currently, he is employed elsewhere but he is still a stockholder of CombinatorX.

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