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Stress-dose corticosteroids hinder bacterial clearance but improve survival after high-dose bacterial challenges in a canine model of septic shock

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Abstract

Purpose—The effects of stress-dose corticosteroid therapy were studied in a staphylococcal pneumonia model of septic shock.

Methods—Immediately following intrabronchial bacterial challenge, purpose-bred beagles were treated with stress doses of desoxycorticosterone (DOC), a mineralocorticoid agonist and dexamethasone (DEX), a glucocorticoid agonist, or placebo for 96 hours. Oxacillin (30 mg/kg q8h) was started 4 hours after infection onset. Bacterial dose was titrated to achieve 80–90% lethality (n=20) using an adaptive design; additional animals (n=18) were investigated using the highest bacterial dose.

Results—Initial analysis of all animals (n=38) demonstrated that effects of DOC+DEX were significantly altered by bacterial dose (p=0.04). Dividing the animals into high- and low-bacterial dose groups, treatment effects of DOC+DEX were different for survival (p=0.05), shock reversal

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($p=0.02$), IL-6 levels ($p=0.02$), and temperature ($p=0.01$). DOC+DEX significantly improved the above parameters (all, $p < 0.03$) and lung injury scores ($p=0.02$) after high-dose bacterial challenges, but not after lower challenges (all, $p=ns$). Oxacillin trough levels were below the minimum inhibitory concentration of the infecting organism, and DOC+DEX increased the frequency of persistent staphylococcal bacteremia [OR 3.09 (1.05–9.11); $p=0.04$].

Conclusions—Stress-dose corticosteroids were only beneficial in sepsis with high risk for death and may interfere with host mechanisms of bacterial clearance.

Keywords

Shock: experimental studies; SIRS/Sepsis: experimental studies; antimicrobial agents; cardiopulmonary resuscitation; host defenses against pathogens; pulmonary nosocomial infections

Introduction

Over the last decade, the concept that relative adrenal insufficiency contributes to the poor outcome of septic shock prompted clinical trials of physiologic stress-dose corticosteroid therapy [i.e. 200 to 300 mg of hydrocortisone (or equivalent) per day given for 5 to 11 days]. The effect of corticosteroids on survival varied across these trials [1, 2]. Meta-regression analysis suggested that stress-dose corticosteroids might confer a survival benefit only in septic shock patients at high risk for death [3, 4]. Notably, sepsis-associated risk of death has been shown to similarly influence the efficacy of mediator-specific anti-inflammatory therapies [5]. In the case of physiologic stress-dose corticosteroids however, the finding that treatment efficacy was dependent on severity of illness was based on 12 relatively small trials (median 44 patients, IQR 41–44) potentially confounded by publication bias [3]. Therefore despite an apparent ameliorative effect on vasopressor-dependent shock during sepsis, the lack of a reproducible survival benefit has made the use of stress-dose corticosteroids in sepsis controversial.

Glucocorticoid-associated increases in secondary infections or impairment of bacterial clearance may underlie the variable results on survival observed with corticosteroid therapy. Neutrophils from murine models subjected to stress alone exhibited impaired bacterial killing *ex vivo* [6]. Restraint stress in murine models delayed wound healing and increased the bacterial load of opportunistic pathogens such as *Staphylococcus aureus* [7]. In this model, pre-treatment with a glucocorticoid receptor antagonist reduced bacterial wound infections [7], suggesting that glucocorticoids released by the hypothalamic-pituitary-adrenal (HPA) axis may be in part responsible for these observations. Corticosteroids over a wide range of glucocorticoid activity have been shown to impair host bacterial clearance from the lung [8] and blood [9] in animal models, although their precise effects in clinical sepsis are less clear [3, 10, 11]. Particularly in low-risk septic shock patients, an increased risk of infection with corticosteroid therapy may outweigh any benefits from reduced dependence on vasopressors. This could in part explain the variable mortality benefit reported in clinical sepsis trials [2, 3].

In an attempt to better understand the beneficial effects of corticosteroid therapy in septic shock, we previously investigated the effects of two different selective corticosteroid

agonists in a canine model of *Staphylococcus aureus* pneumonia [12]. When administered alone, the glucocorticoid dexamethasone (DEX) given at the onset of infection reversed shock and produced a beneficial trend on survival. However, the mineralocorticoid desoxycorticosterone (DOC) was only beneficial when administered a few days before bacterial challenge. In the present study, we examined the effects of combination corticosteroid therapy (DOC + DEX) in the same model. DOC + DEX were given together at the onset of sepsis to simulate common clinical practice [13] (i.e. hydrocortisone has mixed mineralocorticoid and glucocorticoid agonist effects [14]). In the initial phase of these experiments, the dose of bacterial challenge was titrated using a prospective adaptive design to create a highly lethal model (lethality rate of 80 to 90%).

Methods

Study design

All studies described below were approved by the Animal Care and Use Committee of the Clinical Center at the National Institutes of Health. Thirty-eight purpose-bred beagles (12–28 month, 10–12 kg) were studied prospectively comparing dexamethasone and desoxycorticosterone administered together (DOC + DEX) to placebo. In one study, animals were challenged with increasing doses of *S. aureus* (n=20) to achieve a final control mortality of 80–90%. In a second study, animals were given the same corticosteroid regimen but challenged only with the high mortality-producing dose of *S. aureus* (1.5×10^9 cfu/kg; n=18) to directly address the hypothesis that corticosteroids are efficacious in severe sepsis with high lethality. These studies were analyzed together to use all available data from animals receiving this regimen (total n = 38; see online Supplementary Methods). Bacteria challenges were administered intrabronchially at time 0 (T0) as previously described [13], and DOC + DEX or placebo treatment was started according to randomization allocation (n=19 for each group). Corticosteroids were started immediately after bacterial challenge in a therapeutic design to increase our chances of finding an effect. Corticosteroid dosing was designed to be comparable to physiologic stress doses in humans [11] (see online e Supplementary Methods). Technicians and veterinarians responsible for randomizing and caring for animals and for performing all measures were blinded to the study design. A maximum of four animals were randomized in any study week.

Study Protocol

Prior to infectious challenge, animals had a tracheostomy, external jugular vein introducer, and percutaneous femoral artery and urinary catheters placed under general anesthesia [13]. Following these procedures, animals were weaned off anesthesia; continuous sedation (fentanyl, midazolam, and medetomidine; see online Supplementary Methods) and mechanical ventilation via the tracheostomy tube were initiated; and a balloon-tipped pulmonary arterial thermodilution catheter was placed via the introducer. At time 0 (T0), animals underwent bronchoscopy and inoculation of the right lower (caudal) lobe with *S. aureus* (see online Supplementary Methods).

During the first 4 h after *S. aureus* inoculation, phenylephrine was titrated to maintain mean arterial pressure (MAP) >80 mmHg as sedation was optimized and sepsis developed. After 4

h, when symptoms of sepsis were more fully developed, phenylephrine administration was discontinued and intravascular hemodynamic measures and blood samples were obtained (see online Supplementary Methods). Treatment for sepsis was then initiated with fluid boluses and norepinephrine (NE) infusion titrated based on algorithms driven by measurements of pulmonary artery occlusion pressure (PAOP) and MAP, respectively (see online Supplementary Methods). Fractional inspired oxygen concentration (FiO₂), respiratory rate (RR), and positive end expiratory pressure (PEEP) levels were titrated based on algorithms incorporating scheduled arterial blood gas measurements to determine partial pressure of CO₂ and oxygen saturation (SaO₂) as previously described [13]. Oxacillin (30 mg/kg IV q 8 h), dosed based on standard veterinary practice, [15] was started 4 h after bacterial inoculation and continued until the end of the 96 h experiment. Conventional intensive care unit support employed during the ventilation of critically ill large animals was administered as previously described [13]. Animals alive at 96 h were considered survivors and then euthanized (Beuthanol; 75 mg/kg IV) while still sedated.

Statistical Methods

Data from the two studies were pooled to use all available data. A likelihood ratio test demonstrated an interaction between *S. aureus* challenge dose and treatment on survival, and so bacterial doses were divided into two groups that minimized the within-group variation (see online Supplementary Methods). Data was analyzed using exact log rank tests (Figure 1); F tests (baseline characteristics); and linear mixed models (Figures 2–4). All p-values are two-tailed and considered significant if $p \leq 0.05$.

Results

Survival

The effect of DOC + DEX on survival was significantly altered by bacterial dose ($p = 0.04$, likelihood ratio test). We then divided the animals into high-dose [1.35 to 1.5×10^9 cfu/kg ($n = 26$)] and lower-dose [1.05 to 1.2×10^9 cfu/kg ($n = 12$)] groups that minimized the within-group dose variation. When comparing high vs. lower doses of bacteria, giving DOC + DEX had significantly different effects on survival (Cox model, $p = 0.05$ for interaction): DOC + DEX given after high doses of bacteria improved survival compared to controls (stratified log-rank test $p = 0.03$; Fig. 1A), but not after lower doses (stratified log-rank $p = 0.69$; Fig. 1B).

Bacteriology

DOC + DEX-treated animals compared to controls had more positive *S. aureus* blood cultures after both high- [16/38 (42%) vs. 4/24 (17%), respectively] and low-dose [8/19 (42%) and 3/19 (16%), respectively] bacterial challenges [overall OR 3.09 (95% CI 1.05 – 9.11); $p = 0.04$; Fig. 2]. In contrast, the number of positive tracheal aspirate cultures for *S. aureus* were similar comparing DOC + DEX-treated animals and controls after both high- [30/37 (81%) vs. 19/22 (86%) for DOC + DEX vs. controls, respectively; $p = \text{ns}$] and low-dose [8/19 (42%) vs. 11/19 (58%) respectively; $p = \text{ns}$] bacterial challenges. Likewise, there were no significant differences in bacterial colonization and/or possible superinfections, as determined by urine, catheter insertion site, and tracheal aspirate cultures, comparing DOC +

DEX treated animals to controls at high or lower doses of *S. aureus* alike throughout the study (all, $p = \text{ns}$; data not shown).

Oxacillin levels—Because the large percentage of positive blood cultures was unexpected, oxacillin levels were measured on stored samples from a subset of these experimental animals and supplemented with samples from similarly dosed septic animals in other studies (see *Physiological and Laboratory Measurements*, above). At 10 h after *S. aureus* challenge [i.e. 6 h after receiving the initial oxacillin dose ($n=48$)], oxacillin blood levels were [median (interquartile range, IQR)] 0 (0, 0) $\mu\text{g/mL}$ regardless of *S. aureus* dosing or treatment with DOC + DEX. At 24 h after *S. aureus* challenge [i.e. 4 h after the third oxacillin dose ($n = 35$)], oxacillin blood levels were 2.5 (0.0, 7.1) $\mu\text{g/mL}$. At 48 h after *S. aureus* challenge [i.e. 4 h after the sixth oxacillin dose ($n = 20$)], oxacillin blood levels were 5.0 (2.5, 30) $\mu\text{g/mL}$. The measured MIC for oxacillin was 0.5 $\mu\text{g/mL}$. These data indicate that, despite dosing per standard veterinary practice [15], oxacillin levels were likely inadequate at the end of each dosing interval, particularly early in the experiment. Marginal oxacillin dosing likely unmasked corticosteroid-associated impairment of bacterial clearance as manifested by persistently blood cultures.

Shock reversal and lung injury score

At baseline (T0) before *S. aureus* challenge, there were no significant differences in mean shock reversal scores and lung injury scores comparing treatment groups and controls ($p = \text{ns}$ for all; Fig. 3). When comparing the high vs. lower doses of *S. aureus*, DOC + DEX had different effects on shock reversal ($p = 0.02$ for interaction): from 12 to 96 h DOC + DEX improved mean shock reversal scores compared to controls after high dose *S. aureus* challenge ($p = 0.009$; Fig. 3A), but did not significantly alter shock reversal after lower dose challenge ($p = \text{ns}$; Fig. 3C). DOC + DEX also improved mean lung injury scores from 12 to 96 h with high- ($p = 0.02$; Fig. 3B) but not low-dose ($p = \text{ns}$; Fig. 3D) bacterial challenges. However, the differences in effect of DOC + DEX on lung injury score at high vs. lower doses of *S. aureus* challenge did not reach statistical significance ($p = \text{ns}$). For completeness, the effects of DOC + DEX at high and lower doses of bacteria on individual components of the shock reversal and lung injury scores are shown in Fig. E1 of the online data supplement.

Cytokines and temperature

At high vs. lower doses of *S. aureus* challenge, DOC + DEX had different effects on IL-6 levels and temperature ($p = 0.02$ and $p = 0.01$, respectively): DOC + DEX suppressed the rise in IL-6 levels from 4 to 30 h after high dose *S. aureus* challenge ($p = 0.003$; Fig. 4A) and suppressed the rise in temperature from 12 to 96 h compared to controls ($p = 0.02$; Fig. 4B). However, DOC + DEX did not significantly alter IL-6 levels or temperature with lower dose *S. aureus* challenge (both, $p = \text{ns}$; Fig. 4C and D). Examining all 38 animals studied, IL-6 levels at 10 and 24 h after infection were negatively correlated with survival ($r = -0.46$ $p = 0.004$; and $r = -0.83$, $p < 0.0001$, respectively). This correlation showed a similar strong negative correlation in subgroup analysis regardless of dose of bacteria or treatment group examined (see Fig. E2 of online data supplement). There were no significant effects of DOC

+ DEX at high or lower doses of bacteria on mean IL-10 levels compared to controls throughout the study (all, $p = \text{ns}$; data not shown).

Cardiac function, kidney and liver function, electrolytes, and hematology

Compared to controls, from 12 to 30 h, DOC + DEX decreased mean chloride after lower dose bacteria challenge (119.3 ± 2.1 vs. 124.7 ± 1.8 mmol/L; $p = 0.01$) and increased mean creatinine from 12 to 30 h after high dose bacteria challenge (1.09 ± 0.35 vs. 0.80 ± 0.11 mg/dL; $p = 0.02$) (see Table E1A of Online Data Supplement). There were no other significant differences for DOC + DEX compared to controls at high or lower doses of bacteria alike on mean cardiac output, pulmonary artery occlusion pressure, central venous pressure, blood urea nitrogen, alanine aminotransferase, aspartate transaminase, sodium, potassium, hemoglobin or white blood count throughout the study (all, $p = \text{ns}$).

Discussion

Dexamethasone, a selective glucocorticoid agonist, and desoxycorticosterone, a selective mineralocorticoid agonist, given together therapeutically in physiologic stress doses improved survival with high- but not low-dose *S. aureus* pulmonary challenges. The combined treatment also improved shock reversal and decreased lung injury, IL-6 levels, and fever with high- but not low-dose bacteria. The relationship between risk and survival efficacy of a combined glucocorticoid and mineralocorticoid-based corticosteroid regimen is consistent with the results of clinical corticosteroid trials in sepsis [3], as well as with findings regarding the efficacy of mediator-specific anti-inflammatory agents in sepsis [5]. Of concern, corticosteroid therapy increased bacteremia in a setting of marginal oxacillin trough levels, suggesting that even at stress-doses corticosteroids can interfere with host mechanisms of bacterial clearance. Highly effective antibiotic regimens are likely critical to ensure the safety of using short-term corticosteroids in septic shock.

It has been postulated that the net effects of anti-inflammatory agents in sepsis, including corticosteroids, represents a balance between their ability to beneficially limit potentially harmful host inflammation (e.g. cytokine production) *versus* the suppression of host defenses and microbial clearance [5, 16–20]. Notably, IL-6 levels increase with increased severity of illness [21], and therapeutic dexamethasone only appears to improve outcomes in animals with high but not lower IL-6 levels [22]. Given that IL-6 levels were significantly correlated with risk of death in this study, inhibition of an excessive inflammatory response in high-dose bacteria animals may have reduced temperature, shock, and end-organ injury and improved survival more than in animals receiving lower bacterial challenges. However, DOC + DEX similarly hampered *S. aureus* clearance from blood regardless of inoculation dose. Furthermore, we are unaware of a mechanism that might explain why corticosteroid therapy reduced IL-6 levels after high- but not lower-dose bacterial challenges.

The ability of even short-term stress dose corticosteroids to alter innate immune responses is perhaps underappreciated and even controversial. There is some data to suggest that corticosteroids may actually improve phagocytosis and bacterial killing in both human monocytes [23, 24] and clinical trials [25]. However, the CORTICUS trial of stress-dose hydrocortisone therapy for patients with septic shock found a significant increase in

superinfections in the treatment arm [2]. Stress-induced endogenous corticosteroids alone have also been shown to impair neutrophil function [6] and increase bacterial overgrowth in wounds [7] in rodent models. In addition, animal studies have repeatedly demonstrated that exogenous corticosteroids can impair host neutrophil-mediated bacterial clearance mechanisms [7–9], which might explain the excess of positive blood cultures we observed in DOC + DEX-treated animals. More specific to our own study, corticosteroid therapy has been shown to decrease bacterial clearance from the lung in murine models [8], and is most efficacious when administered with appropriate antibiotic therapy [26–28]. These data suggest that adequately dosed and appropriately selected antibiotic therapy is essential whenever corticosteroids are used in septic shock. Of note, the infectious risks associated with corticosteroids and other immunosuppressive agents may be reduced with adequate antibiotic coverage [29–31]. Thus, with better oxacillin dosing it is possible that corticosteroid therapy would have had greater beneficial effects in both the low- and high-bacterial dose groups in this study.

Our finding that DOC + DEX improved shock reversal scores with high- but not low-dose bacterial challenges may be different from the shock reversal effects of corticosteroid therapy that was found in clinical sepsis trials [3]. Overall, there was a significant improvement in shock reversal with stress-dose corticosteroids regardless of risk of death across these clinical studies. However, only 7 of the 12 trials actually reported results on shock reversal [2, 32–37]. Of these, only 2 studies demonstrated significant shock reversal effects, both of which reported high control mortality rates of 61% and 63%, respectively [32, 35]. One low-risk trial (31% control mortality) reported that treatment with stress-dose hydrocortisone improved shock reversal, but the effect was not statistically significant [2]. Moreover, corticosteroid treatment resulted in more episodes of new sepsis and septic shock secondary to an increased number of superinfections. It is possible that DOC + DEX-treated animals in the low-dose *S. aureus* group in our study did not experience a beneficial effect on shock reversal or survival as a result of immunosuppression (and subsequent worsening bacteremia) in the setting of minimal anti-inflammatory benefits.

Alternatively, there are likely additional variables that alter the risk-efficacy relationship of corticosteroid therapy in sepsis. In mice challenged with increasing intratracheal *E. coli* doses, the benefit of hydrocortisone did not appear to vary across different severities of infection or mortality rates [38]. Notably, this model employed antibiotic coverage but not hemodynamic support. Furthermore, in a mouse model of sepsis, type of infecting organism, and timing of antibiotic coverage in relationship to corticosteroid therapy was found to alter treatment efficacy [30]. Whether these discrepancies are model-based, therapy-based, or a combination of the two requires further investigation.

Hydrocortisone, the corticosteroid most frequently administered in septic shock trials, has both glucocorticoid and mineralocorticoid agonist effects. In order to mimic the combined agonist properties of hydrocortisone, we investigated the use of a selective glucocorticoid (DEX) administered in combination with a selective mineralocorticoid (DOC). However, in a prior study investigating the effects of timing of corticosteroid administration in a highly lethal canine model of *S. aureus* pneumonia [12] we found that DOC improved survival, shock reversal, lung injury and lowered fluid requirements, but only if it was given before

(i.e. prophylactically) and not at the time of (i.e. therapeutically) bacterial challenge. In contrast, while therapeutic DEX reversed shock and showed a trend toward prolonged survival, prophylactic DEX lowered aldosterone and cortisol levels, worsened shock acutely, and provided no overall survival benefit. Impairment of innate immunity by corticosteroids has been most closely associated with their glucocorticoid activity. However, our previous study directly comparing DEX and DOC at similar time points failed to detect significant differences in *S. aureus* blood culture positivity rates [12]. Nonetheless, the putative survival benefit and harmful effects on infection in both clinical trials of septic shock and our current investigation in canines seem most consistent with the glucocorticoid activity of stress-dose corticosteroid regimens. Clearly, the innate immune effects of corticosteroid regimens with different properties and varying degrees of glucocorticoid and mineralocorticoid activity require further investigation.

Comparing the effects of therapeutic DEX alone on survival, shock, and lung injury score in our previous study of high-lethality septic shock [12] with the regimen of therapeutic DOC + DEX given together in the present study, the effects are remarkably similar (Table E1B, online data supplement). Consistent with this notion, the addition of a selective mineralocorticoid to hydrocortisone therapy in a recent clinical sepsis trial failed to further improve outcome [39]. Hydrocortisone therapy itself has mineralocorticosteroid activity [14]. However, prophylactic DEX's suppression of the hypothalamus-pituitary-adrenal (HPA) axis resulted in lower aldosterone and cortisol levels in our previous study [12], suggesting that selective glucocorticoid therapy may eventually have adverse effects in prolonged episodes of septic shock. A better understanding of the specific influence of glucocorticoids on the HPA axis during sepsis may inform the design of future corticosteroid regimens. Since short courses of mineralocorticoids have minimal risks [40], supplemental therapy with a mineralocorticoid agonist (or use of hydrocortisone, which has intrinsic mineralocorticoid activity) would seem like a reasonable approach to the corticosteroid treatment of septic shock.

The present study has limitations. A canine model, regardless of methodology that simulates key aspects of the pathogenesis and management of sepsis clinically, cannot mimic the complexity of a heterogeneous patient population with different types and severity of infections. Moreover, the sample size of our experiment was relatively small, and thus a change in mortality of a few animals could have affected results. In addition, two studies were combined in a post hoc analysis. After we found that the effect of corticosteroids on survival was significantly altered by bacterial dose, we divided the animals into two groups for analysis. Thus, future prospective studies are necessary to confirm the findings suggested by the present study. We have also studied only one corticosteroid regimen, and its activity may not be similar to the effects of hydrocortisone [14] or other corticosteroid regimens and doses. Finally, the oxacillin dose and/or dosing interval used in this study produced marginal trough levels. While this unintentional occurrence likely unmasked the deleterious effect of DOC + DEX on bacterial clearance, more adequate oxacillin dosing would be expected to eliminate this potential harmful effect on corticosteroids and alter mortality rates.

In conclusion, a corticosteroid regimen with both glucocorticoid and mineralocorticoid activity had beneficial effects on shock-reversal, lung injury, inflammatory mediators and

survival in high-risk septic animals only. The findings presented here and in our previous study [12] suggest a need for new randomized clinical trials to further evaluate the potential efficacy of mixed corticosteroid regimens in sepsis. These studies may require new methods to aid the selection of relatively homogeneous, highly specific patient populations, such as high-risk patients with severe septic shock [3]. In addition, our data suggest that if stress-dose exogenous corticosteroids are used in septic patients, adequately dosed and appropriately selected antibiotic therapy is critical to mitigate potential interference with host mechanisms of bacterial clearance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Survival

The treatment effects on survival were different for the therapeutic administration of a mineralocorticoid agonist (DOC) with a selective glucocorticoid agonist (DEX) depending on dose of bacteria challenge given. DOC + DEX improved survival compared to controls with high bacterial doses (panel A), whereas there was no survival benefit at lower bacterial doses (panel B).

Figure 2. Bacteriology

Administration of DOC + DEX resulted in an overall doubling of the number of *S. aureus*-positive blood cultures compared to controls independent of the bacterial dose challenge used ($p = 0.04$).

Figure 3. Shock Reversal and Lung Injury Score

With high bacterial doses, DOC + DEX improved mean shock reversal and lung injury scores (calculated from MAP and NE requirements; and from plateau pressure, oxygen saturation, ventilation rate, alveolar oxygen gradient, and pulmonary artery pressure) compared to controls (panel A & B, respectively). With lower bacterial doses, DOC + DEX had no beneficial effects on mean shock reversal or lung injury scores (panel C & D). The treatment effects of DOC + DEX on shock reversal were significantly different comparing high vs. lower dose bacterial doses.

Figure 4. IL-6 and Temperature

The treatment effects of DOC + DEX on both IL-6 levels and temperature were different comparing high vs. lower bacterial doses. With high bacterial doses, DOC + DEX suppressed the rise in IL-6 levels and temperature compared to controls (panel A and B, respectively). With lower bacterial doses, DOC + DEX had no significant effects on IL-6 levels temperature (panels C and D, respectively).