Intensive Care Med (2012) 38:2063–2071
DOI 10.1007/s00134-012-2735-5

EXPERIMENTAL

Caitlin W. Hicks
Daniel A. Sweeney
Robert L. Danner
Peter Q. Eichacker
Anthony F. Suffredini
Jing Feng
Junfeng Sun
Brad Moriyama
Robert Wesley
Ellen N. Behrend
Steven B. Solomon
Charles Natanson

Beneficial effects of stress-dose corticosteroid therapy in canines depend on the severity of staphylococcal pneumonia

Received: 4 May 2012
Accepted: 3 October 2012
Published online: 31 October 2012

The findings in this study have been partially previously presented as a poster at the Society of Critical Care Medicine 40th Critical Care Congress in San Diego, California (January 2011).

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-012-2735-5) contains supplementary material, which is available to authorized users.

C. W. Hicks
Department of General Surgery, The Johns Hopkins Hospital, Baltimore, MD 21287, USA

C. W. Hicks
National Institutes of Health Research Scholars Program, Howard Hughes Medical Institute, Chevy Chase, MD, USA

C. W. Hicks (✉) · R. L. Danner · P. Q. Eichacker · A. F. Suffredini · J. Feng · J. Sun · S. B. Solomon · C. Natanson
Critical Care Medicine Department, Clinical Center, National Institutes of Health, 9000 Rockville Pike, Bldg 10, Room 2C145, Bethesda, MD 20892, USA
e-mail: cmh@jhmi.edu
Tel.: +410-955-5020
Fax: +888-598-1619

D. A. Sweeney
Medical Intensivist Program, Washington Hospital, Fremont, CA 94538, USA

B. Moriyama
Clinical Center Pharmacy Department, National Institutes of Health, Bethesda, MD 20892, USA

R. Wesley
Office of Deputy Director of Clinical Care, Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA

E. N. Behrend
Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA

Abstract *Purpose:* The effects of stress-dose corticosteroid therapy were studied in a canine 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 with placebo for 96 h. Oxacillin (30 mg/kg every 8 h) was started 4 h 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 the effects of DOC + DEX were significantly altered by bacterial dose ($p = 0.04$). The treatment effects of DOC + DEX were different in animals administered high or relatively lower bacterial doses in terms of survival ($p = 0.05$), shock reversal ($p = 0.02$), interleukin-6 levels ($p = 0.02$), and temperature ($p = 0.01$). DOC + DEX significantly improved the above parameters ($p \leq 0.03$ for all) and lung injury scores ($p = 0.02$) after high-dose bacterial challenges, but not after lower challenges ($p =$ not significant for all). Oxacillin trough levels were below the minimum inhibitory concentration of the infecting organism, and DOC + DEX increased the frequency of persistent staphylococcal bacteremia (odds ratio 3.09; 95 % confidence interval 1.05–9.11; $p = 0.04$). *Conclusions:* Stress-dose corticosteroids were only beneficial in cases of sepsis with high risk for death and even short courses 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

During 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., 200–300 mg of hydrocortisone (or equivalent) per day given for 5–11 days]. The effect of corticosteroids on survival varied across these trials [1, 2]. Meta-regression analysis results suggested that stress-dose corticosteroids might confer a survival benefit only in septic shock patients at high risk of 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, interquartile range (IQR) 41–44] potentially confounded by publication bias [3]. Therefore, despite an apparent ameliorative effect of stress-dose corticosteroids on vasopressor-dependent shock during sepsis, the lack of a reproducible survival benefit has made the use of these medications 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. Corticosteroids over a wide range of glucocorticoid activity have been shown to impair host bacterial clearance from the lung [6] and blood [7] in animal models, although their precise effects in clinical sepsis are less clear [3, 8, 9]. 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 gain a better understanding of 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 [10]. 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 study reported here, 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 [11] (i.e., hydrocortisone has mixed mineralocorticoid and glucocorticoid agonist effects [12]). 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 80–90 %).

Methods

A full description of the methods is given in the Electronic Supplementary Material (ESM). Briefly, immediately following intrabronchial *S. aureus* challenge, mechanically ventilated and sedated purpose-bred beagles were treated with stress doses of DOC, a mineralocorticoid agonist, and DEX, a glucocorticoid agonist, or with placebo for 96 h. All animals received mechanical ventilation, sedation, intravenous fluids, and vasopressors titrated to physiologic endpoints throughout the study. 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. Oxacillin (30 mg/kg every 8 h) was started 4 h after infection onset. 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 bacterial doses were therefore divided into two groups (high- and low-bacterial dose groups, respectively) to minimize the within-group variation. Data were analyzed using exact log rank tests (Fig. 1), F tests (baseline characteristics), and linear mixed models (Figs. 2–4). All p values are two-tailed and considered significant at $p \leq 0.05$.

Results

Survival

The effect of DOC + DEX on survival was significantly altered by bacterial dose ($p = 0.04$, likelihood ratio test). Consequently, the animals were divided into a high-dose group ($1.35–1.5 \times 10^9$ cfu/kg, $n = 26$) and a relatively lower dose group (low-dose group; $1.05–1.2 \times 10^9$ cfu/kg, $n = 12$) to minimize the within-group dose variation. The administration of DOC + DEX had significantly different effects on survival (Cox model, $p = 0.05$ for interaction) in the high- versus low-dose groups: DOC + DEX given after high doses of bacterial challenge 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

Compared to the control animals, DOC + DEX-treated animals had an increased number of positive *S. aureus* blood cultures after both high-dose [4/24 (17 %) vs. 16/38 (42 %), respectively] and low-dose [3/19 (16 %) vs. 8/19 (42 %), respectively] bacterial challenges [overall odds ratio (OR) 3.09; 95 % confidence interval (CI) 1.05–9.11; $p = 0.04$; Fig. 2]. In contrast, the number of positive

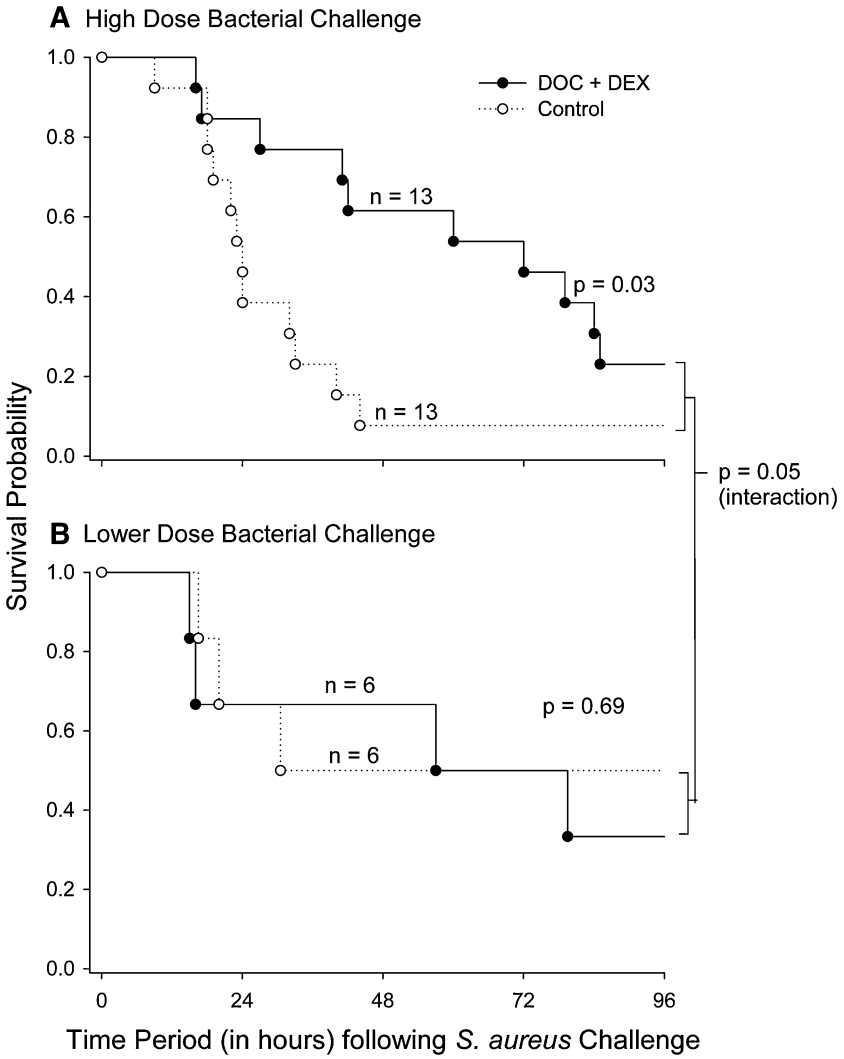

Fig. 1 Survival. The treatment effects of the therapeutic administration of a mineralocorticoid agonist (*DOC*) together with a selective glucocorticoid agonist (*DEX*) on survival were different depending on the dose of bacterial challenge given. DOC + DEX improved survival compared to controls with high bacterial doses (**a**), whereas there was no survival benefit at lower bacterial doses (**b**)

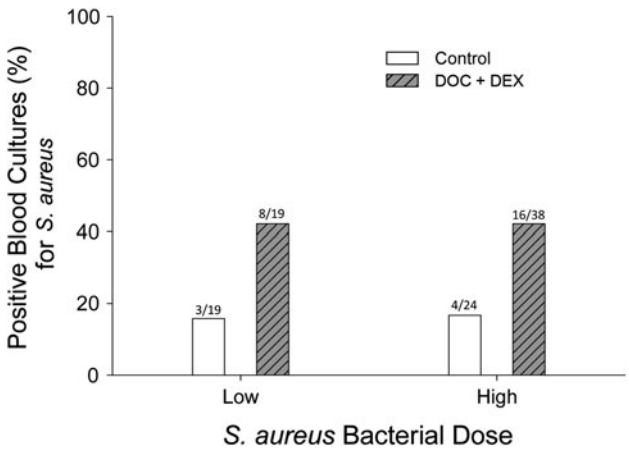

Fig. 2 Bacteriology. Administration of DOC + DEX resulted in a overall doubling of the number of *Staphylococcus aureus*-positive blood cultures compared to controls independent of the bacterial dose challenge used ($p = 0.04$)

tracheal aspirate cultures for *S. aureus* was similar in DOC + DEX-treated animals and controls after both high-dose [30/37 (81 %) vs. 19/22 (86 %), respectively; $p =$ not significant (ns)] and low-dose [8/19 (42 %) vs. 11/19 (58 %) respectively; $p =$ ns] bacterial challenges. Likewise, there were no significant differences in bacterial colonization and/or possible superinfections, as determined by urine and catheter insertion site cultures in the DOC + DEX-reated animals and controls at the high or lower doses of *S. aureus* alike throughout the study ($p =$ ns for all; 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 (ESM). At 10 h after *S. aureus* challenge (i.e., 6 h after receiving the initial oxacillin dose; $n = 48$), the median oxacillin blood level was 0 (IQR 0–0) μg/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, $n = 35$), the median oxacillin blood level was 2.5 (IQR 0.0–7.1) μg/mL. At 48 h after *S. aureus* challenge (i.e., 4 h after the sixth oxacillin dose, $n = 20$), the median oxacillin blood level was 5.0 (IQR 2.5–30) μg/mL. The measured minimum inhibitory concentration for oxacillan was 0.5 μg/mL. These data indicate that despite dosing per standard veterinary practice [13], oxacillin levels were likely to be 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 positive blood cultures.

Shock reversal and lung injury score

At baseline (T0), prior to *S. aureus* challenge, there were no significant differences in mean shock reversal scores and lung injury scores between the two treatment groups and the controls (p = ns for all; Fig. 3). DOC + DEX had different effects on shock reversal in the high- and low-dose *S. aureus* challenged groups ($p = 0.02$ for interaction): from 12 to 96 h following the administration of DOC + DEX the mean shock reversal scores improved in the high-dose *S. aureus* challenged group compared to the controls ($p = 0.009$; Fig. 3a), but shock reversal was not significantly altered in the low-dose challenged group (p = ns; Fig. 3b). The administration of DOC + DEX also improved mean lung injury scores from 12 to 96 h in the high-dose bacterial challenged group ($p = 0.02$; Fig. 3c) but not in the low-dose one (p = ns; Fig. 3d). However, the differences in the effect of DOC + DEX on the lung injury score between the two groups did not

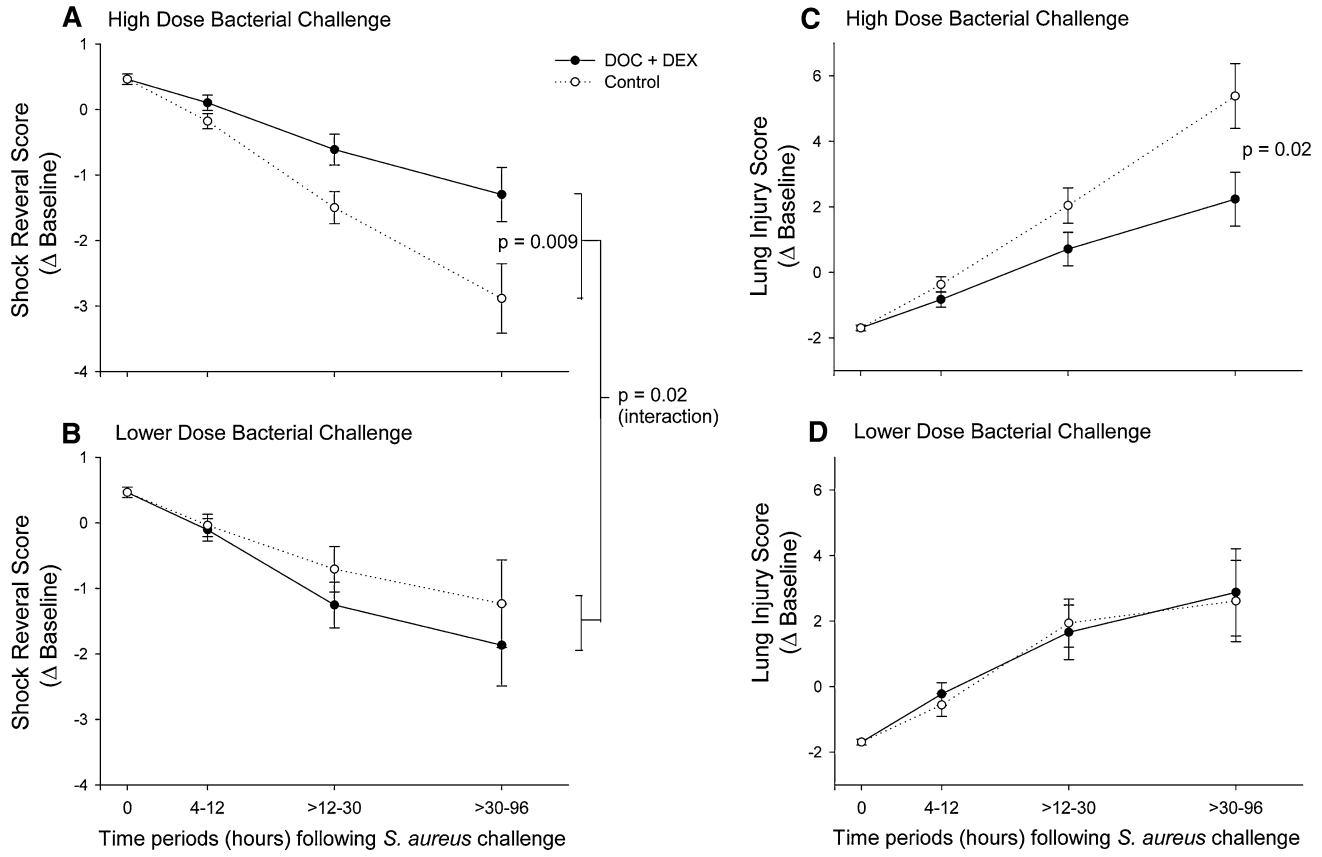

Fig. 3 Shock reversal and lung injury score. At high bacterial doses, DOC + DEX improved mean shock reversal (**a**) and lung injury (**c**) scores (calculated from mean arterial pressure and norepinephrine requirements, plateau pressure, oxygen saturation, ventilation rate, alveolar oxygen gradient, and pulmonary artery pressure). With lower bacterial doses, DOC + DEX had no beneficial effects on mean shock reversal (**b**) or lung injury (**d**) scores. The treatment effects of DOC + DEX on shock reversal were significantly different in the groups challenged with high and relatively lower dose bacterial doses

reach statistical significance (p = 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 ESM.

Cytokines and temperature

At high versus lower doses of *S. aureus* challenge, DOC + DEX had different effects on interleukin-6 (IL-6) levels and temperature ($p = 0.02$ and $p = 0.01$, respectively). Compared to the controls, 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 ($p = 0.02$; Fig. 4b). However, DOC + DEX did not significantly alter IL-6 levels or temperature in animals challenged with the relatively lower dose of *S. aureus* (p = ns for both; Fig. 4c, d). In 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 the subgroup analysis regardless of bacterial dose or treatment group examined (ESM Fig. E2). 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 (p = ns for all; 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 level after the lower dose bacterial challenge (119.3 ± 2.1 vs. 124.7 ± 1.8 mmol/L; $p = 0.01$) and increased the mean creatinine level from 12 to 30 h after high-dose bacterial challenge (1.09 ± 0.35 vs. 0.80 ± 0.11 mg/dL; $p = 0.02$) (ESM Table E1A). Compared to the controls, there were no other significant differences following DOC + DEX administration in the high-dose and low-dose challenged groups on cardiac function, kidney and liver function, electrolytes, or hematology (p = ns for all; ESM).

Discussion

Our results show that DEX, a selective glucocorticoid agonist, and DOC, a selective mineralocorticoid agonist, given together therapeutically in physiologic stress doses

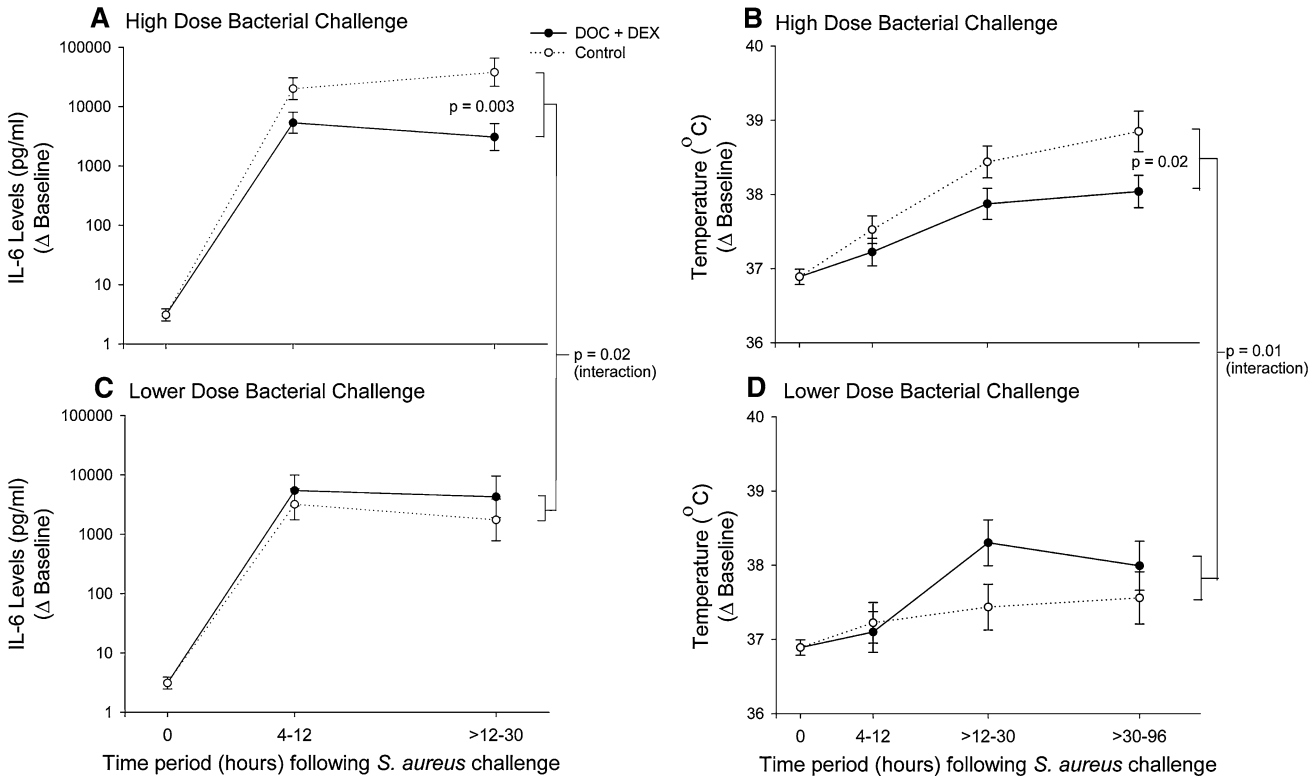

Fig. 4 Interleukin-6 (*IL-6*) levels and temperature. The treatment effects of DOC + DEX on both IL-6 levels and temperature were different in the groups challenged with high and relatively lower bacterial doses, respectively. At high bacterial doses, DOC + DEX suppressed the rise in IL-6 levels (**a**) and temperature (**b**) compared to controls. At relatively lower bacterial doses, DOC + DEX had no significant effects on IL-6 levels (**c**) or temperature (**d**)

improved the survival of canines subjected to high-dose *S. aureus* pulmonary challenge but not that in animals subjected to lower-dose *S. aureus* pulmonary challenge. The combined treatment also improved shock reversal and decreased lung injury, IL-6 levels, and fever in the high-dose group, but not in the low-dose group. 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 is the finding that 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, represent 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, 14–18]. Notably, IL-6 levels increase with increased severity of illness [19], and therapeutic DEX only appears to improve outcomes in animals with high but not lower IL-6 levels [20]. Given that IL-6 levels were significantly correlated with risk of death in this study, inhibition of an excessive inflammatory response in animals challenged with high doses of bacteria 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 the blood regardless of inoculation dose. Furthermore, we are unaware of a mechanism that might explain why corticosteroid therapy reduced IL-6 levels after high-dose but not relatively lower dose bacterial challenges.

The ability of even short-term stress-dose corticosteroids to alter innate immune responses is perhaps underappreciated and even controversial. Studies on human monocytes [21, 22] and clinical trials [23] have provided some data suggesting that corticosteroids may actually improve phagocytosis and the killing of bacteria. 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 [24] and increase bacterial overgrowth in wounds [25] in rodent models. In addition, animal studies have repeatedly demonstrated that the administration of exogenous corticosteroids can impair host neutrophil-mediated bacterial clearance mechanisms [6, 7, 25], which might explain the excess of positive blood cultures we observed in the 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 [6] and is most efficacious when administered with appropriate antibiotic therapy [26–28]. These data suggest that appropriately selected antibiotic therapy is essential whenever corticosteroids are used in the treatment of septic shock. Of note, the infectious risks associated with corticosteroids and other immunosuppressive agents may be reduced with 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 at high-dose but not lower-dose bacterial challenge may differ 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 seven of the 12 trials actually reported results on shock reversal [2, 32–37]. Of these, only two 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 the 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 *Escherichia 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 [10], we found that DOC improved survival, shock reversal, and 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 [10]. Nonetheless, the putative survival benefit and harmful effects on infection in both clinical trials of septic shock and our current investigation in canines seem to be 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.

A comparison of the effects of therapeutic DEX alone on survival, shock, and lung injury score in our previous study of high-lethality septic shock [10] with the regimen of therapeutic DOC + DEX given together in the present study reveals that the effects are remarkably similar (ESM Table E1B). 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 [12]. However, in our previous study, prophylactic DEX suppressed the hypothalamus–pituitary–adrenal (HPA) axis, resulting in lower aldosterone and cortisol levels [10] and 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 the 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 a number of 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. Also, we only examined survival over 96 h and our sample sizes were small, so it is unknown if the observed survival benefits would translate into improved long-term survival. Moreover, because the sample size of our experiment was relatively small, a change in the mortality of a few animals could have affected the 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 (low- and high-dose groups) for analysis. Thus, future prospective studies are necessary to confirm the findings suggested by our study. We have also studied only one corticosteroid regimen, and its activity may not be similar to the effects of hydrocortisone [12] 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 [10] 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.

Conflicts of interest None.

References

1. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y (2004) Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. Br Med J 329:480
2. Sprung CL, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, Laterre PF, Reinhart K, Cuthbertson BH, Payen D, Briegel J, CORTICUS Study Group (2008) Hydrocortisone therapy for patients with septic shock. N Engl J Med 358:111–124
3. Minneci PC, Deans KJ, Eichacker PQ, Natanson C (2009) The effects of steroids during sepsis depend on dose and severity of illness: an updated meta-analysis. Clin Microbiol Infect 15:308–318

4. Kalil AC, Sun J (2008) Why are clinicians not embracing the results from pivotal clinical trials in severe sepsis? A bayesian analysis. PLoS ONE 3:e2291
5. Eichacker PQ, Parent C, Kalil A, Esposito C, Cui X, Banks SM, Gerstenberger EP, Fitz Y, Danner RL, Natanson C (2002) Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. Am J Respir Crit Care Med 166:1197–1205
6. Skornik WA, Dressler DP (1974) The effects of short-term steroid therapy on lung bacterial clearance and survival in rats. Ann Surg 179:415–421
7. Heller AR, Heller SC, Borkenstein A, Stehr SN, Koch T (2003) Modulation of host defense by hydrocortisone in stress doses during endotoxemia. Intensive Care Med 29:1456–1463
8. Cronin L, Cook DJ, Carlet J, Heyland DK, King D, Lansang MA, Fisher CJ Jr (1995) Corticosteroid treatment for sepsis: a critical appraisal and meta-analysis of the literature. Crit Care Med 23:1430–1439
9. Minneci PC, Deans KJ, Banks SM, Eichacker PQ, Natanson C (2004) Meta-analysis: the effect of steroids on survival and shock during sepsis depends on the dose. Ann Intern Med 141:47–56
10. Hicks CW, Sweeney DA, Danner RL, Eichacker PQ, Suffredini AF, Feng J, Sun J, Behrend EN, Solomon SB, Natanson C (2012) Efficacy of selective mineralocorticoid and glucocorticoid agonists in canine septic shock. Crit Care Med 40(1):199–207
11. Minneci PC, Deans KJ, Hansen B, Parent C, Romines C, Gonzales DA, Ying SX, Munson P, Suffredini AF, Feng J, Solomon MA, Banks SM, Kern SJ, Danner RL, Eichacker PQ, Natanson C, Solomon SB (2007) A canine model of septic shock: balancing animal welfare and scientific relevance. Am J Physiol Heart Circ Physiol 293:H2487–H2500
12. Becker KL (2001) Principles and practice of endocrinology and metabolism. Lippincott Williams & Wilkins, Philadelphia, pp 2108–2121
13. Plumb DC (2005) Plumb's veterinary drug handbook, 5th edn. PhrmaVet, Stockholm, Iowa, pp 578–80
14. Knaus WA, Harrell FE Jr, LaBrecque JF, Wagner DP, Pribble JP, Draper EA, Fisher CJ Jr, Soll L (1996) Use of predicted risk of mortality to evaluate the efficacy of anticytokine therapy in sepsis. The rhIL-1ra Phase III Sepsis Syndrome Study Group. Crit Care Med 24:46–56
15. Keh D, Boehnke T, Weber-Cartens S, Schulz C, Ahlers O, Bercker S, Volk HD, Doecke WD, Falke KJ, Gerlach H (2003) Immunologic and hemodynamic effects of "low-dose" hydrocortisone in septic shock: a double-blind, randomized, placebo-controlled, crossover study. Am J Respir Crit Care Med 167:512–520
16. Oppert M, Schindler R, Husung C, Offermann K, Graf KJ, Boenisch O, Barckow D, Frei U, Eckardt KU (2005) Low-dose hydrocortisone improves shock reversal and reduces cytokine levels in early hyperdynamic septic shock. Crit Care Med 33:2457–2464
17. Briegel J, Jochum M, Gippner-Steppert C, Thiel M (2001) Immunomodulation in septic shock: hydrocortisone differentially regulates cytokine responses. J Am Soc Nephrol 12[Suppl 17]:S70–S74
18. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E (1993) Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest 103:565–575
19. Novotny AR, Reim D, Assfalg V, Altmayr F, Friess HM, Emmanuel K, Holzmann B (2011) Mixed antagonist response and sepsis severity-dependent dysbalance of pro- and anti-inflammatory responses at the onset of postoperative sepsis. Immunobiology 31(1):201–213
20. Osuchowski MF, Connett J, Welch K, Granger J, Remick DG (2009) Stratification is the key: inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis. Crit Care Med 37:1567–1573
21. van der Goes A, Hoekstra K, van den Berg TK, Dijkstra CD (2000) Dexamethasone promotes phagocytosis and bacterial killing by human monocytes/macrophages in vitro. J Leukoc Biol 67:801–807
22. Meduri GU, Kanangat S, Bronze M, Patterson DR, Meduri CU, Pak C, Tolley EA, Schaberg DR (2001) Effects of methylprednisolone on intracellular bacterial growth. Clin Diagn Lab Immunol 8:1156–1163
23. Kaufmann I, Briegel J, Schliephake F, Hoelzl A, Chouker A, Hummel T, Schelling G, Thiel M (2008) Stress doses of hydrocortisone in septic shock: beneficial effects on opsonization-dependent neutrophil functions. Intensive Care Med 34:344–349
24. Shurin MR, Kusnecov A, Hamill E, Kaplan S, Rabin BS (1994) Stress-induced alteration of polymorphonuclear leukocyte function in rats. Brain Behav Immun 8:163–169
25. Rojas IG, Padgett DA, Sheridan JF, Marucha PT (2002) Stress-induced susceptibility to bacterial infection during cutaneous wound healing. Brain Behav Immun 16:74–84
26. Cetinkaya RA, Gorenek L, Coskun O, Eyigun CP, Senses Z, Ide T, Kilic S (2009) The effect of methylprednisolone on treatment in rats with induced sepsis. Clin Exp Med 9:45–50
27. Sibila O, Luna CM, Agusti C, Baquero S, Gando S, Patron JR, Morato JG, Absi R, Bassi N, Torres A (2008) Effects of glucocorticoids in ventilated piglets with severe pneumonia. Eur Respir J 32:1037–1046
28. Tagliabue C, Salvatore CM, Techasaensiri C, Mejias A, Torres JP, Katz K, Gomez AM, Esposito S, Principi N, Hardy RD (2008) The impact of steroids given with macrolide therapy on experimental Mycoplasma pneumoniae respiratory infection. J Infect Dis 198:1180–1188
29. Flynn PM, Shenep JL, Stokes DC, Hildner WK, Mackert PW, Snellgrove RL, Rehg JE (1986) Effect of methylprednisolone on bacterial clearance and endotoxin liberation during experimental sepsis induced by gram-negative bacteria. Infect Immun 52:26–30
30. Fadel MV, Repka JC, Cunha CL, Leao MT (2008) Inadequate timing between corticosteroid and antibiotics applications increases mortality due to sepsis. Braz J Infect Dis 12:416–422
31. Assfalg V, Huser N, Reim D, Kaiser-Moore S, Rossmann-Bloeck T, Weighardt H, Novotny AR, Stangl MJ, Holzmann B, Emmanuel KL (2010) Combined immunosuppressive and antibiotic therapy improves bacterial clearance and survival of polymicrobial septic peritonitis. Shock 33:155–161
32. Bollaert PE, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A (1998) Reversal of late septic shock with supraphysiologic doses of hydrocortisone. Crit Care Med 26:645–650
33. Briegel J, Forst H, Haller M, Schelling G, Kilger E, Kuprat G, Hemmer B, Hummel T, Lenhart A, Heyduck M, Stoll C, Peter K (1999) Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. Crit Care Med 27:723–732
34. Chawla K, Kupfer Y, Goldman I (1999) Hydrocortisone reverses refractory septic shock. Crit Care Med 27:A33

35. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troche G, Chaumet-Riffaud P, Bellissant E (2002) Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 288:862–871
36. Mussack T, Briegel J, Schelling G, Biberthaler P, Jochum M (2005) Effect of stress doses of hydrocortisone on S-100B vs. interleukin-8 and polymorphonuclear elastase levels in human septic shock. Clin Chem Lab Med 43:259–268
37. Tandan SM, Guleria R, Gupta N (2005) Low dose steroids and adrenocortical insufficiency in septic shock: a double-blind randomized controlled trial from India. Am J Respir Crit Care Med 171:A43
38. Li Y, Cui X, Li X, Solomon SB, Danner RL, Banks SM, Fitz Y, Annane D, Natanson C, Eichacker PQ (2008) Risk of death does not alter the efficacy of hydrocortisone therapy in a mouse *E. coli* pneumonia model: risk and corticosteroids in sepsis. Intensive Care Med 34:568–577
39. COIITSS Study Investigators, Annane D, Cariou A, Maxime V, Azoulay E, D'honneur G, Timsit JF, Cohen Y, Wolf M, Fartoukh M, Adrie C, Santre C, Bollaert PE, Mathonet A, Amathieu R, Tabah A, Clec'h C, Mayaud J, Lejeune J, Chevret S (2010) Corticosteroid treatment and intensive insulin therapy for septic shock in adults: a randomized controlled trial. JAMA 303:341–348
40. Scott WA, Pongiglione G, Bromberg BI, Schaffer MS, Deal BJ, Fish FA, Dick M (1995) Randomized comparison of atenolol and fludrocortisone acetate in the treatment of pediatric neurally mediated syncope. Am J Cardiol 76:400–402