Original Research

The effects of first-line anti-tuberculosis drugs on the actions of vitamin D in human macrophages

Supavit Chesdachai, Susu M. Zughaier, Li Hao, Russell R. Kempker, Henry M. Blumberg, Thomas R. Ziegler, Vin Tangpricha *

Division of Endocrinology, Metabolism and Lipids, Department of Medicine, Emory University School of Medicine and the Atlanta VA Medical Center, 101 Woodruff Circle NE- WMRB1301, Atlanta, GA 30322, USA

ARTICLE INFO

ABSTRACT

Tuberculosis (TB) is a major global health problem. Patients with TB have a high rate of vitamin D deficiency, both at diagnosis and during the course of treatment with anti-tuberculosis drugs. Although data on the efficacy of vitamin D supplementation on *Mycobacterium tuberculosis* (*Mtb*) clearance are uncertain from randomized controlled trials (RCTs), vitamin D enhances the expression of the antimicrobial peptide human cathelicidin (hCAP18) in cultured macrophages *in vitro*. One possible explanation for the mixed (primarily negative) results of RCTs examining vitamin D treatment in TB infection is that anti-TB drugs given to enrolled subjects may impact actions of vitamin D to enhance cathelicidin in macrophages. To address this hypothesis, human macrophage-like monocytic (THP-1) cells were treated with varying doses of first-line anti-tuberculosis drugs in the presence of the active form of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$). The expression of hCAP18 was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). 1,25(OH)$_2$D$_3$ strongly induced expression of hCAP18 mRNA in THP-1 cells (fold-change from control). The combination of the standard 4-drug TB therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) in the cultured THP-1 cells demonstrated a significant decrease in hCAP18 mRNA at the dosage of 10 µg/mL. In 31 subjects with newly diagnosed drug-sensitive TB randomized to either high-dose vitamin D_3 (1.2 million IU over 8 weeks, n = 13) versus placebo (n = 18), there was no change from baseline to week 8 in hCAP18 mRNA levels in peripheral blood mononuclear cells or in plasma concentrations of LL-37, the protein product of hCAP18. These data suggest that first-line anti-TB drugs may alter the vitamin D-dependent increase in hCAP18 and LL-37 human macrophages.

Introduction

Tuberculosis (TB) remains a major worldwide health issue as the second most frequent cause of infection-related mortality [1,2]. The World Health Organization (WHO) estimates that in 2013 there were ≈9 million new cases and ≈1.5 million deaths from TB disease [2]. The first line regimen of anti-TB drugs consists of a combination of isoniazid (INH), rifampicin, pyrazinamide and ethambutol [3], a combination with high success rates in various studies [4]. However, an epidemic of multi-drug resistant TB (MDR-TB) in which the infecting *Mycobacterium tuberculosis* (*Mtb*) organism is resistant to at least INH and rifampicin has emerged, including in the former Soviet Union Republics, such as Georgia [5].

Alternative and complementary therapies are needed in TB to improve clinical outcomes. Before the development of anti-TB drugs, both high-dose vitamin D and sunlight therapy had been used as a major treatment of TB [6,7] although the pleiotropic effects of vitamin D on immune functions were not yet documented [8–10]. Several recent RCTs have re-examined the potential of vitamin D to help improve cure rates in patients with TB and MDR-TB [9,11–19]. Many immune cells express the vitamin D receptor (VDR) including T-cells, B-cells, dendritic cells and macrophages [7,8,20,21]. Both vitamin D and VDR influence the expression of specific endogenous antimicrobial peptides in the macrophage [21]. Vitamin D activation of the VDR is applicable to common infections such as influenza and TB. Further, the active vitamin D hormone can inhibit growth of *Mtb* in cultured human macrophages [22]. TB activates macrophages via the toll-like receptor (TLR) complex (TLR2/1) to stimulate the expression of VDR and 1α-hydroxylase (1α-OHase or CYP271B) [21]. In the presence of sufficient amounts of 25-hydroxyvitamin D_3 (25(OH)D$_3$) in the serum, it is converted to its

* Corresponding author. Fax: (404) 592-6257.
E-mail address: vin.tangpricha@emory.edu (V. Tangpricha).

active form, 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$) [21]. Binding of 1,25(OH)$_2$D$_3$ to the VDR induces transcription and translation of various genes, including the antimicrobial peptide cathelicidin (hCAP18) and its cleaved activated peptide LL-37, and β-defensin-4 (DEFB-4). Both hCAP18 and DEFB-4 stimulate the eradication of TB via the autolysosomal process [23]. Multiple *in vitro* experiments confirm that vitamin D up-regulates expression of hCAP18; however, several recent randomized control trials examining the efficacy of vitamin D in clearing active TB disease [9,11–19] have been largely negative [9,11,12,17–19]. One of the possible hypotheses for the discrepancy between the *in vitro* and *in vivo* data is that anti-TB drugs may interfere with vitamin D metabolism and thus the induction of hCAP18 by macrophages. Therefore, the objective of our study was to examine the impact of first line anti-TB drugs on vitamin D induced expression of hCAP18 in cultured human monocytes *in vitro* and in peripheral blood mononuclear cells in a randomized controlled clinical trial of high-dose vitamin D in adult patients with newly diagnosed TB disease.

Materials and methods

Cell preparation

Human macrophage-like monocytic cells (THP-1) were obtained from American Type Culture Collection (ATCC; Manassas, VA) and grown in Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 10% fetal bovine serum (FBS), 50 IU/mL penicillin and 50 μg/mL streptomycin. The concentration of THP-1 cells was adjusted to a concentration of 1 million cells per mL and then sub-cultured into 6-well tissue culture plates in the presence of 0.25, 0.50, 1.00, 5.00, and 10.00 nM of 1,25(OH)$_2$D$_3$ (Sigma Aldrich; St. Louis, MO). Cells were incubated for 12 hours to establish the optimal concentration of 1,25(OH)$_2$D$_3$ for induction of hCAP18 mRNA. The concentration of 5 nM of 1,25(OH)$_2$D$_3$ was selected for subsequent *in vitro* experiments.

The anti-TB drugs isoniazid, rifampicin, pyrazinamide and ethambutol were diluted in dimethyl sulfoxide (DMSO) and stored at −80 °C until use. The concentrations of each drug were examined over a range of doses known to inhibit *Mtb* growth from previously published studies [24–29] at a final concentration of 1.0 μg/mL, 5.0 μg/mL and 10.0 μg/mL. THP-1 cells were then incubated in 37 °C, 5% CO_2 for 6 hours, following which cell suspensions were centrifuged and supernatants removed. Harvested THP-1 cells were placed in RLT buffer (Qiagen; Hilden, Germany) and passed through QiaShredder columns (Qiagen; Hilden, Germany).

RNA extraction and quantitative real-time polymerase chain reaction

RNeasy mini kits (Qiagen; Hilden, Germany) were used to extract RNA according to the manufacturer's protocol. Extracted RNA was reverse transcribed to cDNA by using iscript cDNA (Bio-Rad Laboratories; Hercules, CA). Relative gene expression was determined by quantitative real-time polymerase chain reaction (RT-qPCR) using TaqMan® Universal PCR Master Mix (Life Technologies; Grand Island, NY). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene to calculate the fold change of gene expression using ΔΔCT method. Primers purchased from Sigma Aldrich were used for RT-qPCR reactions: human hCAP18; CTTCACCAGCCCGTCCTTC and CCAGGACGACACAGCAGTCA, GAPDH; CTTAGCACCCCTGGCCAAG and TGGTCATGAGTCCTTCCACG. TaqMan® MGB Probes 6000 pmol were purchased from Life Technologies: human LL-37; 6-FAM CAG AGG ATT GTG ACT TCA MGBNFQ, GAPDH; VIC-CAT CCA TGA CAA CTT TGG TA MGBNFQ.

Cellular viability measurement using the colorimetric MTT reduction assay

The tetrazolium salt dye is cleaved by live and metabolically active cells but not by dead cells; therefore, the MTT reduction assay was used to measure the viability of THP-1 cells treated with anti-TB drugs as described before [30]. Briefly, freshly grown THP-1 cells adjusted to 1 million cells/mL were treated with anti-TB drug doses ranging from 1 to 10 μg/mL and incubated overnight. Control cells treated with vehicle only were co-incubated under the same experimental conditions. Five mg of thiazolyl blue tetrazolium bromide was dissolved in 1 mL of PBS and 15 μL was added to 150 μL of treated THP-1 cells which were further incubated at 37 °C for one hour. Cells were centrifuged to remove extracellular dye then lysed in 150 μL of DMSO to dissolve the intracellular dye. The absorbance of reduced dye was measured at 591 nm wavelength using a Bio-Tek spectrophotometer.

Data from clinical studies

We obtained plasma and peripheral blood monocytes (PBMC) from pulmonary TB patients participating in a double-blind, randomized, placebo controlled clinical trial of high-dose vitamin D_3 supplemented to newly diagnosed inpatients with pulmonary tuberculosis in Tbilisi, Republic of Georgia [9]. All patients received first-line 4 drug anti-tuberculosis therapy which included isoniazid, rifampicin, pyrazinamide and ethambutol plus either 50,000 IU of vitamin D_3 or placebo orally three times weekly for 8 weeks then 50,000 IU of vitamin D_3 or placebo every other week for the subsequent 8 weeks. The primary outcome of this study was the time to sputum culture conversion from positive to negative, during the 16-week study period, as defined by Martineau et al. [14]. All subjects provided informed consent for use of their blood samples for future sub-studies as outlined (clinicaltrials.gov NCT00918086). Blood for isolation of peripheral blood mononuclear cells (PBMCs) from subjects was collected at enrollment and at 8 weeks after treatment with high-dose vitamin D_3 (1.2 million IU over 8 weeks, n = 13) or placebo (n = 18). Expression of hCAP18 mRNA from PBMC was determined by RT-qPCR. Plasma LL-37 (the protein product of hCAP18 mRNA) was also measured by enzyme-linked immunosorbent assay (ELISA, Hycult Biotech; Uden, The Netherlands) (n = 31).

Statistical analysis

For the *in vitro* study, mean ± standard deviation and p-value from at least three independent experiments were analyzed with Student's t-test. For the data from the clinical study, comparisons between the vitamin D-treated and the placebo groups were analyzed with Chi square for categorical variables and Student's t-tests for continuous variables. Plasma LL-37 concentrations and PBMC hCAP18 mRNA at baseline and at week 8 were analyzed with two-way mixed model repeated measures analysis of variance (ANOVA). Analysis was performed by Microsoft Excel and IBM SPSS Statistics for Windows, version 23.0 (IBM Corp; Armonk, NY).

Results

Expression of hCAP18 mRNA in THP-1 cells

Addition of 1,25(OH)$_2$D$_3$ to cell culture media strongly induced expression of hCAP18 mRNA in THP-1 cells in a dose-dependent fashion (Fig. 1). INH at a concentration of 1.0 μg/mL significantly increased the expression of hCAP18 mRNA (Fig. 2A). In contrast, rifampicin at a concentration of 1.0 μg/mL significantly decreased
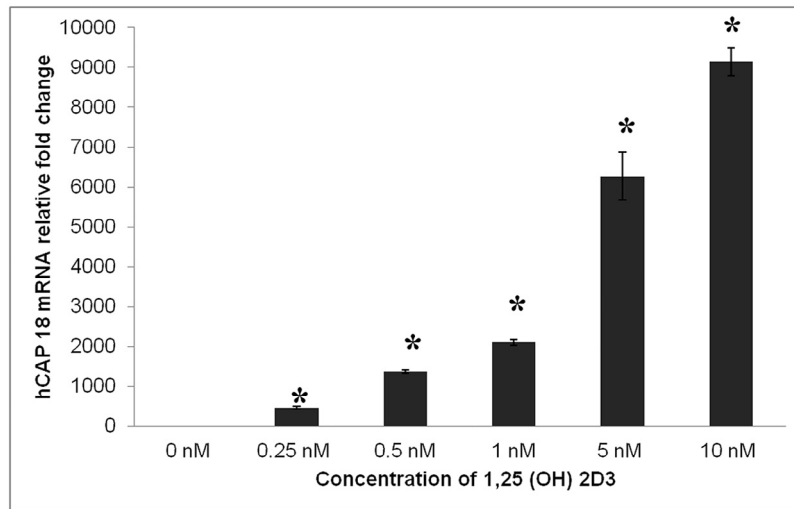
Figure 1. Vitamin D induces expression of human cathelicidin in cultured THP-1 cells. THP-1 cells were cultured for 12 hours in the presence of increasing concentrations of 1,25(OH)$_2$D$_3$ and harvested for RT-PCR for expression of human cathelicidin (hCAP18). The addition of 1,25(OH)$_2$D$_3$ strongly induced the expression of hCAP18 mRNA relative to GAPDH in a dose dependent fashion in the cultured THP-1 cells (*p-value < 0.05).

the expression of hCAP18 (Fig. 2B). At higher concentrations of 5.0 and 10.0 µg/mL, both INH and rifampicin, respectively, did not alter hCAP18 mRNA expression cultured in the presence of 1,25(OH)$_2$D$_3$ (Fig. 2A and B). Neither pyrazinamide nor ethambutol at

concentrations of 1.0, 5.0 and 10.0 µg/mL altered the expression of THP-1 cell hCAP18 mRNA cultured in the presence of 1,25(OH)$_2$D$_3$ (Fig. 2C and D). In contrast, cell culture with the combination of the 4 anti-TB drugs (INH, rifampicin, pyrazinamide and ethambutol)
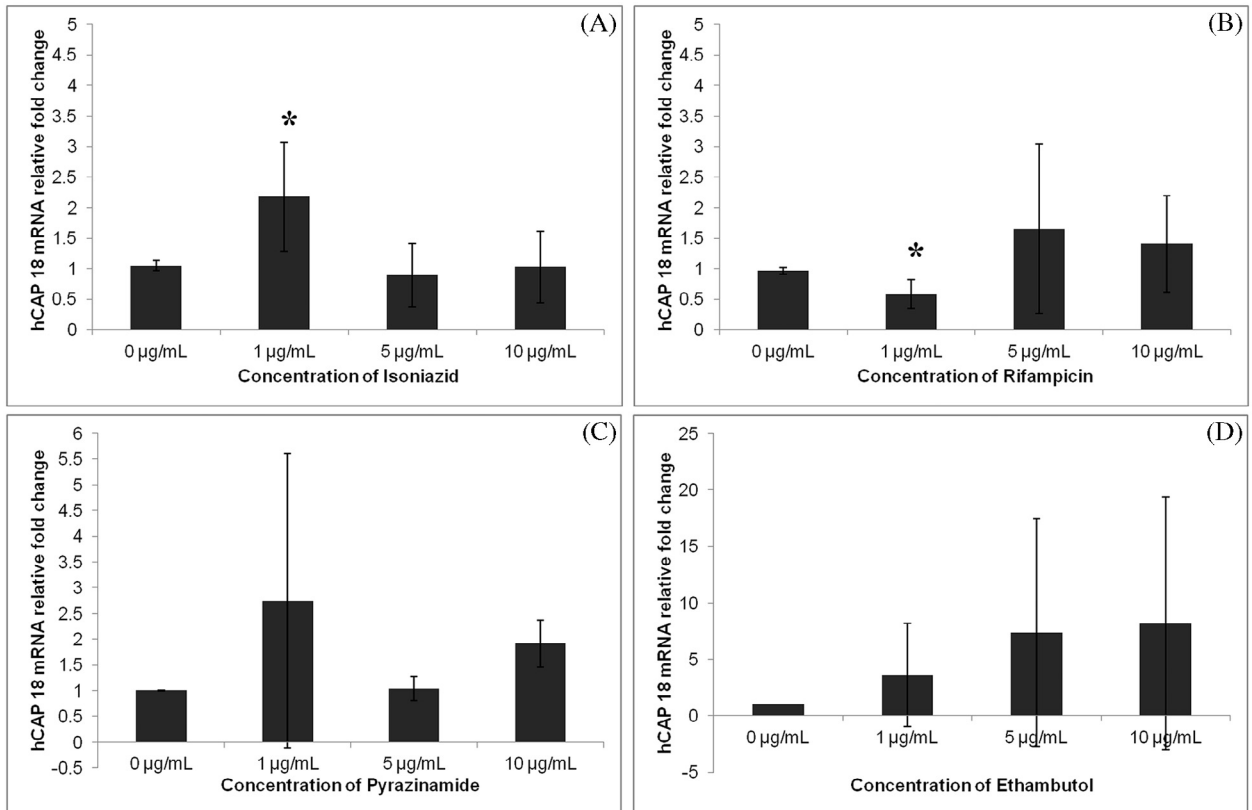

Figure 2. The impact of anti-tuberculosis drugs on induction of hCAP18 by vitamin D. The human monocytic cell line (THP-1) was cultured in the presence of 5 nM of 1,25(OH)$_2$D$_3$ for 12 hours followed by 6 hours of exposure to increasing concentrations of anti-TB drugs including isoniazid (A), rifampicin (B), pyrazinamide (C) and ethambutol (D) (0, 1 µg/mL, 5 µg/mL, and 10 µg/mL). THP-1 cells were harvested and mRNA expression of hCAP18 was evaluated relative to GAPDH. 1 µg/mL of isoniazid significantly increased the expression of hCAP18 mRNA, but the 5 and 10 µg/mL had no effect (A). 1 µg/mL of rifampicin significantly decreased the expression of hCAP18 (B). Pyrazinamide and ethambutol at all of the concentrations evaluated did not significantly alter the expression of hCAP18 in the presence of 1,25(OH)$_2$D$_3$ (C, D) (*p-value < 0.05).
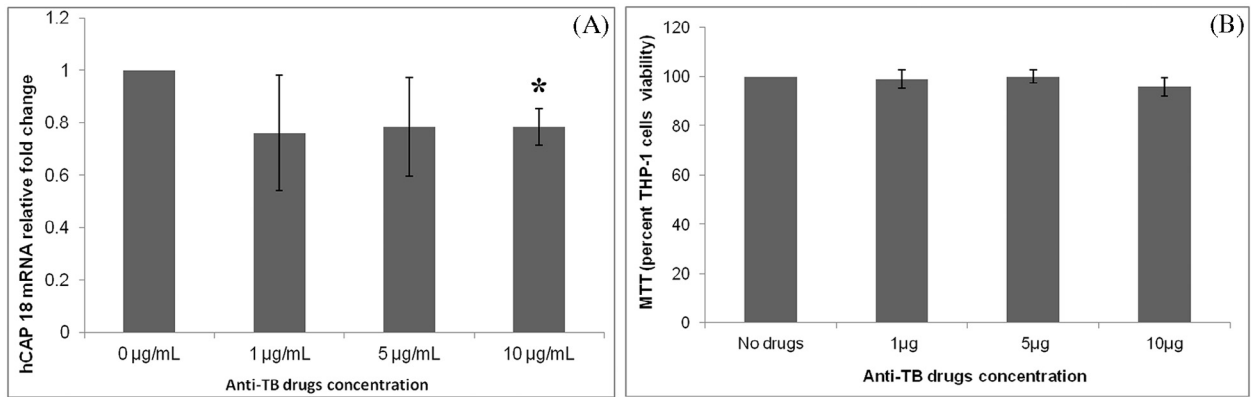
Figure 3. Impact of the 4 drug therapy (isoniazid, rifampicin, pyrazinamide and ethambutol) on vitamin D induced human cathelicidin (hCAP18) mRNA expression relative to GAPDH. The human monocytic cell line (THP-1) was cultured for 12 hours in the presence of $1,25(OH)_2D_3$ and all 4 anti-TB drugs were added at a concentration of 10 μg/mL for 6 hours. The THP-1 cells were harvested and mRNA was extracted for RT-PCR. The 4 drug combination demonstrated a significant decrease in the expression of hCAP18 mRNA at the highest concentration of 10 μg/mL (*p-value < 0.05) (A).

resulted in a significant decrease in the expression of hCAP18 mRNA at the dose of 10.0 μg/mL for each agent (Fig. 3A).

Further, the viability of THP-1 cells exposed to anti-TB drugs was assessed using the MTT assay as described [30]. The combination of the 4 anti-TB drugs treatment at 10.0 μg/mL of each drug did not affect the viability of THP-1 cells in presence of $1,25(OH)_2D_3$, suggesting that the reduction in hCAP18 mRNA is caused by anti-TB drugs (Fig. 3B).

Expression of hCAP18 in PBMC and plasma LL-37 in subjects with pulmonary TB

The demographic information of the subjects participating in this sub-study of the larger double blind RCT [9] is presented in Table 1. There was no difference in the baseline demographic and clinical characteristics between the subjects receiving high-dose vitamin D_3 and the subjects receiving placebo. No subject had MDR-TB and the mean plasma 25(OH)D concentration in each group was in the deficient range, similar to the plasma 25(OH)D concentrations in the full 199-subject RCT cohort [9]. All subjects received conventional dose regimens of INH, rifampicin, pyrazinamide and ethambutol, begun ≤7 days before the baseline blood sample and initiation of high-dose vitamin D_3 or placebo.

Plasma LL-37 concentrations did not differ between the two groups at baseline or after 8 weeks of anti-TB drug and vitamin D_3

Table 1
Participant demographics

Parameter	Placebo (n = 18)	Vitamin D (n = 13)	p-value
Age (yrs) (mean ± SD)	34.1 ± 11.8	33.4 ± 10.9	0.862
Gender (% male)	61.1	69.2	0.641
Employment status (% employed)	50	53.8	0.833
Yearly subject income (%) (<1000 Georgian lari) ($586)	16.7	15.4	0.924
Current smoker (%)	27.8	46.2	0.291
Body mass index (kg/m²) (mean ± SD)	19.8 ± 2.4	21.4 ± 4.3	0.198
Body mass index (kg/m²) (% BMI < 18.5)	38.9	23.1	0.353
Diabetes mellitus history (%)	11.1	7.7	0.751
Plasma 25(OH)D (ng/mL) (mean ± SD)	10.4 ± 7.5	11.2 ± 6.1	0.733
Isoniazid and rifampicin resistance (%)	0	0	1.0
TaqI genotype (%)			0.425
TT	47.1	30.0	
Tt	29.4	40.0	
tt	23.5	30.0	

or placebo administration (P = 0.088) (Fig. 4). The expression of hCAP18 mRNA in PBMC was also similar between the two groups. The expression of hCAP18 mRNA in the PBMC from each group significantly decreased at 8 weeks compared to baseline (P < 0.001) but there was no significant difference between the vitamin D_3 and placebo groups (P = 0.738) (Fig. 5).

Mean plasma 25(OH)D concentration was significantly increased at 8 weeks compared to baseline in the vitamin D group (P < 0.001) whereas there was no significant change in 25(OH)D concentrations at 8 weeks in the placebo group (P = 0.07) (Fig. 6).

Discussion

Several studies have confirmed that vitamin D induces hCAP18 expression in cultured human macrophages [21,24,31,32]. However many clinical studies have failed to show a positive effect of vitamin D on the treatment of tuberculosis infection [33]. One of the differences between the *in vitro* studies and the clinical studies is the potential confounding factor that all subjects receive standard anti-TB therapy in addition to the vitamin D or placebo, as it would be unethical to withhold standard anti-TB drug treatment. Our *in vitro* data confirm past published studies that show $1,25(OH)_2D_3$ strongly induces the expression of hCAP18 mRNA in a human monocytic cell line THP-1. We also found that anti-TB drugs, when added to the culture medium of THP-1 cells, altered the action of vitamin D in terms of vitamin D induced hCAP18 mRNA expression. The major finding of our study was that when all 4 conventional first-line anti-TB drugs were added to culture media together (at 10.0 μg/mL each), the expression of hCAP18 mRNA by the cultured macrophages was significantly decreased in the presence of $1,25(OH)_2D_3$.

These *in vitro* data may help to explain the lack of effect of high-dose vitamin D_3 to enhance *Mtb* clearance drug-sensitive subjects, such as in our recent RCT in the country of Georgia. Our data from a subset of subjects from this RCT shown here demonstrate that high-dose oral vitamin D_3 supplementation did not alter either hCAP18 mRNA expression in peripheral blood mononuclear cells or LL-37 protein expression in blood after 8 weeks. These negative findings may suggest that anti-TB drugs are impacting the action of vitamin D on induction of LL-37 in circulation and in PBMCs. In contrast, in a study of subjects with inflammatory bowel disease, vitamin D_3 supplementation did increase blood levels of cathelicidin (LL-37) [34]. This would be consistent with our hypothesis that anti-TB drugs are impacting the action of vitamin D on induction of cathelicidin as patients with IBD are not on anti-TB drugs.
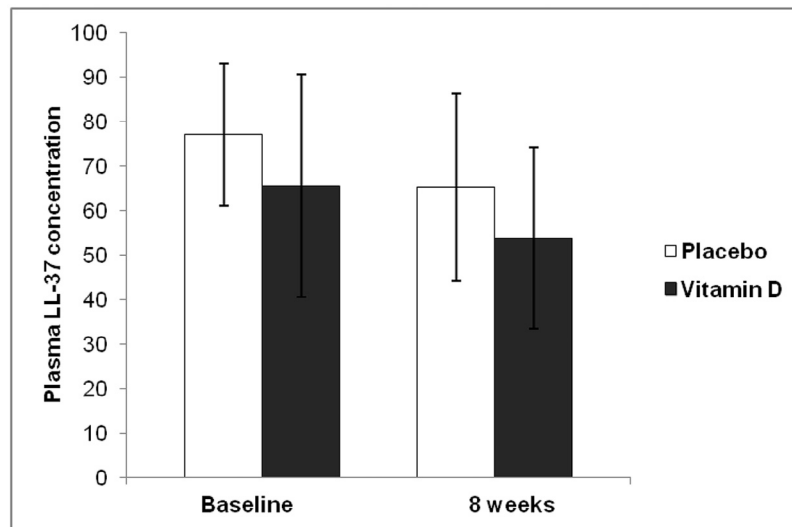
Figure 4. Plasma concentrations of LL-37 (the active human cathelicidin protein) in subjects recently diagnosed with pulmonary TB receiving conventional 4-drug initial anti-TB therapy (INH, rifampicin, pyrazinamide and ethambutol) and receiving at total of 1.2 million units of vitamin D₃ (dark bars, n = 13; 50,000 IU thrice weekly) or placebo (white bars, n = 18) over 8 weeks. There was no significant difference in the change of plasma LL-37 in between the two groups (P = 0.088).

Several drugs may potentially impact the metabolism of vitamin D. Any drug that interferes with vitamin D absorption, metabolism and/or excretion process could potentially alter the action of vitamin D [35]. Several drugs are known to impact one of the two hydroxylation steps of vitamin D: (1) the hydroxylation of vitamin D to 25-hydroxyvitamin D (25(OH)D) via cytochrome P450 vitamin D 25-hydroxylase (25-OHase or CYP27A1) or (2) the hydroxylation of 25(OH)D to 1,25-dihydroxyvitamin D (1, 25(OH)$_2$D) via the cytochrome P450 D-1-α-hydroxylase (1-OHase or CYP27B1) [36]. In a classic endocrine feedback loop, high blood concentrations of vitamin D also induces its own catabolism by increasing the expression of the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) which plays an important role in the catabolism of vitamin D [37]. Anti-convulsive medications such as phenytoin and phenobarbital can increase CYP24A1 expression, which results in the lowering of serum vitamin D [35].

It is currently unclear whether anti-TB drugs may impact vitamin D metabolism directly. Several studies conducted in patients infected with TB and taking the conventional 4 drug anti-TB regimen have suggested a very high prevalence of vitamin D deficiency in these populations [38–41]. The association between TB and vitamin D deficiency suggests that several factors including the anti-TB therapy itself may have impacted the vitamin D status of the patients infected with TB. However, there is no clear mechanism by which anti-TB medications may lower circulating metabolites of vitamin D [42]. Relevant to our data, earlier studies have demonstrated that INH can either induce or inhibit the cytochrome P450 system which may result in the alterations of 25-OHase and 1-OHase [43,44]. Furthermore, rifampicin can increase CYP3A4-dependent 25(OH)D₃ metabolism in liver but not CYP24A1 [25,45]. There are no available data on the impact of ethambutol and pyrazinamide on vitamin D metabolism or the effect of the 4-drug standard
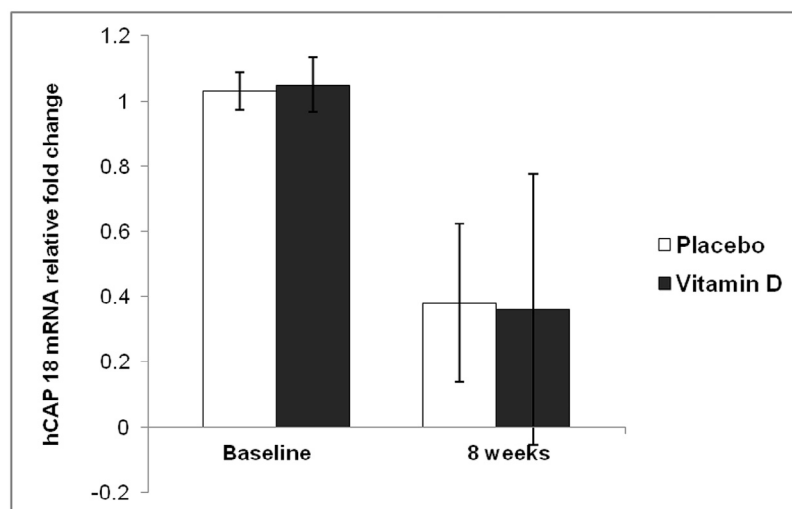

Figure 5. Expression of human cathelicidin (hCAP18) mRNA in peripheral blood mononuclear cells collected from subjects as outlined in Fig. 4. hCAP18 mRNA fell significantly in both groups from baseline to week 8 after entry (P < 0.001). There was no significant difference in the expression of hCAP18 mRNA relative to GAPDH between the two groups at either time point (P = 0.738).
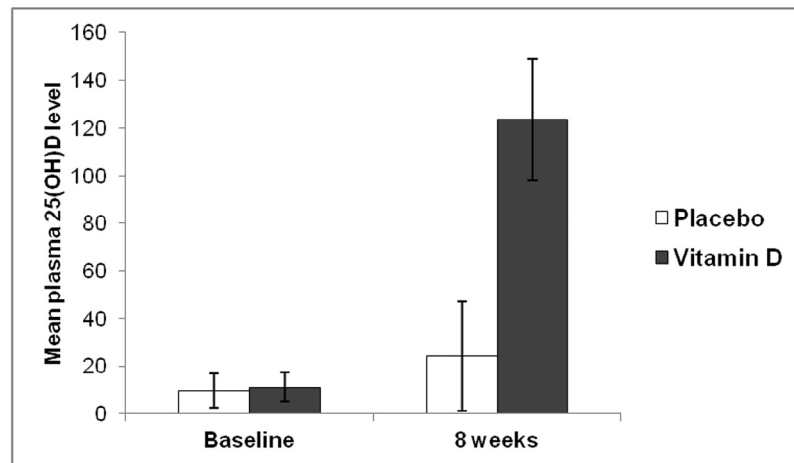
Figure 6. Mean plasma 25(OH)D from subjects as outlined in Fig. 4. Mean plasma 25(OH)D concentration was significantly increased at 8 weeks compared to baseline in the vitamin D group (P < 0.001) whereas there was no significant change in 25(OH)D concentrations at 8 weeks in the placebo group (P = 0.07).

regimen studied in this report. In our study, hCAP18 mRNA in PBMCs fell significantly in both the vitamin D_3-treated and the placebo cohorts from baseline to 8 weeks; LL-37 protein levels in plasma also declined over time, but not significantly. Whether this is due to the effect of the 4-drug regimen to clear *Mtb* and/or an impact on the anti-TB drug regimen on induction of hCAP18 mRNA and LL-37 in the circulation is unclear from our study. Moreover, there is no clear evidence that anti-TB drugs can have direct effects on cathelicidin independent of vitamin D in the literature.

While it is well established that vitamin D increases the expression of cathelicidin *in vitro* and *in vivo* in macrophages infected with TB, there may be other effects of vitamin D independent of cathelicidin and there may be other independent effects of TB medications. Salamon et al. [46] found that vitamin D may alter the growing environment of TB via PPAR by enhancing the antimicrobial effect that was induced by VDR. Tousif et al. [47] found that INH may decrease the function of CD4 T-cells via inducing apoptosis. These other potential vitamin D regulated pathways were worthy of further exploration.

Multiple RCTs have investigated the role of vitamin D as an adjunctive therapy in patients with active pulmonary TB. Most of clinical studies failed to show the improvement of treatment outcome. Our recent RCT of 199 pulmonary TB patients in Tbilisi, Georgia showed no difference in median sputum-culture conversion times between the high-dose vitamin D_3 group and the placebo group [9]. Other negative clinical studies have other limitations as well, which include varying and insufficient doses of vitamin D [13,14], small sample size, missing data [19] and absence of serum 25(OH)D concentrations [16]. The reasons why the results from the large RCTs have been negative are unclear despite very strong preclinical data that vitamin D triggers killing of *Mtb* via induction of LL-37 *in vitro*. However, one potential confounding factor in the investigation of vitamin D on clearance of *Mtb* in prospective clinical trials may be the use of anti-TB drugs by the patients enrolled in these trials. However, removing the potential confounding effect of anti-TB drugs may be challenging as it would be unethical to hold such effective drug therapy in these patients.

Our study has several strengths and limitations. One strength is that *in vitro* evaluation of 4 drugs commonly used in TB treatment has not been previously evaluated in terms of these drugs' effect on vitamin D induction of cathelicidin. We also linked our results to a recently conducted RCT of subjects with TB treated with the same 4 anti-TB drugs. Our study is limited in regard to the exact mechanism by which anti-TB impacts the action of vitamin D in

macrophages and the lack of LL-37 protein concentration measures in either the cultured cells or in media. One possible explanation was the first-line anti-tuberculosis drugs might directly or indirectly interfere the enzymes in the vitamin D metabolism pathway such as CYP27A1 or CYP24A1 which may interfere with the activation of the vitamin D receptor in macrophages. Another limitation is the concentrations of the anti-TBs were based on the minimal inhibitory concentration (MIC) from published *in vitro* THP-1 cell culture and TB experiments in the literature [25–28]. Also our THP-1 cells were not infected by *Mtb*. Although we cannot translate our *in vitro* findings to humans receiving anti-TB drugs, we do confirm that vitamin D induces hCAP18 in THP-1 cells *in vitro* and the impact of vitamin D to up-regulate hCAP18 mRNA and that hCAP18 induction by vitamin D is abolished when cultured in the presence of anti-TB drugs at 10 μg/mL. We speculate that the anti-TB medications given to subjects participating in our clinical RCT impacted the induction of LL-37 in blood or the mRNA expression of hCAP18 by the PBMCs from patients receiving high-dose vitamin D_3.

In conclusion, our study is the first, to our knowledge, to demonstrate the effects of first-line anti-TB drugs on the induction of cathelicidin by vitamin D. We showed that 4 anti-TB drugs used in combination may alter the vitamin D-dependent increase in hCAP18 expression in cultured human macrophages. These findings suggest that further studies should be conducted to determine the mechanism of effect of anti-TB drugs on the action of vitamin D in macrophages.

Conflict of interest

The authors declare they have no conflicts of interest.

Acknowledgments

References

[1] Palwatwichai A. Tuberculosis in Thailand. Respirology 2001;6(1):65–70.
[2] WHO. Global Tuberculosis Report 2014. WHO Press, World Health Organization, Geneva. 2014 (ISBN 978 92 4 156480 9).

[3] WHO. Treatment of Tuberculosis Guidelines. WHO Press, World Health Organization, Geneva. 2010 (ISBN 10: 9241547839).

[4] Mitchison DA. Antimicrobial therapy of tuberculosis: justification for currently recommended treatment regimens. Semin Respir Crit Care Med 2004;25(3): 307–15.

[5] Lew W, Pai M, Oxlade O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. Ann Intern Med 2008;149(2):123–34.

[6] Luong K, Nguyen LT. Impact of vitamin D in the treatment of tuberculosis. Am J Med Sci 2011;341(6):493–8.

[7] Battersby AJ, Kampmann B, Burl S. Vitamin D in early childhood and the effect on immunity to Mycobacterium tuberculosis. Clin Dev Immunol 2012;2012: 430972.

[8] Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocr Pract 2009;15(5):438–49.

[9] Tukvadze N, Sanikidze E, Kipiani M, Hebbar G, Easley KA, Shenvi N, et al. High-dose vitamin D3 in adults with pulmonary tuberculosis: a double-blind randomized controlled trial. Am J Clin Nutr 2015;102(5):1059–69.

[10] Sutaria N, Liu CT, Chen TC. Vitamin D status, receptor gene polymorphisms, and supplementation on tuberculosis: a systematic review of case-control studies and randomized controlled trials. J Clin Transl Endocrinol 2014; 1(4):151–60.

[11] Nursyam EW, Amin Z, Rumende CM. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. Acta Med Indones 2006;38(1):3–5.

[12] Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, et al. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 2007;176(2):208–13.

[13] Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2009;179(9):843–50.

[14] Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 2011;377(9761):242–50.

[15] Kota SK, Jammula S, Kota SK, Tripathy PR, Panda S, Modi KD. Effect of vitamin D supplementation in type 2 diabetes patients with pulmonary tuberculosis. Diabetes Metab Syndr 2011;5(2):85–9.

[16] Ralph AP, Waramori G, Pontororing GJ, Kenangalem E, Wiguna A, Tjitra E, et al. L-arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial. PLoS ONE 2013;8(8):e70032.

[17] Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis. BMC Infect Dis 2013;13:22.

[18] Wang Q, Ma A, Bygbjerg IC, Han X, Liu Y, Zhao S, et al. Rationale and design of a randomized controlled trial of the effect of retinol and vitamin D supplementation on treatment in active pulmonary tuberculosis patients with diabetes. BMC Infect Dis 2013;13:104.

[19] Daley P, Jagannathan V, John KR, Sarojini J, Latha A, Vieth R, et al. Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2015;15(5):528–34.

[20] Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J Mol Med 2010;88(5):441–50.

[21] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311(5768):1770–3.

[22] White JH. Vitamin D metabolism and signaling in the immune system. Rev Endocr Metab Disord 2012;13(1):21–9.

[23] Hewison M. Vitamin D and the intracrinology of innate immunity. Mol Cell Endocrinol 2010;321(2):103–11.

[24] Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, et al. Vitamin D-directed rheostatic regulation of monocyte antibacterial responses. J Immunol 2009;182(7):4289–95.

[25] Larsson MC, Lerm M, Angeby K, Nordvall M, Jureen P, Schon T. A luciferase-based assay for rapid assessment of drug activity against Mycobacterium tuberculosis including monitoring of macrophage viability. J Microbiol Methods 2014; 106:146–50.

[26] Verma RK, Kaur J, Kumar K, Yadav AB, Misra A. Intracellular time course, pharmacokinetics, and biodistribution of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to mice. Antimicrob Agents Chemother 2008;52(9):3195–201.

[27] Kos J, Nevin E, Soral M, Kushkevych I, Gonec T, Bobal P, et al. Synthesis and antimycobacterial properties of ring-substituted 6-hydroxynaphthalene-2-carboxanilides. Bioorg Med Chem 2015;23(9):2035–43.

[28] Stokes RW, Doxsee D. The receptor-mediated uptake, survival, replication, and drug sensitivity of Mycobacterium tuberculosis within the macrophage-like cell line THP-1: a comparison with human monocyte-derived macrophages. Cell Immunol 1999;197(1):1–9.

[29] Sato E, Imafuku S, Ishii K, Itoh R, Chou B, Soejima T, et al. Vitamin D-dependent cathelicidin inhibits Mycobacterium marinum infection in human monocytic cells. J Dermatol Sci 2013;70(3):166–72.

[30] Zughaier SM, Stauffer BB, McCarty NA. Inflammation and ER stress downregulate BDH2 expression and dysregulate intracellular iron in macrophages. J Immunol Res 2014;2014:140728.

[31] Torres-Juarez F, Cardenas-Vargas A, Montoya-Rosales A, Gonzalez-Curiel I, Garcia-Hernandez MH, Enciso-Moreno JA, et al. LL-37 immunomodulatory activity during mycobacterium tuberculosis infection in macrophages. Infect Immun 2015;83(12):4495–503.

[32] Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6(3):231–43.

[33] Kearns MD, Alvarez JA, Seidel N, Tangpricha V. Impact of vitamin D on infectious disease. Am J Med Sci 2015;349(3):245–62.

[34] Raftery T, Martineau AR, Greiller CL, Ghosh S, McNamara D, Bennett K, et al. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: results from a randomised double-blind placebo-controlled study. United European Gastroenterol J 2015;3(3):294–302.

[35] Robien K, Oppeneer SJ, Kelly JA, Hamilton-Reeves JM. Drug-vitamin D interactions: a systematic review of the literature. Nutr Clin Pract 2013;28(2): 194–208.

[36] Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D metabolism. Endocrinol Metab Clin North Am 2010;39(2):243–53. table of contents.

[37] Holick MF. Vitamin D deficiency. N Engl J Med 2007;357(3):266–81.

[38] Keflie TS, Nolle N, Lambert C, Nohr D, Biesalski HK. Vitamin D deficiencies among tuberculosis patients in Africa: a systematic review. Nutrition 2015;31(10):1204–12.

[39] Desai NS, Tukvadze N, Frediani JK, Kipiani M, Sanikidze E, Nichols MM, et al. Effects of sunlight and diet on vitamin D status of pulmonary tuberculosis patients in Tbilisi, Georgia. Nutrition 2012;28(4):362–6.

[40] Sloan DJ, Mwandumba HC, Kamdolozi M, Shani D, Chisale B, Dutton J, et al. Vitamin D deficiency in Malawian adults with pulmonary tuberculosis: risk factors and treatment outcomes. Int J Tuberc Lung Dis 2015;19(8):904–11.

[41] Yamshchikov AV, Kurbatova EV, Kumari M, Blumberg HM, Ziegler TR, Ray SM, et al. Vitamin D status and antimicrobial peptide cathelicidin (LL-37) concentrations in patients with active pulmonary tuberculosis. Am J Clin Nutr 2010;92(3):603–11.

[42] Self TH, Chrisman CR, Baciewicz AM, Bronze MS. Isoniazid drug and food interactions. Am J Med Sci 1999;317(5):304–11.

[43] Brodie MJ, Boobis AR, Hillyard CJ, Abeyasekera G, MacIntyre I, Park BK. Effect of isoniazid on vitamin D metabolism and hepatic monooxygenase activity. Clin Pharmacol Ther 1981;30(3):363–7.

[44] Brodie MJ, Boobis AR, Hillyard CJ, Abeyasekera G, Stevenson JC, MacIntyre I, et al. Effect of rifampicin and isoniazid on vitamin D metabolism. Clin Pharmacol Ther 1982;32(4):525–30.

[45] Wang Z, Lin YS, Zheng XE, Senn T, Hashizume T, Scian M, et al. An inducible cytochrome P450 3A4-dependent vitamin D catabolic pathway. Mol Pharmacol 2012;81(4):498–509.

[46] Salamon H, Bruiners N, Lakehal K, Shi L, Ravi J, Yamaguchi KD, et al. Cutting edge: vitamin D regulates lipid metabolism in Mycobacterium tuberculosis infection. J Immunol 2014;193(1):30–4.

[47] Tousif S, Singh DK, Ahmad S, Moodley P, Bhattacharyya M, Van Kaer L, et al. Isoniazid induces apoptosis of activated CD4+ T cells: implications for post-therapy tuberculosis reactivation and reinfection. J Biol Chem 2014;289(44):30190–5.