Efficacy of selective mineralocorticoid and glucocorticoid agonists in canine septic shock

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Objective: **Corticosteroid regimens that stimulate both mineralocorticoid and glucocorticoid pathways consistently reverse vasopressor-dependent hypotension in septic shock but have variable effects on survival. The objective of this study was to determine whether exogenous mineralocorticoid and glucocorticoid treatments have distinct effects and whether the timing of administration alters their effects in septic shock.**

Design, Setting, Subjects, and Interventions: **Desoxycorticosterone, a selective mineralocorticoid agonist; dexamethasone, a selective glucocorticoid agonist; and placebo were administered either several days before (prophylactic) or immediately after (therapeutic) infectious challenge and continued for 96 hrs in 74 canines with staphylococcal pneumonia.**

Measurements and Main Results: **Effects of desoxycorticosterone and dexamethasone were different and opposite depending on timing of administration for survival ($p = .05$); fluid requirements ($p = .05$); central venous pressures ($p \leq .007$); indicators of hemoconcentration (i.e., sodium [$p = .0004$], albumin [$p = .05$], and platelet counts [$p = .02$]); interleukin-6 levels ($p = .04$); and cardiac dysfunction ($p = .05$). Prophylactic desoxycorticosterone treatment significantly improved survival, shock, and all the other outcomes stated, but therapeutic desoxycorticosterone did not. Conversely, prophylactic dexamethasone was much less effective for improving these outcomes compared with therapeutic dexamethasone with the exception of shock reversal. Prophylactic dexamethasone given before sepsis induction also significantly reduced serum aldosterone and cortisol levels and increased body temperature and lactate levels compared with therapeutic dexamethasone ($p \leq .05$), consistent with adrenal suppression.**

Conclusions: **In septic shock, mineralocorticoids are only beneficial if given prophylactically, whereas glucocorticoids are most beneficial when given close to the onset of infection. Prophylactic mineralocorticoids should be further investigated in patients at high risk to develop sepsis, whereas glucocorticoids should only be administered therapeutically to prevent adrenal suppression and worse outcomes. (Crit Care Med 2012; 40:199–207)**

Key Words: animal; corticosteroids; infection; models; sepsis

Sepsis is a substantial cause of morbidity and mortality worldwide. In the United States alone, 751,000 hospitalizations (3.0 per 1000 population) and 215,000 deaths annually are attributable to this syndrome (1). Despite numerous clinical trials over the past 50 yrs, new drug therapies have been largely unsuccessful in reducing sepsis-related deaths (2, 3). One possible exception is corticosteroid administration (4–7). Over the last 35 yrs, the doses, regimens, and extent of corticosteroid use have varied, likely in response to inconsistent results from clinical trials. Most recently, use of physiological doses equivalent to the stress cortisol response (i.e., most commonly hydrocortisone in doses of 200–300 mg/day for 7–10 days) have been advocated, in part because of clinical trials demonstrating shock reversal with such use (7). However, this approach is not universally accepted because effects on survival have varied and some studies have reported an increased risk of secondary infection and myopathy (8, 9).

Corticosteroid administration in septic shock has been based on at least three different rationales: 1) suppression of an excessive inflammatory response; 2) direct reversal of sepsis-induced vascular hyporeactivity; and 3) treatment of relative adrenal insufficiency (8). However, serum levels of cortisol, the main endogenous corticosteroid, generally increase with sepsis severity and the entity of relative adrenal insufficiency, or "critical illness-related corticosteroid insufficiency," has been difficult to define or characterize clinically (10, 11). Likewise, the benefits of anti-inflammatory therapy were not confirmed in studies using high-dose glucocorticoids or other agents that specifically target components of the innate immune response (12, 13). Further advances in this field may require more precise knowledge of the mechanisms

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underlying the purported benefits and risks of corticosteroids in septic shock.

Hydrocortisone, the corticosteroid used most commonly for sepsis, has both glucocorticoid and mineralocorticoid activity (14). In contrast to the strong anti-inflammatory effects of glucocorticoids (15), mineralocorticoid receptor-specific ligands have relatively weaker anti-inflammatory effects (16) and have not been associated with overt immune suppression. However, the regulation of sodium metabolism, intravascular volume, and endothelial function by mineralocorticoid receptors (17) may be more important in the treatment of septic shock than suppression of the inflammatory response (18). Regimens of pure mineralocorticoid agonists devoid of glucocorticoid activity have not been investigated in clinical trials (5, 19), and a literature review did not reveal any animal studies examining mineralocorticoid therapy alone in septic shock. Therefore, to our knowledge, the safety and efficacy of mineralocorticoid agonists alone in septic shock have not been tested, and the therapeutic contribution of mineralocorticoid activity, if any, to corticosteroid regimens with mixed activity is unclear.

Based on the different activity of mineralocorticoid and glucocorticoid ligands, we hypothesized that the effects of corticosteroids in sepsis would be different depending on agonist activity and timing of administration. Using intrapulmonary challenges of *Staphylococcus aureus* to produce septic shock in canines (20, 21), stress doses of dexamethasone (DEX), a specific glucocorticoid agonist, or desoxycorticosterone (DOC), a specific mineralocorticoid agonist, were compared for effects on pathophysiology and survival. To simulate therapeutic interventions commonly used in human septic shock, animals were managed with standardized sedation, antibiotic therapy, mechanical ventilation support adjusted to correct gas exchange abnormalities, and need-based cardiovascular support using vasopressors and fluids to normalize intravascular pressures. Glucocorticoid- and mineralocorticoid-specific regimens were both evaluated using two different timeframes of administration. Prophylactic therapy was started several days before the onset of infection to fully establish any phenotypic changes that may result from downstream gene regulation. Therapeutic treatment was started immediately after the onset of infection to simulate the traditional manner in which corticosteroids have been used to treat septic shock. We demonstrate that the timing and activity of different corticosteroid regimens fundamentally affect the therapeutic benefits of glucocorticoids and mineralocorticoids in septic shock.

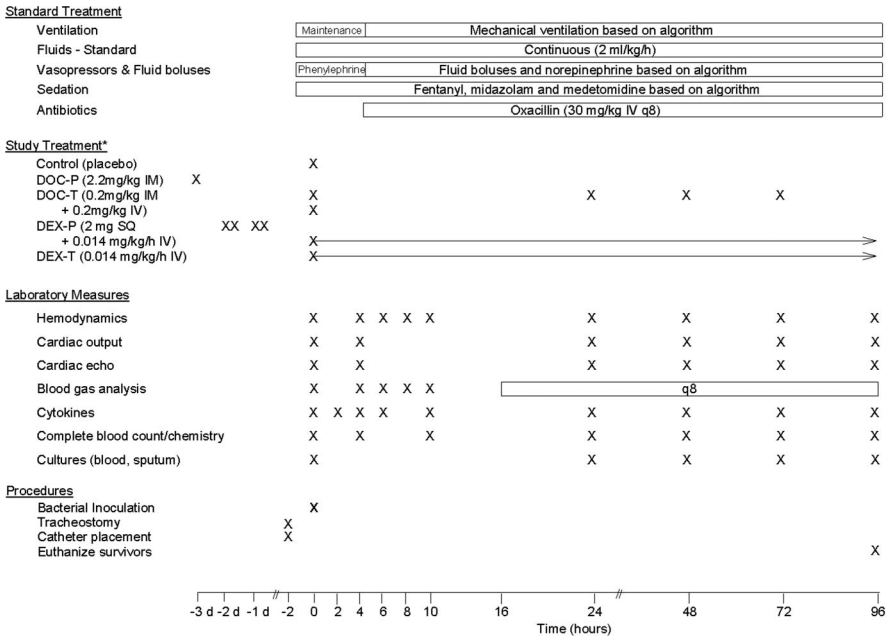

Figure 1. Study protocol. Treatments, laboratory measures, and procedures performed during the course of the 96-hr study. *DOC*, desoxycorticosterone; *DEX*, dexamethasone; *P*, prophylactic; *T*, therapeutic.

METHODS

Study Design. All experiments were performed under protocol approved by the Animal Care and Use Committee of the Clinical Center at the National Institutes of Health. Seventy-four purpose-bred beagles (12–18 months, 10–12 kg) were studied prospectively for 96 hrs using a canine *S. aureus* pneumonia model of sepsis separated in one of four sets of experiments designed to examine and compare the individual effects of DOC and DEX given prophylactically (P) or therapeutically (T) (Fig. 1). To study DOC-P, animals were randomized to receive DOC subcutaneously 72 hrs before bacterial inoculation in a depot preparation that releases drug throughout the experiment (DOC-P; n = 14) or placebo (control; n = 14). For DOC-T, animals were randomized to receive an infusion of DOC or placebo immediately after bacterial challenge followed by daily subcutaneous injections of DOC for the duration of the experiment (DOC-T; n = 6) or placebo (controls; n = 6). To study DEX-P, animals were randomized to receive DEX twice daily subcutaneously or placebo for 48 hrs before bacterial inoculation followed by continuous infusions of DEX (DEX-P; n = 13) or placebo (control; n = 13) for the duration of study. For DEX-T, animals were randomized to receive a continuous infusion of either DEX (DEX-T; n = 12) or placebo (controls; n = 6) starting immediately after bacterial challenge and continuing for the duration of study. DOC was administered in a dose equivalent to that used clinically to treat adrenal insufficiency in canines (22). DEX was administered in a dose that is comparable to the stress dose cortisol therapy (300 mg/day hydrocortisone) used to treat sepsis clinically (5). For a more detailed description of the treatment regimens and dose selection, see "Corticosteroid Dosing" in the Supplementary Methods (see Supplemental Digital Content 1, http://links.lww.com/CCM/A320).

In each set of experiments, animals were allocated such that two treatment animals were always studied with one to two concurrent controls each study week. Technicians and veterinarians responsible for randomizing and caring for animals and for performing all treatments, making clinical decisions, and recording and laboratory measures (Supplementary Methods [see Supplemental Digital Content 1, http://links.lww.com/CCM/A320]) were unaware of (blinded to) the study design. As a result of intensive care unit resources constraints, a maximum of four animals were enrolled in any study week.

Study Protocol. The protocol followed in these experiments has been previously described (20, 21, 23) (Fig. 1 and Supplementary Methods [see Supplemental Digital Content 1, http://links.lww.com/CCM/A320]). At time 0 (T0), baseline blood samples and hemodynamic profiles were obtained (Supplementary Methods [see Supplemental Digital Content 1, http://links.lww.com/CCM/A320]), and then

animals received an inoculation of *S. aureus* (1.5–7.5 × 10^9 colony-forming units/kg) into the right caudal lobe through bronchoscopy (20). During the first 4 hrs after *S. aureus* inoculation, phenylephrine was titrated to maintain mean arterial pressure >80 mm Hg as sedation was optimized and sepsis developed. After 4 hrs, treatment for sepsis was initiated based on algorithms to maintain pressures by titrating norepinephrine, oxygenation by adjusting fractional inspired oxygen concentration, and positive end-expiratory pressure levels and acid-base status by adjusting respiratory rate measured by arterial blood gas. Preload was maintained with fluid boluses based on scheduled pulmonary artery occlusion pressure measures (20). Oxacillin (30 mg/kg intravenously every 8 hrs) was started 4 hrs after bacterial inoculation and administered every 8 hrs thereafter. Conventional intensive care unit support used during the ventilation of critically ill large animals was administered as previously described (20). Animals alive at 96 hrs were considered survivors and subsequently euthanized (Beuthanol [Intervet/Schering-Plough, Summit, NJ]; 75 mg/kg intravenously).

Statistical Methods. Data were analyzed using a Cox proportional hazards model and stratified log rank tests (survival effects); principal component analyses (shock reversal score and pulmonary function score); and linear mixed models (Supplementary Methods [see Supplemental Digital Content 1, http://links.lww.com/CCM/A320]). All p values are two-tailed and considered significant if $p \leq .05$. We report interactions based on two-tailed p values as large as $p = .06$ to limit type 2 errors.

RESULTS

Effects of DOC and DEX Given Prophylactically or Therapeutically on Survival

DOC given prophylactically improved survival compared with controls (stratified log-rank $p = .01$; Fig. 2*A*) but lost its benefit if given therapeutically (stratified log-rank $p = .41$; Fig. 2*C*). In contrast, DEX given prophylactically (DEX-P) had no significant effect on survival compared with controls (stratified log-rank $p = .78$; Fig. 2*B*) but given therapeutically had a survival effect that approached significance for benefit (stratified log-rank $p = .08$; Fig. 2*D*). Notably, when comparing timing of treatment (prophylactic and therapeutic), mineralocorticoids and glucocorticoids had significantly different and opposite effects on survival ($p = .05$ for interaction; Table 1).

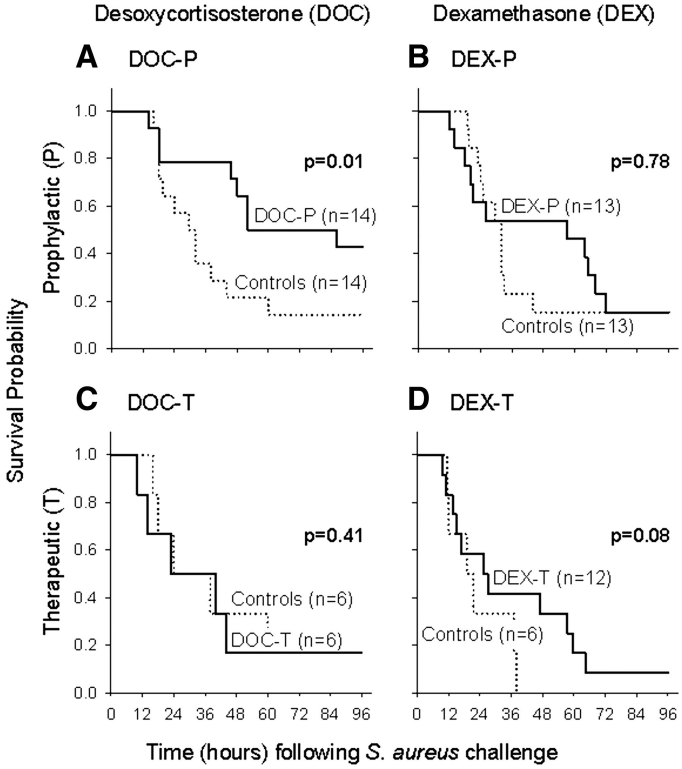

Figure 2. Survival. The treatment effects on survival were different and opposite for mineralocorticoids (DOC) and glucocorticoids (DEX) depending on the timing of administration (see also Table 1; survival log hazards ratio): DOC-P improved survival compared with controls (*A*), whereas DOC-T had no survival benefit (*C*). In contrast, DEX-P had no significant effect on survival compared with controls (*B*), but there was a survival benefit with DEX-T that approached significance (*D*). *DOC*, desoxycorticosterone; *DEX*, dexamethasone; *P*, prophylactic; *T*, therapeutic.

Effects on Shock Reversal

From baseline (0 hr) to 32 hrs after *S. aureus* challenge, there were no significant differences in mean shock reversal score (mean arterial pressure and norepinephrine requirements; see "Statistical Methods" for details) comparing all treatment groups and controls (all, p = nonsignificant; Fig. 3). However, from 32 to 96 hrs after *S. aureus* challenge, DOC-P improved the mean shock reversal score compared with controls ($p = .007$; Fig. 3*A*) and compared with DOC-T-treated animals ($p = .05$; Fig. 3*E*). DOC-T had no significant effect compared with controls on shock reversal during that time period (p = nonsignificant; Fig. 3*C*).

Like DOC-P, DEX-P improved the mean shock reversal score from 32 to 96 hrs compared with controls ($p = .005$; Fig. 3*B*). Unlike DOC-T, DEX-T also tended to improve shock reversal from 32 to 96 hrs, an effect that approached significance compared with controls ($p = .06$; Fig. 3*D*). Shock reversal with DEX-P compared with DEX-T was markedly similar from 32 to 96 hrs ($p = .41$; Fig. 3*F*), and when combined, shock reversal attributable to DEX regardless of time administered was significantly improved compared with controls ($p = .003$; Fig. 3*F*).

For completeness, the effects of each corticosteroid treatments on individual components of the score are shown in Figure E1 in the online data supplement (see Supplemental Digital Content 2, http://links.lww.com/CCM/A321).

Effects on Pulmonary and Cardiac Function

Pulmonary. Before *S. aureus* challenge (0 hr) to 12 hrs after, there were no significant differences in mean lung injury score ($A\text{-}aO_2$, plateau pressure, peak airway pressure, SaO_2, and respiratory rate; see "Statistical Methods" for details) comparing all treatment groups and controls (all, p = nonsignificant; data not shown). However, from 12 to 96 hrs after *S. aureus* challenge, the effect of corticosteroids on the mean lung injury score changed when given prophylactically vs. therapeutically ($p = .06$ for interaction; Table 1). Specifically, DOC-P-treated animals had less severe lung injury

Table 1. Parameters in which treatment effects after *Staphylococcus aureus* challenge changed in a different manner with mineralocorticoid vs. glucocorticoid therapy depending on timing of administration (prophylactically vs. therapeutically)

Parameter, Mean ± SEM or 95% Confidence Interval	DOC			DEX				
	Prophylactic (DOC–prophylactic)	Therapeutic (DOC–therapeutic)	Mean Difference	Prophylactic (DEX–prophylactic)	Therapeutic (DEX–therapeutic)	Mean Difference	*p* Value Interaction	All Controls Mean Value
Survival log hazards ratio	−0.96 ± 0.55	0.25 ± 0.72	−1.21	−0.17 ± 0.54	−1.17 ± 0.71	+1.00	$p = .05$	N/A
Pulmonary function score 12–96 hrs	0.95 ± 1.35	2.21 ± 1.18	−1.26	2.21 ± 0.98	1.89 ± 1.10	+0.32	$p = .06$	2.08 ± 1.22
Fluid input, mL/kg/hr, over 96 hrs	5.7 ± 0.3	6.5 ± 0.7	−0.8	6.0 ± 0.4	4.8 ± 0.5	+1.2	$p = .05$	6.0 ± 0.3
Central venous pressure, mm Hg, 4–12 hrs	5.1 ± 0.4	3.9 ± 0.3	+1.2	4.2 ± 0.3	5.4 ± 0.4	−1.2	$p = .007$	4.6 ± 0.3
Central venous pressure, mm Hg, 12–30 hrs	5.6 ± 0.5	4.4 ± 0.6	+1.2	4.6 ± 0.6	6.4 ± 0.7	−1.8	$p = .005$	4.7 ± 0.4
Serum sodium, mEq/L, at 10 hrs	142.9 ± 1.2	147.3 ± 0.5	−4.4	145.3 ± 0.8	141.9 ± 0.7	+3.4	$p = .0004$	143.0 ± 0.5
Lactate, mmol/L, at 10 hrs	1.21 ± 0.24	0.72 ± 0.11	+0.49	2.74 ± 0.44	1.02 ± 0.10	+1.72	$p = .03$	1.00 ± 0.10
Hemoglobin, g/dL, at 10 hrs	14.7 ± 0.8	16.9 ± 1.6	−2.2	15.4 ± 0.6	14.3 ± 1.6	+1.1	$p = .06$	16.8 ± 0.6
Albumin, g/dL, at 10 hrs	1.96 ± 0.08	1.98 ± 0.012	−0.02	2.18 ± 0.05	1.96 ± 0.05	+.22	$p = .05$	2.04 ± 0.05
Platelets, cells $\times 10^3$, at 10 hrs	245 ± 27	313 ± 31	−68	248 ± 16	170 ± 30	+78	$p = .02$	231 ± 14
Left ventricular ejection fraction, %, at 10 hrs	0.45 ± 0.04	0.39 ± 0.03	+0.06	0.39 ± 0.02	0.49 ± 0.02	−0.10	$p = .05$	0.41 ± 0.2
Interleukin-6, pmol/L, $\log_{10}$ scale, at 10 hrs	3.91 ± 0.08	4.0 ± 0.03	−0.09	4.21 ± 0.06	3.94 ± 0.13	+0.27	$p = .04$	4.06 ± 0.04

DOC, desoxycorticosterone; DEX, dexamethasone.

from 12 to 96 hrs after *S. aureus* challenge compared with DOC-T-treated animals, whereas DEX-P-treated animals had worse lung injury than DEX-T-treated animals during that same time period. For completeness, the effects of each corticosteroid treatment for individual components of the score are shown in Figure E2 in the online data supplement (see Supplemental Digital Content 3, http://links.lww.com/CCM/A322).

Cardiac. The effects of corticosteroids on mean left ventricular ejection fraction were also different given prophylactically compared with therapeutically by 10 hrs after *S. aureus* challenge ($p = .05$ for interaction; Table 1). Specifically, DOC-P-treated animals had a higher mean left ventricular ejection fraction than DOC-T-treated animals at 10 hrs, whereas DEX-P-treated animals had a lower mean left ventricular ejection fraction than DEX-T-treated animals at this time point. There were no other significant differences on mean left ventricular ejection fraction throughout the study (all, p = nonsignificant; data not shown).

Effects on Fluid Status and Hemoconcentration

Fluid Status. Mean fluid requirements and central venous pressure changed when corticosteroids were given prophylactically vs. therapeutically ($p = .05$ and $p \leq .005$ for interaction, respectively; Table 1): DOC-P-treated animals had higher mean central venous pressures from 4 to 24 hrs and required less fluids throughout the study to maintain systemic and cardiac filling pressures at predetermined levels than DOC-T, whereas DEX-P-treated animals had lower central venous pressures and required more fluids than DEX-T to maintain these pressures. DOC-P-treated animals also retained more fluids than both controls and DOC-T-treated animals over the 96 hrs after *S. aureus* challenge (101.5 ± 16.4 mL/kg/24 hrs vs. 63.4 ± 11.3 mL/kg/24 hrs and 19.0 ± 1.6 mL/kg/24 hrs, respectively; $p = .05$ and $p = .03$, respectively), but there were no other significant differences in mean fluid retention throughout the study (all, p = nonsignificant; data not shown). Because pulmonary artery occlusion pressure was to be maintained using fluid boluses at ≥10 mm Hg in all animals throughout the 96-hr experiment study per protocol, as expected, there were few differences in this measure throughout the study.

Hemoconcentration. Consistent with these fluid status findings, DOC-P-treated animals had lower mean hemoglobin, sodium, and albumin concentrations by 10 hrs after infection compared with DOC-T-treated animals, whereas DEX-P-treated animals had higher concentrations of hemoglobin, sodium, and albumin compared with DEX-T-treated animals at this time point ($p = .06$, $p = .0004$, and $p = .05$ for interaction, respectively; Table 1). In addition, DOC-P-treated animals had a lower mean hemoglobin concentration compared with controls at 24 hrs (14.8 ± 0.9 g/dL vs. 17.5 ± 0.7 g/dL; $p = .03$), and DEX-P-treated animals had lower mean serum sodium concentrations than controls from 12 to 96 hrs after infection (139.6 ± 1.0 mEq/L vs. 143.3 ± 1.0 mEq/L; $p = .02$). There were no other significant differences in mean hemoglobin, sodium, or albumin concentrations throughout the study (all, p = nonsignificant; data not shown).

Effects on Adrenal Function

Before *S. aureus* challenge (0 hr), there were no significant differences in mean aldosterone or cortisol levels comparing all treatment groups and controls (all, p = nonsignificant; Fig. 4).

Aldosterone. DOC-P-treated animals had lower mean aldosterone levels at 10 hrs ($p = .01$) and 24 hrs ($p = .05$) after *S. aureus* challenge compared with controls (Fig. 4*IA*). In contrast, DOC-T-treated an-

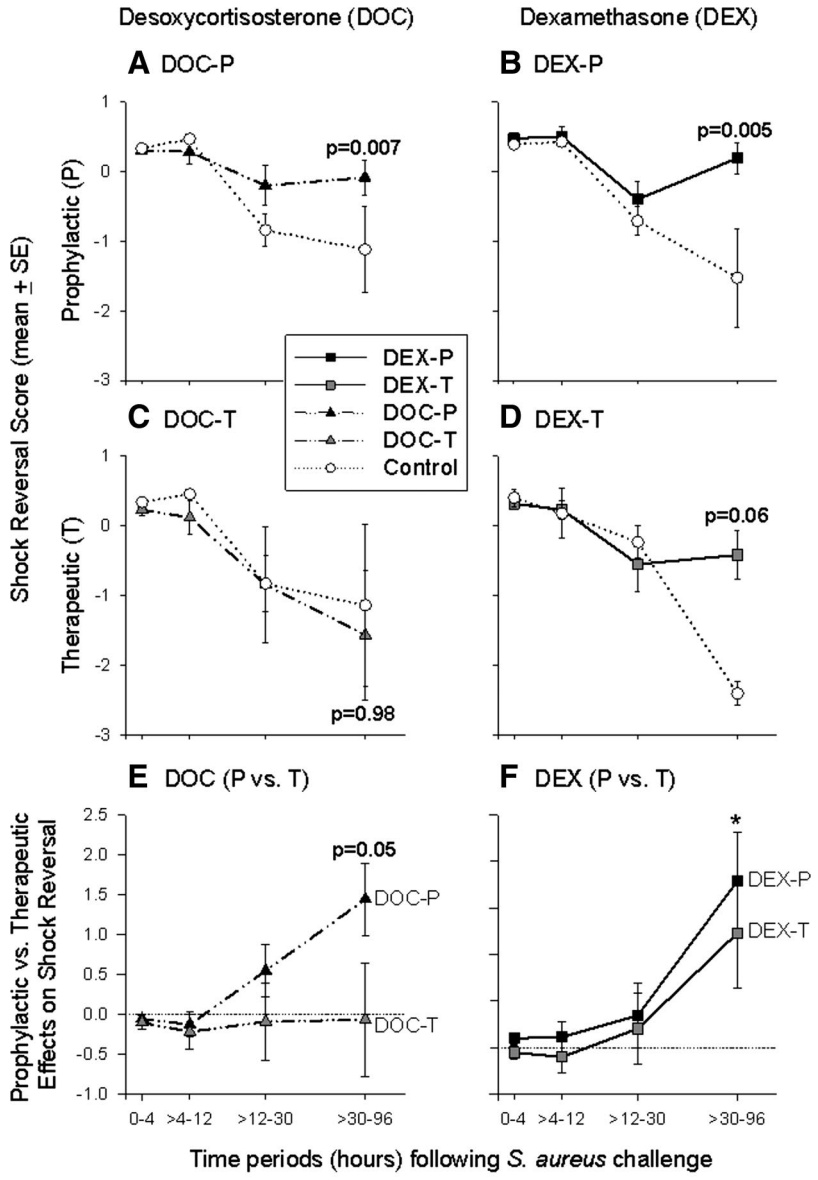

Figure 3. Shock reversal. DOC-P improved the mean shock reversal score (calculated from mean arterial pressure and norepinephrine requirements; see "Statistical Methods" for details) compared with both controls (*A*) and DOC-T-treated animals (*E*) from 32 to 96 hrs after *Staphylococcus aureus* challenge, whereas DOC-T had no significant effect compared with controls on shock reversal during that time period (*C*). DEX-P improved the mean shock reversal score from 32 to 96 hrs compared with controls (*B*), and there was also an improvement in the shock reversal score with DEX-T that approached significance compared with controls during this time period (*D*). Shock reversal with DEX-P compared with DEX-T was markedly similar from 32 to 96 hrs and when combined was significantly improved compared with controls (*F*). (See Figure E1 for the individual components of shock reversal score over time by treatment group [Supplemental Digital Content 2, http://links.lww.com/CCM/A321].) *DOC*, desoxycorticosterone; *DEX*, dexamethasone; *P*, prophylactic; *T*, therapeutic.

imals had no significant differences in mean aldosterone levels compared with both controls and DOC-P-treated animals throughout the study (all, p = nonsignificant; Fig. 4*IC* and *E*). DEX-P significantly reduced mean aldosterone concentration at 10 hrs after *S. aureus* challenge compared with both controls ($p = .002$; Fig. 4*IB*) and DEX-T-treated animals ($p = .003$; Fig. 4*IF*). DEX-T had no significant effects on mean aldosterone concentration compared with controls throughout the study (all, p = nonsignificant; Fig. 4*ID*).

Cortisol. DOC-P- and DOC-T-treated animals had no significant differences in mean cortisol concentrations compared with controls (all, p = nonsignificant; Fig. 4*IIA, C*, and *E*). In contrast, DEX-P reduced mean cortisol levels compared with controls at 10 hrs ($p < .0001$) and 24 hrs ($p = .006$) after *S. aureus* challenge (Fig. 4*IIB*) and also compared with DEX-T-treated animals at 10 hrs after *S. aureus* challenge ($p = .003$; Fig. 4*IIF*). DEX-T had no significant effects on mean cortisol levels compared with controls throughout the study (all, p = nonsignificant; Fig. 4*IID*).

Effects on Platelet Counts, Temperature, Lactate, and Cytokines

Mean platelet counts, serum lactate concentrations, and interleukin-6 (IL-6) levels changed when corticosteroids were given prophylactically vs. therapeutically ($p = .02$, $p = .03$, and $p = .04$ for interaction, respectively; Table 1): DOC-P-treated animals had lower platelet counts and IL-6 levels and similar lactate concentrations compared with DOC-T-treated animals at 10 hrs after infection, whereas DEX-P-treated animals had higher platelet counts and IL-6 levels and markedly increased mean serum lactate concentrations compared with DEX-T-treated animals at this time point. DEX-P-treated animals also had higher mean body temperatures compared with controls at 10 hrs ($p = .05$) and DEX-T-treated animals at 10 hrs and 24 hrs (both, $p \leq .03$); and higher mean IL-10 levels compared with DEX-T at 10 hrs ($p = .01$). However, both DEX-P- and DEX-T-treated animals had lower mean IL-10 concentrations than controls at 24 hrs (both, $p = .05$). For the sake of brevity, a more detailed explanation of the treatment effects on platelet counts and inflammatory measures (i.e., lactate concentrations, body temperature, and IL-6 and IL-10 levels) as well as a presentation of white blood cell counts can be found in the Supplementary Results (see Supplemental Digital Content 1, http://links.lww.com/CCM/A320).

Effects on Bacteriology

During the 96 hrs after *S. aureus* challenge, the number of positive blood cultures over the number collected for each treatment group were as follows: DEX-P: two of 34 (6%); DEX-T: seven of 25; (28%); DOC-P: five of 44 (11%); DOC-T: zero of 12 (0%); and controls: 11 of 53

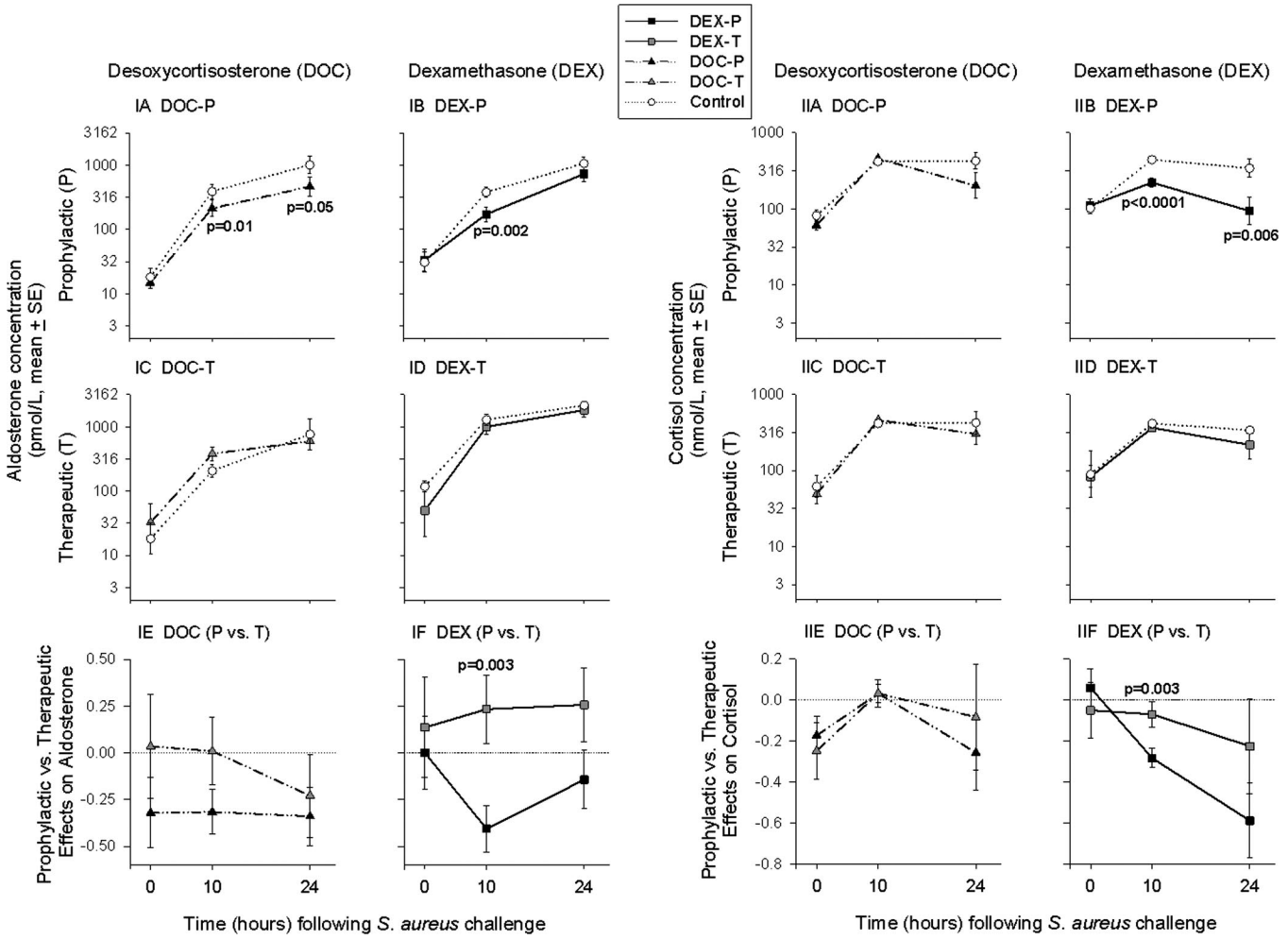

Figure 4. Aldosterone and cortisol. DOC-P-treated animals had lower mean aldosterone levels at 10 hrs and 24 hrs after *Staphylococcus aureus* challenge compared with controls (*IA*), whereas DOC-T-treated animals had no significant differences in mean aldosterone levels compared with both controls (*C*) and DOC-P-treated animals (*E*) throughout the study. DOC-P- and DOC-T-treated animals had no significant differences in mean cortisol concentrations compared with controls or with each other throughout the study (*IIA, C, E*). DEX-P significantly reduced mean aldosterone concentration at 10 hrs after *S. aureus* challenge compared with both controls (*IB*) and DEX-T-treated animals (*IF*), whereas DEX-T had no significant effects on mean aldosterone concentration compared with controls throughout the study (*ID*). Similarly, DEX-P reduced mean cortisol levels compared with controls at 10 hrs and 24 hrs after *S. aureus* challenge (*IIB*) and also compared to DEX-T-treated animals at 10 hrs after *S. aureus* challenge (*IIF*). DEX-T had no significant effects on mean cortisol levels compared with controls throughout the study (*IID*). *DOC*, desoxycorticosterone; *DEX*, dexamethasone; *P*, prophylactic; *T*, therapeutic.

(21%). DEX-T-treated animals had a higher rate of positive blood cultures compared with DEX-P ($p = .03$), but there were no other significant differences in the rate of positive blood cultures or the number of colony-forming units in the blood over the 96-hr study. There were also no significant differences in the probability of positive sputum cultures for *S. aureus* comparing all treatment groups and controls throughout the study (p = nonsignificant for all).

DISCUSSION

In a canine model of bacterial pneumonia and septic shock, two different corticosteroid therapies were tested: a selective mineralocorticoid agonist and a selective glucocorticoid agonist, each administered both prophylactically and therapeutically. The mineralocorticoid was only beneficial when administered prophylactically, whereas the glucocorticoid was primarily beneficial when given therapeutically close to the onset of infection.

DOC given for 3 days before infection not only increased overall survival, but also lowered fluid requirements, improved central venous pressure, reversed shock, lessened pulmonary and cardiac dysfunction, and caused greater fluid retention. Furthermore, prophylactic mineralocorticoid therapy reduced signs of hemoconcentration, lowering serum sodium, hemoglobin, platelet, and albumin concentrations. Taken together, these data suggest that DOC-P-treated animals were better able maintain intravascular volume compared with those that received DOC later, indicating that important changes may have occurred before bacterial challenge as a result of early DOC pretreatment.

Several known effects of mineralocorticoids may explain the benefits of early mineralocorticoid treatment observed in the present study. Mineralocorticoid receptor signaling regulates sodium channels and other genes in the vasculature (17) that maintain endothelial function and integrity (24), possibly reducing capillary leak and increasing intravascular volume in severe sepsis. In addition, DOC may increase venous smooth muscle tone

through actions on endothelin-1 expression, calcium channel signaling, and sympathetically mediated venoconstriction (25, 26). Because intravascular volume depletion is a potent stimulus for aldosterone production and secretion, DOC-P-associated reductions in this hormone are consistent with increased intravascular volume. Improved lung function may also reflect a mineralocorticoid-stimulated increase in alveolar sodium and water clearance (27); improving alveolar sodium clearance has been proposed as a therapeutic strategy for treating acute lung injury (28). DOC-P also had modest anti-inflammatory effects, manifested by reduced IL-6 concentrations, which may have contributed to improved hemodynamics and organ function. These findings are consistent with *in vitro* work demonstrating mineralocorticoid receptor-mediated suppression of nuclear factor-κB signaling (16), a major transducer of the innate immune response, and aldosterone-mediated inhibition of neutrophil-induced intercellular adhesion molecule-1 expression on endothelial cells (29). Such targeted anti-inflammatory effects may have limited endothelial injury and organ dysfunction, thereby contributing to improved cardiovascular function and survival.

Lack of benefit with DOC starting late after infectious challenge may relate to the length of time required to establish some mineralocorticoid effects in the vasculature. Importantly, continuous intravenous and intracerebroventricular aldosterone infusions do not produce significant blood pressure increases in healthy canines until 72 hrs after administration (30, 31), indicating that mineralocorticoid-induced changes in vascular function take time to develop. Of note, the onset of shock in our canine sepsis model is rapid, developing within hours of bacterial challenge. The short latency to severe illness, together with the prolonged time required for mineralocorticoids to increase mean arterial pressure in normal animals, suggests that starting DOC after bacterial challenge would likely be too late to affect outcome.

In contrast to our findings with DOC, treatment with DEX significantly reversed shock regardless of timing of administration. Furthermore, directly opposite to our mineralocorticoid findings, glucocorticoid therapy improved survival and reduced pulmonary and cardiac dysfunction when given therapeutically rather than prophylactically. DEX-T-treated animals required less fluids to maintain predetermined systemic pressures, had higher cardiac filling pressures (i.e., central venous pressure), and showed reduced signs of hemoconcentration (i.e., lower hemoglobin, platelet, and albumin concentrations) compared with control animals or those that received DEX-P. Adrenal suppression with loss of mineralocorticoid activity (32) may in part explain why prophylactic DEX failed to provide benefit. Starting almost immediately after bacterial challenge, animals that received DEX-P had significantly lower total basal cortisol and aldosterone concentrations compared with controls. In contrast, these stress hormones were not significantly affected by DEX given immediately after bacterial challenge. Although giving DEX provides full glucocorticoid activity, it cannot replace the profound loss of mineralocorticoid activity caused by the suppression of both cortisol and aldosterone (33). Notably, clinical trials have demonstrated that critically ill patients with hypoaldosteronism suffer from persistent hypotension and higher mortality rates both with and without corticosteroid therapy (34–36). Animals pretreated with DEX not only were less able to maintain intravascular volume, but also had significantly higher serum lactate levels and body temperatures compared with controls at 10 hrs after infection, all consistent with suppressed adrenal function and worse shock. Thus, the suppression of endogenous mineralocorticoid activity after infection as a result of prolonged glucocorticoid therapy may have counteracted the benefits of DEX observed with DEX-T therapy.

Unexpectedly, the differential effects of DEX given prophylactically compared with therapeutically were also evident with regard to measures of anti-inflammatory activity: DEX given after infection somewhat lowered circulating IL-6 and IL-10 concentrations compared with DEX given prophylactically. Despite higher rates of positive blood cultures, overall outcomes were still better with DEX-T compared with DEX-P. During septic shock, the anti-inflammatory effects of glucocorticoids have been reported to play a significant role in their beneficial effects (37), and DEX-T may likewise have had a similar benefit in our study.

Consistent with our shock reversal findings with DEX given pre- vs. postinfectious challenge, a study by Mansart et al (38) found that dexamethasone given early or late was associated with reversal of hypotension in a cecal ligation and perforation murine model of sepsis. Also similar to our study, the improvement of blood flow in this sepsis model was more substantial with late treatment. In contrast, a study by Ottoson et al (39) showed that single doses of either dexamethasone given 2 hrs before *Escherichia coli* challenge in a rat model increased survival time, and this effect decreased linearly with later administration of dexamethasone up to 8 hrs after bacterial challenge. More recently, in a rat subcutaneous group B streptococcal-challenged model, a study by Tran et al (40) showed a significant mortality benefit for animals receiving dexamethasone 24 hrs before bacterial challenge but no survival benefits with dexamethasone given either 30 mins or 24 hrs after challenge. However, pretreatment in these studies was given closer to bacterial challenge than in our study and the type of pathogen used, site of infection, ancillary therapies given, and animal species studied were also different.

Our finding that DEX given therapeutically demonstrated a trend toward a survival benefit is different from the results of Corticosteroid Therapy of Septic Shock (CORTICUS), the largest and most recent sepsis steroid trial to date (9). The CORTICUS trial (n = 499) reported no overall survival benefit of hydrocortisone for sepsis. However, metaregression analysis has suggested that corticosteroids have a survival benefit only in septic patients at high risk of death (7). The control mortality of the CORTICUS study was only 31% within 28 days compared with an expected 40% based on clinical sepsis trials reporting salutatory effects of corticosteroids and 90% within 4 days in the current study. The CORTICUS study presents strong evidence that, in low-risk sepsis patients, physiological-dose corticosteroids provide no benefit and may increase risk of morbidity. The effect of corticosteroids in high-risk sepsis patients is unresolved. Of note, the CORTICUS trial did report a shorter time to shock reversal among all patients receiving hydrocortisone (9), which is consistent with the shock reversal effects that we observed with DEX.

The limitations of this study warrant discussion. Only two agents were tested, one for each corticosteroid receptor, at fixed doses, and only one pre- and one postinfectious timing of administration were used. Different doses of DEX or DOC or other glucocorticoid or mineralocorticoid receptor ligands might alter the efficacy of these approaches. Notably, different ligands can produce selective conforma-

tional changes in steroid receptors that alter their gene targets and biological activity (41). Likewise, the activation of glucocorticoid and mineralocorticoid receptors simultaneously might have effects different from expected from studying either in isolation. Regardless, the efficacy of selective mineralocorticoid and glucocorticoid agonists in septic shock differs in regard to optimal timing.

In conclusion, in a canine model of *S. aureus* pneumonia-induced septic shock using pulmonary and cardiovascular support measures similar to those used clinically, mineralocorticoids lessened fluid requirements, increased central venous pressures and fluid retention, prevented hemoconcentration, reversed shock, and improved survival and cardiopulmonary dysfunction, but only if given prophylactically. In contrast, glucocorticoids reversed shock independent of timing of administration but had markedly less beneficial effects on survival and other measures of organ function if they were given before rather than after infectious challenge. Applied clinically, our data suggest that selective mineralocorticoids should be investigated as a potential prophylactic agent for patients at high risk for septic shock (e.g., patients with neutropenic cancer receiving chemotherapy, high-risk surgery patients with abdominal infections, etc.) to improve salt and water metabolism, prevent shock, and lessen cardiopulmonary dysfunction and mortality. In contrast, glucocorticoids appear to be essential for reversing shock in septic patients. However, prolonged early glucocorticoid therapy should be avoided as a result of a risk of adrenal suppression-associated worse outcomes. Furthermore, supplemental therapy with mineralocorticoid agonists should be considered for patients at high risk to develop sepsis who are on glucocorticoids for other reasons.

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