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REVIEW ARTICLE

Vitamin D as Adjunctive Host-Directed Therapy in Tuberculosis: A Systematic Review

Robert S. Wallis[1,2,3] and Alimuddin Zumla[4]

[1]Aurum Institute, Johannesburg, and [2]Advancing Care for TB/HIV, Johannesburg, South African Medical Research Council, South Africa; [3]Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio; and [4]Division of Infection and Immunity, University College London and NIHR Biomedical Research Centre, UCLH NHS Foundation Trust, United Kingdom

Vitamin D plays an important role in innate defenses against intracellular pathogens. Seasonal vitamin D insufficiency (VDI) due to reduced sun exposure far from the equator increases tuberculosis risk. Eight randomized controlled trials examined vitamin D as adjunctive therapy during tuberculosis treatment. The studies varied substantially regarding patient genetic backgrounds, the extent of baseline VDI, the administered dose, the study endpoints, and the quality of the reported data. One carefully performed study in which moderately large vitamin D doses were given to markedly VDI patients found a benefit sufficient to support shortening treatment from 6 to 4 months, although other similar studies did not. Vitamin D is thought to have anti-inflammatory effects. However, 2 studies reported 3 vitamin D recipients with severe paradoxical inflammatory reactions. Future studies of vitamin D in tuberculosis in patients with specific genetic backgrounds must monitor these events closely to determine their risks and underlying mechanisms.

Keywords. clinical trial; host-directed therapy; tuberculosis; vitamin D.

Although vitamin D is best recognized as a regulator of calcium and bone homeostasis, it has diverse additional cellular functions, affecting differentiation, proliferation, activation, and death. In humans, vitamin D mainly results from ultra violet B-induced cleavage of 7-dehydrocholesterol in skin (reviewed in Ref. [1]). Dietary animal-derived vitamin D_3 (cholecalciferol) and plant-derived D_2 (ergocalciferol) can supplement that produced in the skin (Figure 1). Vitamin D is converted in the liver to calcidiol (25[OH]D), which then undergoes 1α-hydroxylation in the kidney by the cytochrome P450 enzyme CYP27B1 to form calcitriol ($1,25[OH]_2D$), the active form. Calcitriol acts on the kidneys, gut, and bones to regulate serum calcium. It also regulates its own catabolism via induction of the 24-hydroxylase CYP24A1 and its metabolism, through negative regulation of parathyroid hormone, which induces CYP27B1 in response to hypocalcaemia.

Serum concentrations of calcidiol are commonly measured to assess nutritional status. Concentrations >20 ng/mL (50 nM) are required to maintain calcium homeostasis and to prevent secondary hyperparathyroidism; higher levels may be required for other functions. Serum concentrations of calcidiol exceed those of calcitriol by 3 logs. Multiple gene polymorphisms have been associated with vitamin D insufficiency (VDI), although their relationship to tuberculosis (TB) risk seems complex [2]. There is no clear evidence that human immunodeficiency virus (HIV)-1 infection per se reduces vitamin D levels, although antiretroviral drugs may do so [3]. Vitamin D levels fluctuate seasonally in regions far from the equator. Martineau et al [4] have argued that in Cape Town (34°S), summer peaks in calcidiol levels are the cause for the autumn nadirs in TB notifications. Seasonal variation in notifications has also been reported in Birmingham, UK (52°N) [5], and in southernmost Australia (40–44°S), but not in equatorial regions of that country [6].

The ability of calcitriol to restrict the growth of virulent *Mycobacterium tuberculosis* in human macrophages was first reported by Crowle et al [7]. In that study, calcitriol 4 μg/mL (5 logs higher than in blood) increased the intracellular doubling time of virulent *M tuberculosis* from 1 to 3 days, although it did not produce overt bactericidal activity. Several mechanisms have since been proposed, including production of nitric oxide (NO) and promotion of phagolysosome fusion [8]. Similarly high concentrations are required to inhibit *M tuberculosis* growth in mixed mononuclear cell cultures, which also lack overt bactericidal activity [9]. These observations seem to indicate that monocyte-lymphocyte interactions do not contribute substantially to vitamin D effects. Recent studies have focused on induction by vitamin D of antimicrobial peptides, particularly cathelicidin antimicrobial peptide, an inducer of autophagy in macrophages [10, 11]. Cathelicidin antimicrobial peptide is cleaved by proteinase 3 (PR3) to form cathelicidin (LL37), which exerts direct antimicrobial activity by binding to and disrupting bacterial cell wall phosphatidylglycerol monolayers [12]. Proteinase 3 is mainly produced by neutrophils, implying

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Correspondence: R. S. Wallis, Chief Science Officer, Aurum Institute, 29 Queens Rd, Parktown, Johannesburg 2193, South Africa (rwallis@auruminsitute.org, rswallis@gmail.com).

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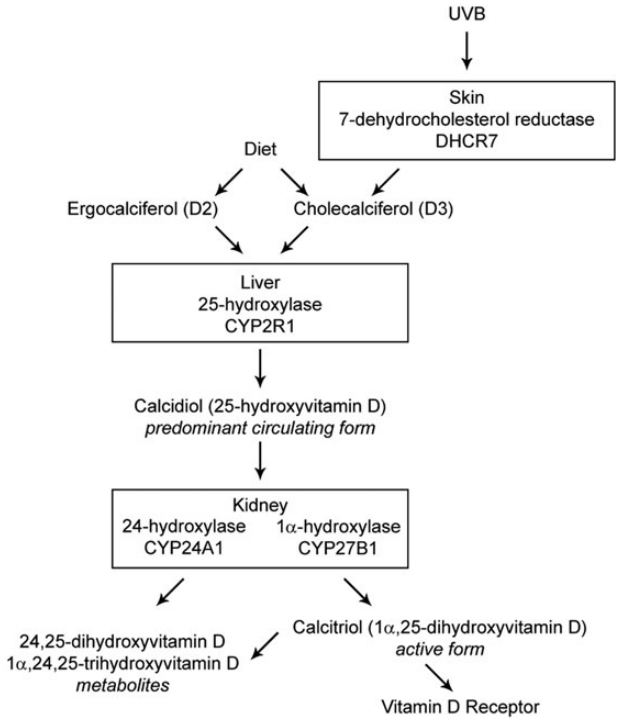

Figure 1. Vitamin D metabolism. Adapted from Coussens et al. Anti-inflammatory and antimicrobial actions of vitamin D in combating TB/HIV. *Scientifica (Cairo)* **2014**; 2014:903680.

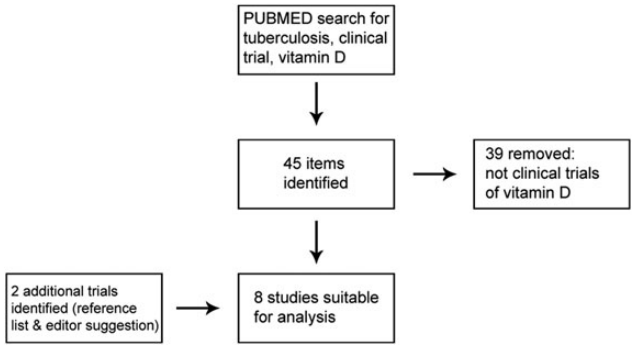

Figure 2. PRIZMA flow diagram of study selection.

that greater antimicrobial activity may be expressed in mixed cell culture models that include both neutrophils and macrophages. One controlled trial of vitamin D supplementation in healthy London TB household contacts found superior killing during the first 24 hours of whole blood culture in ergocalciferol recipients [13]. However, antimycobacterial activity was not sustained in these cultures, which showed net mycobacterial growth during the subsequent 3 days. Neutrophils contribute to antimycobacterial activity early in whole blood culture [14], but these cells quickly lose viability in vitro due to apoptosis.

High concentrations of calcitriol added to *M tuberculosis*-infected blood mononuclear cell cultures inhibit production of interferon (IFN)-γ, tumor necrosis factor, and interleukin-12p40, as well as multiple matrix metalloproteinases [9]. Together, these findings support the concept that adjunctive host-directed treatment with vitamin D in TB patients might accelerate cure, reduce relapse, and reduce inflammation, thereby preventing permanent lung damage.

METHODS

PubMed was searched using the terms TUBERCULOSIS, CLINICAL TRIAL, and VITAMIN D or its synonyms. One additional study was found from the references of identified studies, and a second additional recent study was suggested by an editor (Figure 2). Studies were included if they were randomized and controlled and if sputum smear or culture results were reported. Sputum culture status after 8 weeks of treatment was the preferred endpoint because this is the sole TB biomarker meeting the criteria of Chau et al [15] as a "known valid" predictor of relapse [16, 17] and failure [18, 19]. Sputum acid-fast smear conversion was substituted for culture in 3 studies in which culture was not reported. Odds ratios for conversion of sputum culture or smear were calculated using an online calculator (https://www.medcalc.org/calc/odds_ratio.php) according to the method of Altman [20]. The extent of VDI at baseline was categorized as low (>70 nM mean serum calcidiol), intermediate (30–70 nM), and high (<30 nM). Total vitamin D doses during the first 8 weeks of treatment were categorized as low (2.5 mg), intermediate (7.5–10.5 mg), and high (30 mg).

RESULTS

Eight studies were identified for this review (Table 1). Unless otherwise noted, they (1) enrolled HIV-uninfected adult patients with sputum acid-fast smear positive fully drug-sensitive TB at a single-study site, (2) administered daily isoniazid, rifampin, ethambutol plus pyrazinamide for 2 months, followed by daily isoniazid plus rifampin for 4 months (2HREZ/4HR), (3) compared oral cholecalciferol versus placebo in a double-blinded manner, and (4) analyzed the primary endpoint in a modified intent-to-treat (ITT) population. Serum calcidiol concentrations were measured in 6 trials. Values at baseline correlated highly with study site latitude ($R = -0.971$, $P = .006$). From this it may be surmised that calcidiol levels likely were high in patients in the remaining 2 trials due to their equatorial proximity. Odds ratios for sputum conversion at 4 and 8 weeks for each study appear in Figure 3. The trials are discussed in order overall effect, from low to high.

Ralph et al [21] conducted a 4-arm study in 200 randomized patients in Timika, Indonesia. Patients infected HIV were included. The study examined L-arginine and vitamin D

Table 1. Randomized Controlled Trials of Adjunctive Vitamin D in Tuberculosis, Arranged in Order of Vitamin D Dose

Study	Country	Latitude	Baseline Serum Calcidiol nM	N[a]	Vitamin D Dose to wk 8	Vitamin D Dose Total
Ralph et al [21]	Indonesia	5°S	ND	200	2.5 mg	2.5 mg
Martineau et al [22]	UK	52°N	21	146	10 mg	10 mg
Wejse et al [23]	Guinea Bissau	12°N	78	367	2.5 mg	7.5 mg
Daley et al [24]	India	13°N	62	247	10 mg	10 mg
Salahuddin et al [25]	Pakistan	25°N	53	259	30 mg	30 mg
Tukvadze et al [26]	Georgia	42°N	35	199	30 mg	35 mg
Mily et al [27]	Bangladesh	24°N	27	144	7.5 mg	7.5 mg
Nursyam et al [28]	Indonesia	6°S	ND	67	10.5 mg	10.5 mg

Abbreviations: ND, not determined; UK, United Kingdom.

[a] N refers to the number of subjects randomized to vitamin D or control arms.

supplementation separately and in combination in a double placebo design. The rationale for L-arginine was to promote NO production. Cholecalciferol 1.25 mg was given at baseline and after 4 weeks. Patients received thrice-weekly isoniazid plus rifampin during the continuation phase ($2HRZE/4H_3R_3$) and were observed for 24 weeks. The primary outcome measures were (1) sputum culture after 4 weeks using liquid medium and (2) a composite clinical score that included change in weight, forced expiratory volume in 1 second (FEV_1), cough, sputum, and hemoptysis after 8 weeks. Other endpoints included other spirometry, exercise capacity (6-minute walk test [6MWT]), and lung symptoms (the St. George Respiratory Symptom Questionnaire [SGRSQ]). One hundred fifty-five patients (78%) could be analyzed for the first primary endpoint. Neither treatment affected the primary outcome measures nor was any interaction between treatments detected. There was no effect on time to sputum smear conversion nor on sputum culture status at 4 or 8 weeks. There was no effect on composite clinical score, FEV_1, chest x-ray score, or 6MWT at week 8 (all $P > .4$). There was superior resolution of SGSRQ scores in the placebo arm ($P = .02$), and, in a subset analysis of HIV-negative cases, superior culture conversion at week 8 in the placebo arm ($P = .05$), both contrary to the study hypothesis. Both treatments appeared safe and well tolerated. This study appears to have been hampered by testing a low vitamin D dose in a relatively small patient population unlikely to be profoundly VDI.

Martineau et al [22] conducted a multicenter trial in 146 randomized patients in London, UK. Patients received 2.5 mg cholecalciferol or placebo every 2 weeks for 4 doses (total 10 mg over 2 months). Patient follow up was limited to 8 weeks. Analyses were performed according to assigned treatment (including 4 subjects with rifampin-resistant isolates assigned to the

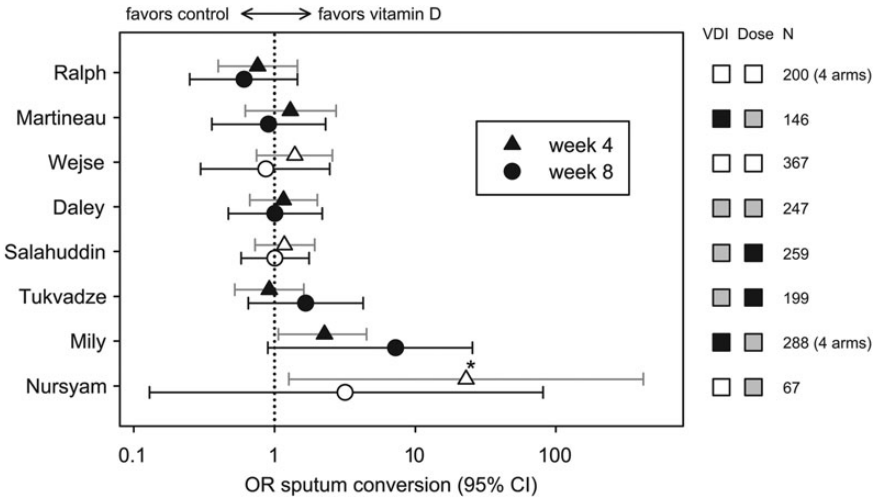

Figure 3. (Left) Odds ratio (OR) for sputum conversion at weeks 4 and 8 according to study. Black symbols indicate sputum culture; white symbols indicate sputum smear. Error bars indicate 95% confidence interval (CI). The asterisk indicates smear conversion at week 6. (Right) Classification of baseline vitamin D insufficiency (VDI), administered vitamin D dose during the first 8 weeks, and number of subjects. White = low; gray = intermediate; black = high. For VDI, low = >70 nM mean serum calcidiol, intermediate = 30–70 nM, and high = <30 nM. For dose, low = 2.5 mg, intermediate = 7.5–10.5 mg, and high = 30 mg.

control arm). Participants had profound VDI, with serum calcidiol <20 nM in 75 of 126. Serum calcidiol levels and renal calcium excretion were significantly augmented by vitamin D supplementation. One hundred twenty-six patients (86%) were evaluated for the primary endpoint of time to sputum culture conversion. There was no effect on sputum culture, smear, or time to detection in automated liquid culture in univariate or multivariate analyses. Vitamin D had no effect on erythrocyte sedimentation rate (ESR), body mass index (BMI), or chest radiographs. Vitamin D was generally well tolerated; however, 2 D-treated patients experienced symptomatic paradoxical reactions ([PRs], disease worsening despite microbiologic improvement, attributed to changes in immune function) that required therapeutic drainage of paraspinal and psoas muscle abscesses. A third patient developed asymptomatic hypercalcemia. Coussens et al [29] conducted an immunologic analysis on a per-protocol subset of 95 patients from the trial, removing, among others, the 2 PR cases. There was no effect on time to sputum culture conversion after adjusting for multiple baseline factors (35 vs 46.5 days; hazard ratio [HR], 1.27; 95% confidence interval [CI], .76–2.13). However, in striking contrast to the ITT analysis, vitamin D accelerated normalization of ESR and serum C-reactive protein ([CRP] $P \leq .0072$) in this population. Vitamin D also reduced chemokine production, but it had no effect on IFN-γ.

Wejse et al [23] conducted a trial in 367 randomized subjects in Guinea Bissau. One third of patients were infected with HIV-1 or 2. Patients were given 2.5 mg cholecalciferol or placebo on entry and at 5 and 8 months. Patients did not receive rifampin in the continuation phase (2HRZE/6HE). Antiretroviral therapy was not available in Guinea Bissau at the time of the study. There was no effect on serum calcidiol levels, which increased from approximately 75nM to >100 nM equally in both arms, although greater increases tended to occur in vitamin D recipients with VDI at baseline. The main outcome measure was a composite disease severity score ("TBscore") [30] that included cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung-auscultation finding, fever, low BMI, and low mid-upper arm circumference, which was monitored during 8 months of treatment. Three hundred four patients (83%) could be evaluated at month 2; 281 (77%) could be evaluated for mortality at month 12. There was no effect on TBscore at any time point; values declined equally in both arms from approximately 6.7 at diagnosis to 2.2 by week 8 and to 1.5 by end-of-treatment. Post hoc subset analyses according to baseline VDI and HIV status showed no effect of vitamin D on this score. There was no effect on sputum smear at any time point. Sputum culture was not reported. There was no effect on survival over 12 months either in the full cohort or in the HIV-infected subset. There was no effect on CD4 T-cell counts in HIV-infected subjects. After adjusting for baseline VDI, the mortality risk ratio in HIV-infected subjects was 2.4 (95% CI, .95–6.5), tending to favor placebo. Although patients were monitored until end-of-treatment, there was no analysis of treatment failure. In retrospect, this study was hindered by high baseline calcidiol levels, a dosing schedule in which most cholecalciferol was given after sputum conversion had occurred, and a primary endpoint with limited capacity to distinguish symptoms of TB from those of untreated acquired immune deficiency syndrome.

Daley et al [24] conducted a multicenter trial in 247 randomized patients in Tamil Nadu, India, 211 (85%) of whom could be evaluated in the primary efficacy analysis, time to sputum culture conversion. Patients received 2.5 mg cholecalciferol or placebo every 2 weeks for 8 weeks (10 mg total). The mean serum calcidiol concentration at baseline was 62 nM. By day 180, calcidiol concentrations had increased 14 nM from baseline in vitamin D recipients vs 7 nM in the control arm, a modest effect. There was no effect on time to sputum smear or culture conversion using any of the 3 definitions of conversion. Ten percent of subjects in both arms of this trial remained culture positive throughout treatment. There was no effect on BMI or performance score.

Salahuddin et al [25] conducted a multicenter trial in 259 randomized patients in Karachi, Pakistan. Patients received 15 mg cholecalciferol or placebo intramuscularly on entry and at 4 weeks, and they were monitored for 12 weeks. The mean serum calcidiol level on entry was 50 nM. All 259 randomized patients could apparently be evaluated for the primary endpoint, the change from baseline to 12 weeks in TBscore [23]. Vitamin D did not affect this outcome measure at week 4, 8, or 12 (all $P > .2$), although it did improve body mass (+1.4 kg) and chest x-ray involvement (−0.5 zones) at week 12. There was no effect on sputum smear in 211 subjects. In post hoc subset analyses in patients with baseline serum calcidiol <75 nM, vitamin D was reported to improved TBscore and smear conversion, but the latter was not described further. There was 1 death in the vitamin D arm due to rapidly progressive respiratory failure of undetermined etiology within 2 weeks of randomization, which the authors speculated may have been due to a PR.

Tukvadze et al [26] conducted a trial in Tblisi, Georgia, in 199 randomized subjects, including 23 with multidrug-resistant TB (MDR-TB) and 4 with HIV-1 infection. Subjects received 1.25 mg cholecalciferol or placebo thrice weekly for 8 weeks, followed by 1.25 mg every 2 weeks for 8 additional weeks. Patients had moderately severe VDI on entry. Calcidiol levels rose from 35 nM at baseline to 250 nM by week 8 in the vitamin D arm, but they remained unchanged in controls. There was no effect on median time to sputum culture conversion ($P = .99$) or on culture status after 8 weeks. The high vitamin D dose seemed well tolerated.

Mily et al [27] conducted a 4-arm trial in 288 randomized patients in Bangladesh. The study examined vitamin D and phenylbutyrate (a cathelicidin inducer [31]) separately and in

combination. Cholecalciferol 0.125 mg daily and phenylbutyrate 500 mg twice daily were administered for 60 days. Follow-up observations continued for 24 weeks. Two hundred fifty-six patients (89%) were available for analysis at week 8, and 219 (76%) were available at week 24. The main outcome measures were sputum microbiology and TBscore. There was marked VDI on entry (mean serum calcidiol 26 nM). Values increased to the 80–90 nM range in the vitamin D arms, but they remained unchanged in the nonvitamin D arms. Superior culture conversion compared with controls occurred in D + phenylbutyrate and D recipients ($P = .001$ and .032, respectively) at week 4 and in D recipients ($P = .032$) at week 8. At this time point, vitamin D reduced the proportion culture positive from 10.9% to 1.6%. Vitamin D plus phenylbutyrate produced an intermediate effect (5.6%); phenylbutyrate alone had no effect at either time point ($P > .60$) (Figure 4). The TBscores followed a trajectory similar to that described by Wejse et al [23]. However, only the phenylbutyrate alone arm showed superior resolution of scores during the period of adjunctive treatment, with scores approximately 0.5 lower than other arms at 2, 4, and 8 weeks. There was no effect on ESR or CRP. Both agents appeared well tolerated. The authors had previously reported in abstract form that sputum culture conversion occurred equally in all arms [32]; further investigation by the study team revealed the abstract to be in error, in fact describing smear rather than culture results (R. Raqib, International Center for Diarrheal Disease Research, written communication, December 2015). The discordance between culture and symptom scores somewhat lessens the potential impact of this study.

Nursyam et al [28] conducted a trial in 67 patients in Jakarta, Indonesia, in which vitamin D 250 μg/day was given for the first 6 weeks (10.5 mg total over 1.5 months). All 67 patients could be evaluated for the main study endpoints: sputum smear status at weeks 6 and 8. Superior smear conversion occurred at week 6 in vitamin D recipients, but not at week 8, at which time all but 1 subject (a placebo recipient) had converted to negative. The study had numerous shortcomings. The manuscript described neither ethical review nor informed consent. Sputum smear results at week 4 were not reported, and cultures were not reported at any time point. No information was provided regarding the vitamin D composition or manufacturer. The analysis populations were not defined. The methods section referred to follow-up chest x-rays being performed at 6 months, whereas the text referred to them at 6 weeks. Approximately half of randomized patients were lost to follow-up before a planned 6-month evaluation.

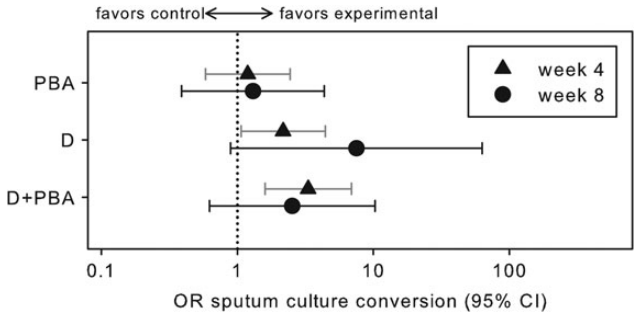

Figure 4. Odds ratio (OR) for sputum culture conversion at weeks 4 and 8 for patients receiving phenylbutyrate (PBA), vitamin D, and both agents. Adapted from Mily et al. Significant effects of oral phenylbutyrate and vitamin D3 adjunctive therapy in pulmonary tuberculosis: a randomized controlled trial. *PLoS One* **2015**; 10: e0138340. Abbreviation: CI, confidence interval.

Two studies performed subset analyses of potential interest despite their small size. Martineau et al [22] reported a significant benefit of vitamin D evident in 12 subjects with the tt Taq1 VDR genotype (HR, 8.09; 95% CI, 1.36–48.01), with proportions culture positive at week 8 of 0% and 57% in the vitamin D and control arms, respectively. There was no benefit of vitamin D in other VDR genotypes. However, these findings were not replicated by Tukvadze et al [26], who found no effect of VDR genotype on vitamin D in an analysis that included 30 tt genotype subjects. Tukvadze et al [26] also conducted a subset analysis in 18 patients who were found after enrollment to have MDR-TB. Treatment with second-line drugs began after a mean of 51 days for placebo recipients and 62 days for vitamin D recipients (ie, close to or shortly after the week 8 time point). Six of 10 recipients remained culture positive at week 8 in the placebo arm versus 1 of 8 in the vitamin D arm ($P = .07$).

DISCUSSION

These 8 studies of adjunctive vitamin D treatment in TB differed substantially with respect to study design, baseline patient characteristics (including extent of vitamin D lack), calciferol dose and dosing schedule, and study endpoints. Only 2 of the 8 studies found a significant microbiologic effect of vitamin D, and, of these, only 1 can be considered to have been conducted in a rigorous manner. High concentrations of calcitriol are required to produce antimycobacterial effects in cultured macrophages [7]. Therefore, it is tempting to hypothesize that the greatest clinical benefit would be seen when high calciferol doses were administered to patients with marked deficiency at baseline. This does not appear to be the case, because studies sharing these characteristics here yielded markedly different results. The cause of this variation remains unexplained. Genetic polymorphisms in the vitamin D receptor, or in the multiple enzymes involved in vitamin D metabolism, remain attractive candidates, because the 2 studies to date to address this question have been limited in terms of subject numbers and scope of genetic testing. Measurement of calcitriol-induced antimycobacterial activity in ex vivo whole blood culture in future studies may help us better understand the functional effects of specific genetic polymorphisms.

Even in the best case, however, the benefits of vitamin D in drug-sensitive TB may well fall short of expectations. One of the goals of host-directed adjunctive therapies is to facilitate shorter TB regimens without increasing the risk of relapse. If the finding by Mily et al [27] that vitamin D reduced to 1.6% the proportion culture positive at 8 weeks proves to be correct, the model developed by Wallis et al [16, 17] predicts that if administered for 4 months, this regimen would yield a relapse rate of 6% and have approximately a 15% chance of relapse rate >10% in a typical phase 3 trial. For vitamin D plus phenylbutyrate, the reported 5.6% positive rate would most likely yield 10.1% relapses. The former rate may well be viewed favorably by TB control programs, whereas the latter likely would not. None of the other studies described here approached this modest level of effect. Further studies in MDR-TB may be warranted, because the reduced activity of current MDR-TB regimens may offer a greater window of opportunity for a modestly active adjunctive therapy to demonstrate clinical benefit. That being said, the findings of the Tukvadze et al [26] MDR-TB subset analysis, in which vitamin D increased from 40% to 88% the proportion converting to negative at week 8, before the majority had started second-line drug therapy, are difficult to understand. These findings underscore the need for adjunctive host-directed therapies with greater antimicrobial effects in vitro and in vivo.

CONCLUSIONS

A final comment regarding safety is also warranted. These studies generally found vitamin D supplementation to be safe and well tolerated. However, the occurrence of 3 serious PRs, resulting in death or requiring surgical or radiologically guided intervention, occurring only in vitamin D-treated subjects, is a concern. The finding appears contrary to the apparent anti-inflammatory effects of vitamin D described in other patients. Specific attention will be required in future studies to determine whether vitamin D increases PR risk, and, if so, the mechanism of this effect.

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