ORIGINAL ARTICLE

Randomized Controlled Trial of Calcitriol in Severe Sepsis

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Abstract

Rationale: Vitamin D and its metabolites have potent immunomodulatory effects *in vitro*, including up-regulation of cathelicidin, a critical antimicrobial protein.

Objectives: We investigated whether administration of 1,25-dihydroxyvitamin D (calcitriol) to critically ill patients with sepsis would have beneficial effects on markers of innate immunity, inflammation, and kidney injury.

Methods: We performed a double-blind, randomized, placebo-controlled, physiologic study among 67 critically ill patients with severe sepsis or septic shock. Patients were randomized to receive a single dose of calcitriol (2 μg intravenously) versus placebo. The primary outcome was plasma cathelicidin protein levels assessed 24 hours after study drug administration. Secondary outcomes included leukocyte cathelicidin mRNA expression, plasma cytokine levels (IL-10, IL-6, tumor necrosis factor-α, IL-1β, and IL-2), and urinary kidney injury markers.

Measurements and Main Results: Patients randomized to calcitriol (n = 36) versus placebo (n = 31) had similar plasma cathelicidin protein levels at 24 hours ($P = 0.16$). Calcitriol-treated patients had higher cathelicidin ($P = 0.04$) and IL-10 ($P = 0.03$) mRNA expression than placebo-treated patients 24 hours after study drug administration. Plasma cytokine levels (IL-10, IL-6, tumor necrosis factor-α, IL-1β, and IL-2) and urinary kidney injury markers were similar in calcitriol- versus placebo-treated patients ($P > 0.05$ for all comparisons). Calcitriol had no effect on clinical outcomes nor were any adverse effects observed.

Conclusions: Calcitriol administration did not increase plasma cathelicidin protein levels in critically ill patients with sepsis and had mixed effects on other immunomodulatory markers. Additional phase II trials investigating the dose and timing of calcitriol as a therapeutic agent in specific sepsis phenotypes may be warranted. Clinical trial registered with www.clinicaltrials.gov (NCT 01689441).

Keywords: 1,25-dihydroxyvitamin D; vitamin D; cathelicidin; innate immunity; critical illness

At a Glance Commentary

Scientific Knowledge on the Subject: Observational studies have shown associations between low 25-hydroxyvitamin D levels and increased mortality among critically ill patients. Administration of vitamin D and its metabolites elicits potent immunomodulatory effects *in vitro*, but its *in vivo* effects have not been evaluated.

What This Study Adds to the Field: In this double-blind, randomized, placebo-controlled study among critically ill patients with sepsis, we demonstrate that administration of 1,25-dihydroxyvitamin D (calcitriol) did not increase plasma levels of cathelicidin, a critical antimicrobial protein, but did increase leukocyte mRNA expression of cathelicidin. Additional phase II trials investigating the dose and timing of calcitriol as a therapeutic agent in specific sepsis phenotypes may be warranted.

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Sepsis is common among critically ill patients and associated with considerable morbidity and mortality (1). Observational studies among critically ill patients, many of whom have sepsis, have shown strong associations between decreased 25-hydroxyvitamin D (25D) levels and adverse outcomes, including increased length of stay (2), infection (3), acute kidney injury (AKI) (4), and mortality (2–4). Additionally, studies from our group have documented an inverse association between 25D levels and severity of sepsis (5, 6).

Whether these associations are causal in nature is unknown; however, a biologically plausible role for vitamin D in sepsis is suggested by the important effects of its active metabolite, 1,25-dihydroxyvitamin D (1,25D), on host defense (7). 1,25D enhances innate immunity primarily by signaling monocytes, macrophages, and epithelial cells to increase production of cathelicidins, a major family of antimicrobial peptides (8). Cathelicidins constitute an important component of immunologic defense and have activity against gram-positive (9–11) and gram-negative bacteria (12, 13) and some viruses and fungi (14, 15). In animal models, deficiency of cathelicidin is associated with increased susceptibility to bacterial infection (9, 10, 12), whereas overexpression confers protection (13). Among patients undergoing dialysis, plasma levels of the only known human cathelicidin antimicrobial peptide (hCAP-18) are directly associated with 1,25D plasma levels and inversely associated with 1-year infection-related mortality (16).

In addition to its important effects on innate immunity, 1,25D inhibits production of proinflammatory cytokines produced by T-helper cells including IL-2, IL-6, IL-8, and tumor necrosis factor (TNF)-α (17, 18); increases production of antiinflammatory cytokines, such as IL-10 (19, 20); and may attenuate AKI (21, 22), an important complication of sepsis. Most of these studies, however, were performed *in vitro* or in animal models or were observational in nature.

We performed a double-blind, randomized, placebo-controlled, physiologic study among critically ill patients with sepsis to test whether 1,25D (calcitriol) administration has beneficial effects on markers of immunity, inflammation, and kidney injury. We hypothesized that calcitriol would increase markers of innate immunity (hCAP-18), decrease markers of inflammation (IL-6), and decrease markers of kidney injury (neutrophil gelatinase-associated lipocalin [NGAL] and kidney injury molecule-1 [KIM-1]).

Methods

Study Design

We conducted a double-blind, randomized physiologic study of calcitriol versus placebo among patients admitted to intensive care units (ICUs) at Brigham and Women's Hospital and Beth Israel Deaconess Medical Center (both in Boston, MA). Patients or their surrogates provided written informed consent and all protocols were approved by the institutional review boards of the respective hospitals.

Randomization was performed centrally by the Brigham and Women's Hospital research pharmacy with the use of a computer-generated assignment sequence, using permuted blocks of four. Because AKI is associated with altered vitamin D physiology (6, 23),

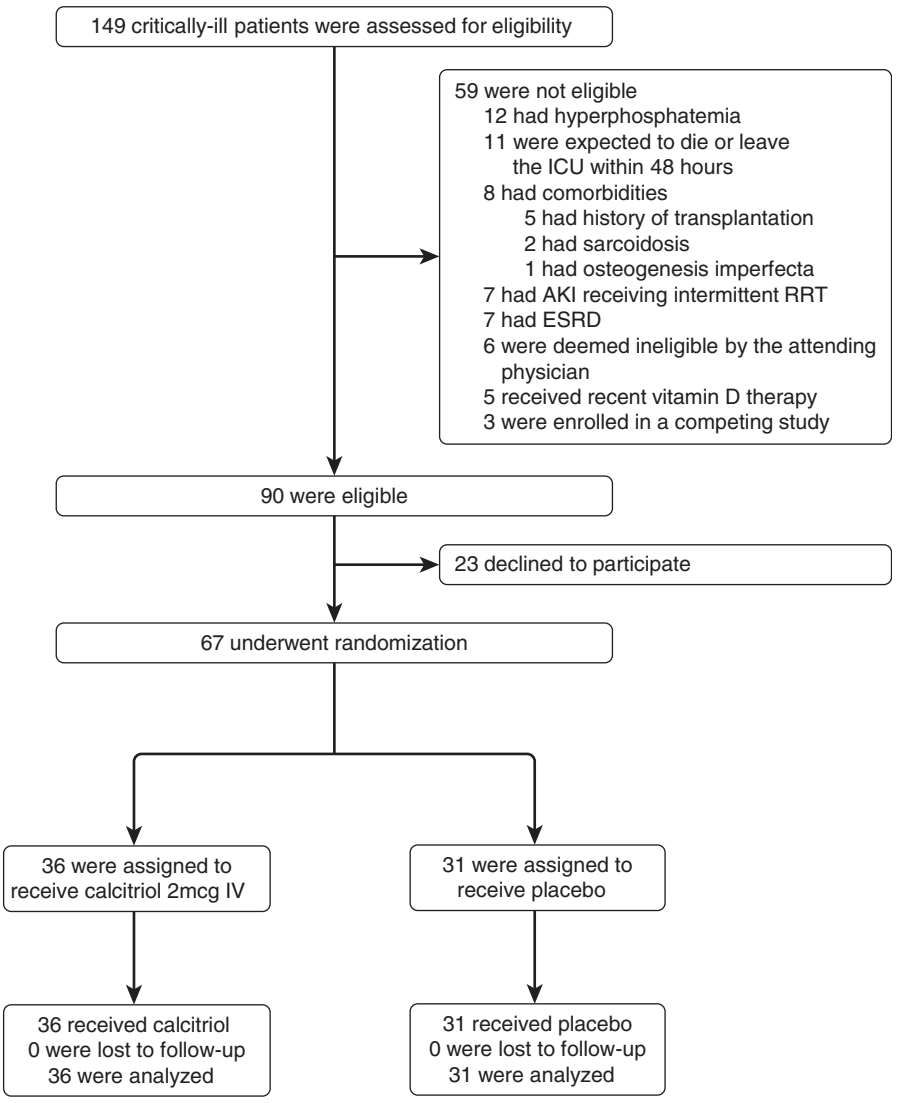

Figure 1. Enrollment flow chart. The slight imbalance in number of patients randomized to calcitriol versus placebo resulted from a clerical error in one of the permuted blocks generated by the research pharmacy. This error did not affect blinding, and all patients received the drug to which they were randomized. AKI = acute kidney injury (defined in the METHODS); ESRD = end-stage renal disease; ICU = intensive care unit; RRT = renal-replacement therapy.

randomization was stratified by the presence or absence of AKI, defined as an increase in serum creatinine greater than or equal to 0.3 mg/dl within 48 hours, a 50% increase in 7 days, oliguria (<0.5 ml/kg/h for >6 h), or requirement for renal-replacement therapy (RRT) (24). Physicians, nurses, patients, and study investigators were masked to treatment assignment. A formal data and safety monitoring board was deemed unnecessary by the institutional review boards, and the conduct of the trial was overseen by investigators D.E.L. and S.S.W.

Patients

Inclusion criteria were age greater than or equal to 18 years, severe sepsis or septic shock, and presence of an arterial or central venous catheter (for blood drawing). Severe sepsis and septic shock were defined as per consensus definition (25), with minor modifications made to the criteria for severe sepsis (*see* the METHODS section in the online supplement).

Exclusion criteria were serum calcium greater than or equal to 10.0 mg/dl or phosphate greater than or equal to 6.0 mg/dl within the previous 48 hours; current or recent therapy (within the previous 7 d) with nutritional vitamin D at doses greater than 1,000 IU per day or 1,25D at any dose; history of primary parathyroid disease, metabolic bone disease, sarcoidosis, or end-stage renal disease; AKI receiving intermittent RRT (patients receiving continuous RRT were eligible); expected to die or leave the ICU within 48 hours; and pregnancy.

Study Treatments and Sample Collections

Patients were randomly assigned to receive, in 1:1 fashion, a single intravenous dose of calcitriol, 2 μg, or an identically appearing equal volume of saline (2 ml). We chose 2 μg for the dose of calcitriol based on multiple randomized controlled trials of calcitriol in patients with chronic kidney disease in which this dose was shown to be well tolerated (minimal changes in serum calcium or phosphate) and effective at reducing parathyroid hormone levels (26, 27). Additionally, in the only pilot study that evaluated calcitriol among critically ill patients, a dose of 2 μg was used and no adverse effects were reported (28).

We collected plasma and urine samples at four time points: time 0 (immediately before study drug administration) and 6, 24, and 48 hours later. We stored plasma and urine aliquots at −80°C within 1 hour of collection. Additionally, whole-blood leukocyte RNA was collected at time 0 and 24 hours later using Tempus blood RNA tubes containing stabilizing reagent, which inactivates cellular RNases (Applied Biosystems/Life Technologies, Grand Island, NY).

Outcomes

The primary outcome was plasma hCAP-18 protein levels assessed at 24 hours. Prespecified secondary outcomes included change in leukocyte mRNA expression of hCAP-18, IL-6, IL-10, and TNF-α, assessed at 0 and 24 hours; and plasma cytokine levels (IL-6, IL-10, TNF-α, IL-1β, and IL-2) and urinary kidney injury markers (NGAL and KIM-1) assessed at 0, 6, 24, and

Table 1. Baseline Demographic and Clinical Characteristics

Characteristic	Calcitriol (*n = 36*)	Placebo (*n = 31*)
Age, yr, median (IQR)	68 (54–70)	58 (49–69)
Female sex, n (%)	14 (39)	16 (52)
White race, n (%)	35 (97)	27 (87)
Comorbidities, n (%)		
Hypertension	20 (56)	19 (61)
Diabetes mellitus	11 (31)	7 (23)
Congestive heart failure	9 (25)	6 (19)
Chronic kidney disease	3 (8)	2 (6)
COPD	7 (19)	3 (10)
Cirrhosis	2 (6)	0 (0)
Cancer	5 (14)	3 (10)
Type of ICU, n (%)		
Surgical	19 (53)	19 (61)
Nonsurgical	17 (47)	12 (39)
Days in ICU, median (IQR)	5 (3–6)	5 (3–9)
Severity of illness		
APACHE II score, median (IQR)*	18 (15–20)	21 (15–24)
SOFA-CV score, median (IQR)†	1 (1–3)	1 (0–3)
Mechanical ventilation, n (%)	25 (69)	22 (71)
Shock, n (%)	16 (44)	14 (45)
AKI, n (%)‡	13 (36)	13 (42)
RRT, n (%)	3 (8)	5 (16)
Enrollment laboratory values		
White cell count, per mm^3	11.0 (9.0–14.9)	15.4 (9.5–19.4)
Hemoglobin, g/dl	9.0 (7.9–10.2)	9.0 (8.0–10.1)
Creatinine, mg/dl	1.1 (0.7–1.4)	0.9 (0.6–1.6)
Albumin, g/dl	2.4 (2.0–2.9)	2.4 (2.0–2.6)
Arterial pH	7.43 (7.38–7.46)	7.44 (7.40–7.47)
Lactate, mmol/L	1.4 (1.2–1.7)	1.8 (1.5–2.4)
Medications, n (%)		
#Vasopressors/inotropes		
0	18 (50)	19 (61)
1	11 (31)	6 (19)
⩾2	7 (19)	6 (19)
#Antibiotics		
0	0 (0)	1 (3)
1	5 (14)	5 (16)
⩾2	31 (86)	25 (81)
Glucocorticoids, n (%)§	4 (11)	2 (7)
Antiepileptic agents, n (%)‖	5 (14)	8 (26)

Definition of abbreviations: AKI = acute kidney injury; APACHE II = Acute Physiology and Chronic Health Evaluation II; COPD = chronic obstructive pulmonary disease; ICU = intensive care unit; IQR = interquartile range; RRT = renal-replacement therapy; SOFA-CV = Sequential Organ Failure Assessment (Cardiovascular).

*APACHE II, an ICU severity of illness scoring system ranging from 0 to 71, with higher scores corresponding to more severe disease.

†SOFA-CV, an ICU severity of illness scoring system ranging from 0 to 4, with higher scores corresponding to more severe hypotension and greater vasopressor requirements.

‡AKI defined in the METHODS.

§Glucocorticoids included hydrocortisone and prednisone.

‖Antiepileptic agents included levetiracetam, phenytoin, valproic acid, and topiramate.

Table 2. Primary Site of Infection

Primary Site of Infection	Calcitriol (*n = 36*) [*n (%)*]	Placebo (*n = 31*) [*n (%)*]	*P* Value
Respiratory	20 (56)	18 (58)	1.00
Gastrointestinal/biliary	7 (19)	5 (16)	0.76
Bloodstream	4 (11)	1 (3)	0.36
Urinary tract	1 (3)	0 (0)	1.00
Abscess	1 (3)	1 (3)	1.00
Unknown or other	3 (8)	6 (19)	0.28

48 hours. In exploratory analyses we also measured leukocyte mRNA expression of additional immune and antiinflammatory markers that are up-regulated by 1,25D in cell culture: the p38 inhibitor mitogen-activated protein kinase phosphatase-1, inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells, and toll-like receptor 4. The major safety endpoint was development of hypercalcemia (>10.7 mg/dl) or hyperphosphatemia (>6.0 mg/dl) within 48 hours of study drug administration, and was reviewed by study investigators D.E.L. and S.S.W. after enrollment of every 10 patients.

Laboratory Analyses

RNA expression. Leukocyte mRNA expression was assessed at time 0 and 24 hours later using real-time polymerase chain reaction. Additional details are provided in the online supplement.

Plasma protein levels. hCAP-18 protein levels were measured at all four time points in singulate using a commercial ELISA kit (Hycult Biotech, Uden, Netherlands). Plasma cytokine levels were measured at all four time points in duplicate using a bead-based multiplex assay (Millipore, Billerica, MA). Vitamin D metabolites were measured in singulate at time 0 and 6 hours later using immunoaffinity enrichment and liquid chromatography–tandem mass spectrometry.

Urinary kidney injury markers. Urinary NGAL and KIM-1 levels were measured in duplicate at all four time points using a microbead-based sandwich ELISA, and were normalized to urinary creatinine concentrations to account for the influence of urinary dilution on biomarker concentrations.

Additional assay characteristics, including coefficients of variation, are provided in Table E1 in the online supplement.

Clinical Data

Although the study was not powered to detect a difference in clinical outcomes, the following data were recorded on all patients: ICU, hospital, and 28-day mortality; ICU and hospital length of stay; duration of mechanical ventilation; Sequential Organ Failure Assessment (Cardiovascular) score at 0, 24, and 48 hours after study drug administration; urine output; and new RRT requirement. To avoid the confounding effect of mortality, we assessed ICU and hospital length of stay only among survivors. We also calculated ventilator-free days, defined as 28 minus the number of ventilator-dependent days, assuming survival to 28 days or discharge from the hospital. Patients who died before 28 days were assigned a score of zero (29). Investigator D.E.L. adjudicated all outcomes by reviewing electronic medical records, and was masked to treatment assignment and biomarker results at the time of adjudication.

Statistical Analysis

We determined that a sample of 60 patients would provide the study with 80% power to detect a relative increase of 50% in plasma hCAP-18 protein levels, with a two-sided α of 5%. We therefore enrolled 67 patients to account for missing data. All analyses were conducted on an intention-to-treat basis. Missing data were not imputed. Between-group comparisons of plasma and urinary markers were assessed using the Wilcoxon rank sum test. Correlations between fold-elevation in hCAP-18 and IL-10 mRNA levels with fold-elevation in 1,25D levels were analyzed using Spearman rank correlation coefficient. All comparisons are two-tailed, with *P* less than 0.05 considered significant. Statistical analysis was performed with SAS Version 9.3 (SAS Institute Inc., Cary, NC).

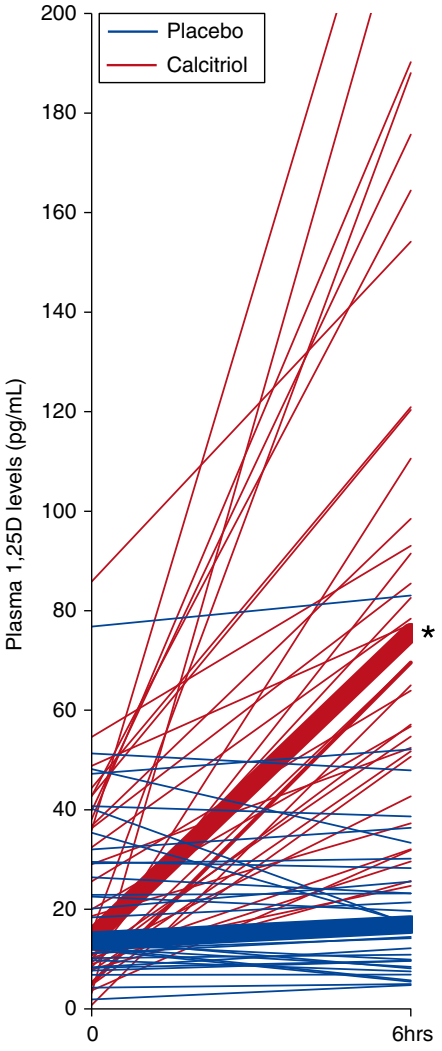

Figure 2. Change in plasma 1,25-dihydroxyvitamin D (1,25D) levels, placebo versus calcitriol. 1,25D levels represent the sum of $1,25D_2$ and $1,25D_3$. *$P < 0.001$, comparison of 1,25D levels in calcitriol- versus placebo-treated patients at 6 hours. n = 36 (calcitriol), n = 31 (placebo). *Thin lines* represent individual patients. *Thick lines* represent median levels.

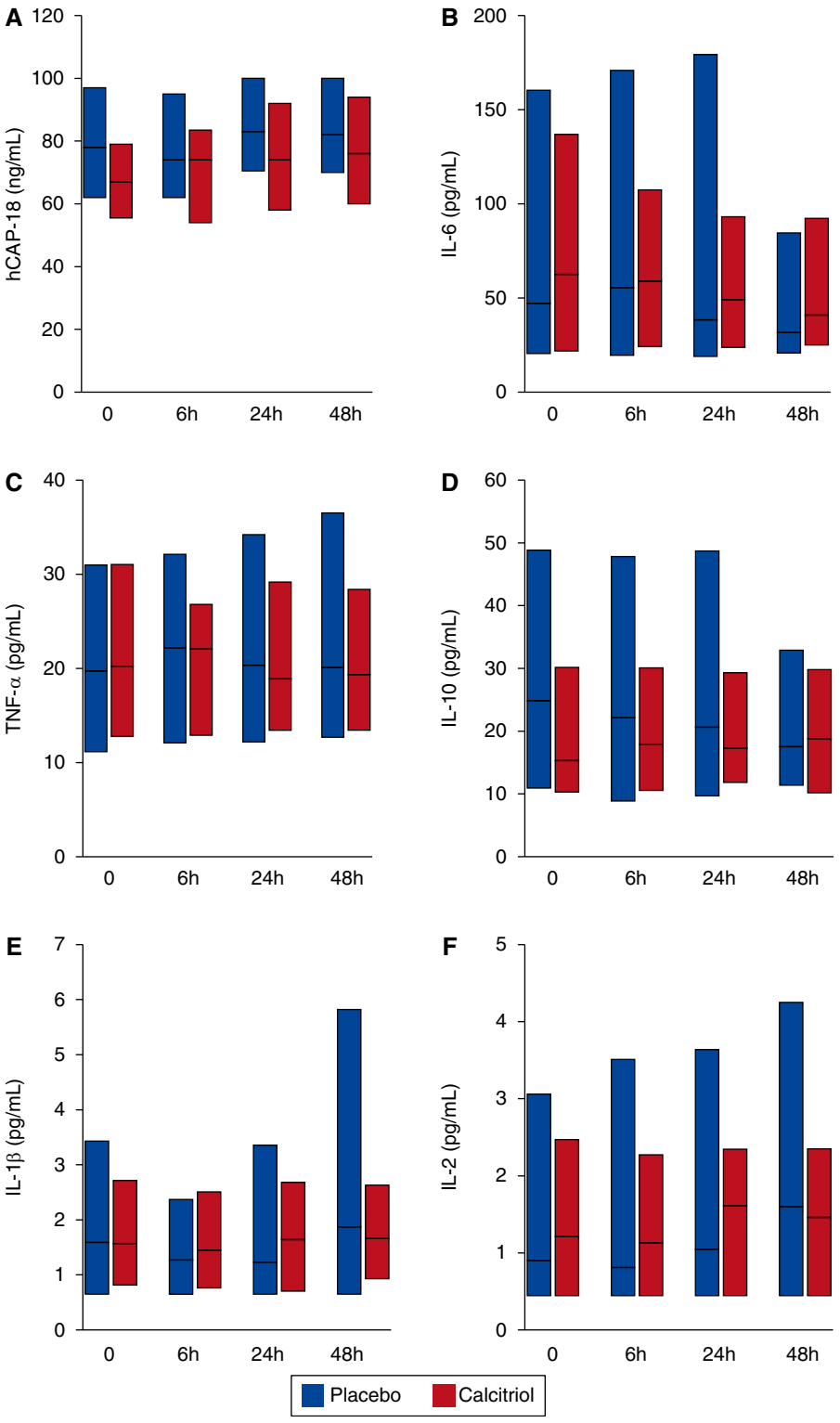

Figure 3. Effect of calcitriol on plasma levels of immune and inflammatory markers. No significant between-group differences were detected in plasma protein levels of (*A*) human cathelicidin antimicrobial peptide (hCAP)-18, (*B*) IL-6, (*C*) tumor necrosis factor (TNF)-α, (*D*) IL-10, (*E*) IL-1β, or (*F*) IL-2. n = 36 (calcitriol) and n = 31 (placebo). *Bars* represent median (25th–75th interquartile range).

Results

Study Population

From January 2013 through November 2013, a total of 67 patients were enrolled. All patients received their assigned study drug, and none were lost to follow-up (Figure 1). Plasma samples were available for protein analyses in all 67 patients, urine samples were available in 62 patients, and leukocyte mRNA was available in 58 patients.

Baseline characteristics were similar between the two groups (Table 1). There were slight imbalances in age, Acute Physiology and Chronic Health Evaluation II score, white blood cell count, and lactate, with patients in the placebo group tending to be younger and with increased severity of illness; however, these differences did not reach statistical significance (Table 1). Use of medications that affect vitamin D metabolism (e.g., glucocorticoids and antiepileptic agents) was similar in the two groups (Table 1). The primary site of infection was similar in the two groups (Table 2).

Effect of Calcitriol on Vitamin D Metabolites

We found significantly increased plasma 1,25D levels at 6 hours in calcitriol- versus placebo-treated patients (75.7 [52.1–115.5] and 16.9 [9.0–26.9] pg/ml; $P < 0.001$) (Figure 2). Levels of other vitamin D metabolites were unchanged and are shown in Table E2.

Effect of Calcitriol on Plasma Protein Levels

We found similar plasma hCAP-18 protein levels (Figure 3A) and similar plasma cytokine levels (IL-6, TNF-α, IL-10, IL-1β, and IL-2) (Figures 3B–3F) at all four time points in calcitrol- versus placebo-treated patients.

Effect of Calcitriol on Leukocyte mRNA Expression

We found significantly increased hCAP-18 mRNA expression at 24 hours in calcitriol- versus placebo-treated patients (1.8 [0.9–3.3] and 1.0 [0.7–1.8] fold-elevation from baseline; $P = 0.04$) (Figure 4A]. Additionally, we found a graded relationship between fold-elevation in 1,25D and fold-elevation in hCAP-18 mRNA expression (P for trend 0.003) (Figure 4B).

Patients who received calcitriol also had higher IL-10 mRNA expression at 24 hours compared with patients who received placebo (Figure 4A). Similar to hCAP-18,

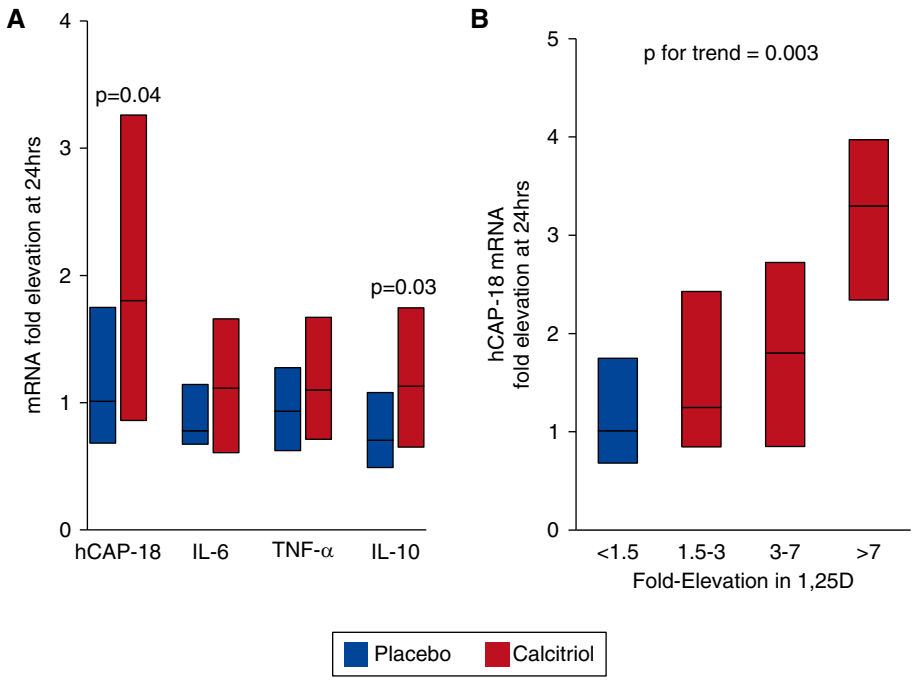

Figure 4. Effect of calcitriol on leukocyte mRNA expression of immune and inflammatory markers. (*A*) mRNA fold elevation at 24 hours for hCAP-18, IL-6, tumor necrosis factor (TNF)-α, and IL-10. (*B*) Fold-elevation in 1,25-dihydroxyvitamin D (1,25D) levels (0–6 h) versus fold-elevation in human cathelicidin antimicrobial peptide (hCAP)-18 mRNA expression (0–24 h). n = 32 (calcitriol) and n = 26 (placebo). *Bars* represent median (25th–75th interquartile range). 1.0-fold elevation is equivalent to no change.

we found a graded relationship between fold-elevation in 1,25D and fold-elevation in IL-10 mRNA expression (*P* for trend 0.004) (*see* Figure E1A). In contrast, we found no significant between-group differences in mRNA expression of IL-6, TNF-α, mitogen-activated protein kinase phosphatase-1, inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells, or toll-like receptor 4 (Figure 4A; *see* Figure E1B).

Effect of Calcitriol on Urinary Kidney Injury Biomarkers

We found similar urinary levels of KIM-1 and NGAL in calcitriol- versus placebo-treated patients at all four time points (Figure 5).

Clinical and Safety Outcomes

Patients who received calcitriol versus placebo had similar mortality, ICU and hospital length of stay, Sequential Organ Failure Assessment (Cardiovascular) score at 24 and 48 hours, and other clinical and physiologic outcomes (Table 3). No patients developed hypercalcemia within 48 hours of study drug administration, and the incidence of hyperphosphatemia was rare and similar between groups (Table 3). Calcium, phosphate, creatinine, and white blood cell count values at times 0, 24, and 48 hours are shown in Table E3.

Discussion

In this doubled-blind, randomized, placebo-controlled physiologic study we report that calcitriol administered to critically ill patients with sepsis did not increase plasma hCAP-18 protein levels at 24 hours. Additionally, we report that calcitriol- versus placebo-treated patients had higher leukocyte mRNA expression of both hCAP-18 and the antiinflammatory cytokine, IL-10, at 24 hours. Finally, patients treated with calcitriol versus placebo had similar plasma cytokine (IL-6, TNF-α, and others) and urinary kidney injury marker levels (KIM-1 and NGAL).

A link between vitamin D and sepsis has long been supported by studies performed in both animals and humans. The use of artificial sunlight to treat cutaneous tuberculosis was pioneered by Niels Finsen in 1895, and by the 1920s phototherapy was used routinely for pulmonary tuberculosis (30). Based on recent studies, the effect of sunlight on infections is now better understood and is related to the effect of 1,25D on the expression of antimicrobial peptides, such as hCAP-18. Low 25D levels are associated with reduced production of hCAP-18 by macrophages infected with *Mycobacterium tuberculosis*, whereas treatment with 1,25D *in vitro* results in enhanced production of hCAP-18 and improved killing of the microorganisms (31). In addition to macrophages, the *in vitro* inducibility of hCAP-18 by 1,25D has been demonstrated in multiple other human cell lines, including respiratory (32), gingival (33), and biliary epithelial cells (34) and keratinocytes (35).

The association between 1,25D and sepsis is not limited to hCAP-18 and tuberculosis. hCAP-18 has antimicrobial effects against a broad range of gram-positive (9–11) and gram-negative bacteria (12, 13) and some viruses and fungi (14, 15). Moreover, 1,25D exerts other immunomodulatory effects unrelated to hCAP-18, such as blunting of an exaggerated inflammatory response. The latter is accomplished by decreasing circulating levels of proinflammatory cytokines, such as IL-6 and TNF-α (17, 18), and increasing levels of antiinflammatory cytokines, such as IL-10 (19, 20). Our findings of increased hCAP-18 and IL-10 mRNA expression in calcitriol- versus placebo-treated patients are consistent with and expand on the established *in vitro* effects of 1,25D, described previously.

Consistent with an important role of 1,25D in host defense, observational studies have consistently documented associations between low levels of both 25D (2–4, 36) and 1,25D (37) and increased mortality in patients with sepsis. Additionally, supplementation with 1,25D is associated with decreased mortality in patients with chronic kidney disease (38) and end-stage renal disease receiving hemodialysis (39). Although fewer studies have directly investigated the association between plasma hCAP-18 protein levels and adverse outcomes, one study among dialysis patients found an inverse association with 1-year infection-related mortality (16).

Despite these compelling preclinical and observational studies, it remains to be determined whether administration of 1,25D results in favorable effects on immunity and inflammation among

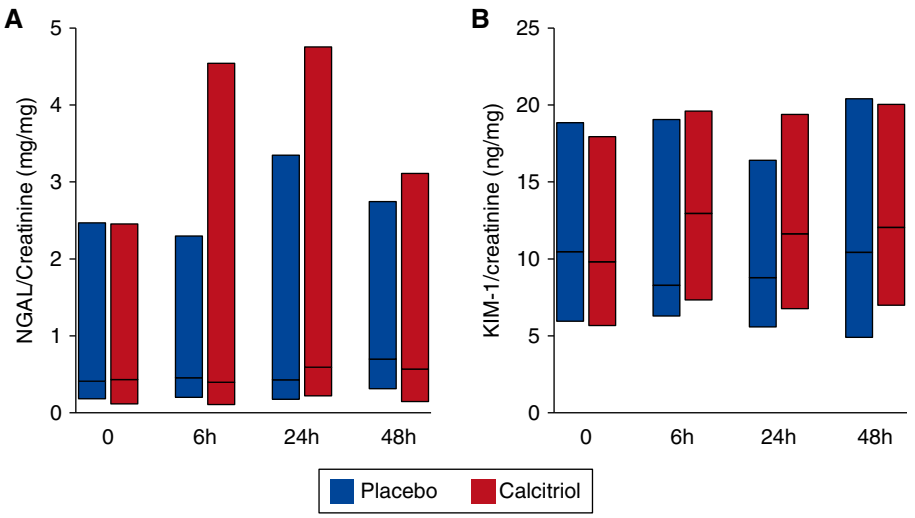

Figure 5. Effect of calcitriol on urinary biomarkers. No significant between-group differences were detected for (*A*) urinary NGAL or (*B*) urinary KIM-1. n = 36 (calcitriol), n = 26 (placebo). NGAL = neutrophil gelatinase-associated lipocalin; KIM-1 = kidney injury molecule-1. *Bars* represent median (25th–75th interquartile range).

critically ill patients with sepsis. Experts have repeatedly highlighted the need for interventional studies of vitamin D in critically ill patients (40–43). To date, no interventional studies have evaluated the effect of any vitamin D metabolite on hCAP-18 expression in patients with critical illness or sepsis. Interventional studies in other contexts, including healthy volunteers (44), patients with chronic obstructive pulmonary disease (45), and patients with cystic fibrosis (18), have failed to show an effect of nutritional vitamin D_3 supplementation on circulating hCAP-18 levels. However, the current study differs from prior interventional studies of vitamin D and hCAP-18 in at least three important ways: (*1*) the use of 1,25D, as opposed to "nutritional" vitamin D_3; (*2*) measurement of hCAP-18 protein levels and mRNA expression, as opposed to protein levels alone; and (*3*) measurement of the effects of 1,25D on immune and inflammatory biomarkers acutely (after 6, 24, and 48 h), as opposed to weeks or months later.

A unique feature of the current study is the use of 1,25D rather than nutritional vitamin D_3. Many cell types, including monocytes and macrophages, express the enzyme 25-hydroxyvitamin D-1α-hydroxylase, and are therefore capable of converting local 25D into 1,25D. Accordingly, some researchers have proposed that the immunomodulatory effects of circulating 1,25D are minimal, and that local 25D is more important (46). We used 1,25D rather than nutritional vitamin D_3 because of its rapid onset of action and because of the likelihood of impaired renal (23) and extrarenal (47) conversion of 25D to 1,25D in the setting of critical illness. Our finding of increased hCAP-18 mRNA expression in response to 1,25D administration challenges the view

Table 3. Effect of Calcitriol on Clinical Outcomes

Clinical Outcome	Calcitriol (*n* = *36*)	Placebo (*n* = *31*)	*P* Value
Mortality, n (%)			
ICU mortality	7 (19)	6 (19)	1.00
Hospital mortality	8 (22)	7 (23)	1.00
28-d mortality	6 (17)	7 (23)	0.56
Length of stay among survivors, d, median (IQR)			
ICU	8 (5–18)	13 (6–16)	0.83
Hospital	22 (17–32)	21 (15–30)	0.39
SOFA-CV score, n (%)			
Improvement at 24 h	10 (28)	7 (23)	0.78
Improvement at 48 h	12 (33)	12 (39)	0.80
Respiratory			
Ventilator-free days, median (IQR)	25 (13–28)	21 (6–27)	0.33
$Pa_{O_2}/F_{I_{O_2}}$, median (IQR)			
24 h	234 (179–264)	249 (198–376)	0.25
48 h	264 (184–319)	246 (212–348)	0.88
Renal			
New RRT requirement, n (%)	1 (3)	1 (3)	1.00
UOP, L, median (IQR)			
0–24 h	2.3 (1.1–3.9)	2.2 (1.1–3.7)	0.65
24–48 h	2.8 (1.5–4.2)	2.0 (1.3–2.8)	0.08
Safety endpoints, n (%)			
Hypercalcemia	0 (0)	0 (0)	N/A
Hyperphosphatemia	1 (3)	1 (3)	1.00

Definition of abbreviations: ICU = intensive care unit; IQR = interquartile range; RRT = renal-replacement therapy; SOFA-CV = Sequential Organ Failure Assessment (Cardiovascular); UOP = urine output.
$Pa_{O_2}/F_{I_{O_2}}$ is reported only for patients receiving mechanical ventilation.
Hypercalcemia and hyperphosphatemia were assessed only a daily basis for 48 hours after study drug administration.

that the immunomodulatory effects of circulating 1,25D are minimal.

We detected significant between-group differences in hCAP-18 leukocyte mRNA expression but not plasma protein levels. Several possible explanations may explain this. First, because hCAP-18 is produced mainly by leukocytes, quantification of leukocyte mRNA expression may be a more reliable and sensitive method than measurement of plasma protein levels, which may be affected by degradation, elimination, and changes in plasma volume. Additionally, hCAP-18 is produced as a propeptide, which is stored intracellularly in granules and then cleaved to produce the biologically active C-terminal fragment, LL-37, which is measured extracellularly in the plasma (48). Thus, plasma LL-37 protein levels may be influenced by such factors as activity of the cleavage enzyme, proteinase 3, and may not be an accurate reflection of intracellular hCAP-18 protein levels. Consistent with this notion, others have shown an absence of correlation between leukocyte hCAP-18 mRNA expression and plasma protein levels (49). Finally, although not statistically significant, patients in the placebo group had a higher white blood cell count and higher hCAP-18 protein levels at baseline than patients in the calcitriol group.

We found increased IL-10 mRNA expression in calcitriol- versus placebo-treated patients but did not detect significant differences in mRNA expression or protein levels of other inflammatory markers, including IL-6 and TNF-α. These findings differ from previous *in vitro* studies, in which 1,25D supplementation decreases proinflammatory cytokines (17, 18). Previous *in vivo* findings, in contrast, have been mixed, with studies finding that nutritional vitamin D decreases (18, 50), increases (51), and produces no change (52, 53) in proinflammatory cytokine levels. In the current study, it is likely that the heterogenous and rapidly evolving nature of sepsis and critical illness created substantial "noise" and diminished our ability to detect an effect of calcitriol on inflammatory markers.

We acknowledge the limitations of this study including modest sample size and single-dose administration of calcitriol. Although 1,25D levels increased approximately fivefold in patients treated with calcitriol, the plasma levels achieved were nonetheless lower than concentrations required to produce immune responses in most *in vitro* studies. Thus, it is possible that higher doses of calcitriol would have produced a greater effect on the biomarkers that were studied. We did not measure levels of vitamin D metabolites at 24 and 48 hours, which might have yielded pharmacokinetic data that could have been informative for future studies. We did not measure all potentially relevant markers of mineral metabolism, such as vitamin D binding protein, parathyroid hormone, and fibroblast growth factor-23. We measured total leukocyte mRNA expression but did not isolate cell-specific populations, such as monocytes. Finally, because of the large number of tests performed, we acknowledge the possibility of a type I error among the secondary endpoints.

In conclusion, our central findings are that administration of a single dose of 1,25D to critically ill patients with sepsis failed to increase plasma hCAP-18 protein levels but may have increased leukocyte hCAP-18 mRNA expression. Based on these findings, larger phase III studies of calcitriol in sepsis would be premature. Additional phase II studies are needed to investigate whether higher and repeated doses of calcitriol or administration of other vitamin D metabolites (e.g., cholecalciferol or calcifediol) can influence immune function in humans. Future interventional studies should also consider using more sensitive techniques to evaluate immune function, including magnetic-activated cell sorting to isolate specific leukocyte populations, flow cytometry to evaluate expression of cell surface markers, and functional *ex vivo* studies. Finally, because sepsis is a remarkably heterogeneous condition, future studies should attempt to identify a specific population of patients most likely to benefit from administration of vitamin D metabolites. ■

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