Impact of Vitamin D in Tuberculosis

Subjects: Infectious Diseases

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Vitamin D plays a crucial role in many infectious diseases, such as tuberculosis (TB), that remains one of the world's top infectious killers with 1.5 million deaths from TB in 2021. Vitamin D suppresses the replication of Mycobacterium tuberculosis in vitro and showed a promising role in TB management as a result of its connection with oxidative balance.

Keywords: vitamin D ; tuberculosis ; TB ; outcome ; prophylaxis

1. Vitamin D and Tuberculosis

1.1. Innate Immunity

MT uses the toll-like receptors (TLRs) present on the surface of macrophages to enter the body. In macrophages, following the activation of the signaling pathway mediated by TLRs and exposure to inflammatory cytokines, there is an increase in the expression of CYP27B1 oxidase, responsible for the oxidation of 25(OH)D to the active form $1,25(OH)_2D$. This, through an autocrine mechanism, activates the signaling pathway mediated by the VDR/RXR receptors present on the macrophages themselves, with a consequent increase in the synthesis of cathelicidin hCAP-18 from which the Leucine-Leucine-37 peptide (LL-37) is derived. It destroys the bacterial cell by interacting with the molecules of the bacterial wall and by perforating the cytoplasmic membrane [1][2][3][4].

Other studies have also pointed out that vitamin D seems to induce autophagy in infected macrophages [5]. In fact, if on the one hand MT tries to block the autophagic process, through which the infected macrophages eliminate the bacteria contained in the phagolysosomes, on the other hand, the mycobacterial lipoproteins activate the signaling pathways TLR2/1, CD14 and VDR, which stimulate autophagy [6]. Moreover, several studies have demonstrated the role of LL-37 in autophagy; in particular, it has been highlighted that the autophagic process is blocked if the expression of LL-37 is silenced [7]. Another study showed that the administration of vitamin D and phenylbutyrate is associated with a marked increase in LL-37 levels in macrophages and lymphocytes and with increased intracellular killing of MT [8].

Vitamin D also appears to inhibit the growth of MT in infected macrophages through the production of nitrogen and oxygen reactants: in fact, a study has shown that macrophages, under the stimulation of LPS and $1,25(OH)_2D_3$, increase the expression of inducible nitric oxide synthase (NOS2), acquiring the ability to produce a large amount of NO [9].

1.2. Acquired Immunity

Some studies have reported the absence of some antimycobacterial activities mediated by T-helper lymphocytes in case of vitamin D deficiency: for example, the production of INF-γ by Th1 lymphocytes, which enhances antibacterial activity of macrophages and stimulation of the antibody-mediated response by Th2 lymphocytes via the secretion of IL4 and IL5 [10][11].

1.3. Anti-Inflammatory Activity

If on the one hand vitamin D seems to carry out all these pro-inflammatory and antimicrobial activities aimed at counteracting the MT infection, on the other, it also seems to perform anti-inflammatory functions that limit an excessive inflammatory response.

In fact, several studies have shown that vitamin D has an anti-inflammatory activity, through various mechanisms, including the induction of the expansion of T-reg lymphocytes, which in turn limit the activity of Th1 and the regulation of the expression of the genes that encode for metalloproteinases (MMPs) [12]. MMPs are known to be associated with tissue remodeling and the formation of tubercular granulomas. An in vitro study using MT-infected human leukocytes showed that $1,25(OH)_2D$ significantly attenuated MT-induced increases in expression of MMP-7 and MMP-10, and

suppressed secretion of MMP-7 by MT-infected PBMC, whilst MMP-9 gene expression, secretion and activity were significantly inhibited by 1,25$(OH)_2D_3$ irrespective of infection [12][13]. It appears that vitamin D and its hydroxylated derivatives also promote the stabilization of the endothelium and of the barrier function in the presence of inflammatory mediators [14], as summarized in **Table 1**. Moreover, it has been observed that vitamin D, through LL-37, induces a modulation of the expression of pro-inflammatory and anti-inflammatory cytokines (reduction of pro-inflammatory cytokines TNFα and IL-17 and increase of anti-inflammatory ones IL-10 and TGF-β), without reducing the antimycobacterial activity [15]. This is essential to reduce the inflammatory state and tissue damage that characterize the pathophysiology of TB.

Table 1. Summary of antimicrobial and anti-inflammatory actions of Vitamin D.

Pro-Inflammatory/Antimicrobial Actions of Vit. D	Anti-Inflammatory Actions of Vit. D
Induces destruction of the bacterial cell by activating the cathelicidin/LL-37 system in infected macrophages	**Induces the expansion of T-reg lymphocytes, which in turn limit the activity of Th1**
Induces autophagy in infected macrophages	**Attenuates *M. tuberculosis*-induced expression of MMP**
Inhibits the growth of MT in infected macrophages through the production of nitrogen and oxygen reactants	**Stabilization of the endothelium and of the barrier function in the presence of inflammatory mediators**
Stimulates production of INF-γ by Th1 lymphocytes, which enhances antibacterial activity of macrophages	**Reduction of pro-inflammatory cytokines and increase of anti-inflammatory ones, without reducing the antimycobacterial activity**
Enhances the antibody-mediated response by Th2 lymphocytes via the secretion of IL4 and IL5	

2. The Role of Vitamin D in Prevention of Tuberculosis

Given the properties and mechanisms of action of vitamin D currently known, several studies have questioned the link between vitamin D levels and the risk of progression to active TB in patients exposed to MT. First, a lower concentration of 25(OH)D has been demonstrated in patients with active TB in comparison with healthy patients [16][17][18]. However, it is not clear whether the vitamin D deficiency is due to infection or whether the progression of the infection is favored by the vitamin D deficiency. Moreover, there is evidence of the ability of vitamin D to inhibit the replication of MT in vitro [9][19]. For example, a study has shown that a concentration of 4 µg/mL of cholecalciferol is sufficient to slow down the proliferation of the bacillus inside cultured human macrophages. This value is significantly higher than the normal circulating levels of 1,25$(OH)_2D$; however, as already mentioned, the infected macrophages are capable of autonomously producing this active form of vitamin D and could be therefore capable of reaching such concentrations [19]. On the other hand, the studies carried out in vivo are discordant and have not led to unequivocal conclusions about the efficacy of vitamin D supplementation in preventing the development of the disease.

In a randomized controlled clinical trial conducted on 8851 children in Mongolia with latent tuberculosis infection (LTBI) determined by QuantiFERON(®), et al. found no significant difference in the reduction of the risk of tuberculosis infection and tuberculosis disease among children who were given a vitamin D supplementation (14,000 UI of vitamin D for week) and those treated with placebo [20]. However, a meta-analysis, conducted on studies focused on various aspects of the relations between vitamin D and TB, found that a low level of 25(OH)D is associated with an increased risk of developing active TB. On the other hand, the same study showed a trend of higher levels of 1,25$(OH)_2D$ (the bioactive form) in subjects with active TB, supporting the theory that the 25(OH)D deficiency is due to an increase in its conversion into the bioactive form in response to infection [17]. Another meta-analysis of prospective studies carried out by Aibana O, Huang C-C et al. [21] confirmed the results of the previous one, showing in fact, a positive dose-dependent correlation between pre-existent low levels of 25(OH)D in the bloodstream and an increased risk of developing active disease in high risk groups (LTBI subject/household contacts of active TB patients). This risk was higher among HIV-positive patients with severe vitamin D deficiency. Nevertheless, both meta-analyses have important limitations, including the variety that exists in the definition of vitamin D deficiency among the studies on which they are based. So, there is a need for further studies and clinical trials to evaluate the effectiveness of the vitamin D supplement in the prevention of active TB.

3. The Role of Vitamin D in Treatment of Tuberculosis

Given the high spread of TB worldwide, the long duration of therapy, the scarce availability of anti-tuberculosis drugs and the increasing presence of infections caused by resistant to first-line drugs MT, it is necessary to search for new medications or supplements that could reduce the time of administration of therapy and enhance the effect of already

existing drugs. Although several studies have confirmed in vitro the important role of active vitamin D against MT [21][22], randomized controlled trials (RCTs) did not confirm what was expected, and the results of the in vivo studies are conflicting, so the debate is still open. Jing Zhang et al. conducted an in vivo study on mice in which the therapeutic synergy between vitamin D and pyrazinamide (PZA) was analyzed. The study demonstrates how using calcitriol and PZA concurrently results in an interruption of bacterial growth and a faster resolution of MT-related lung lesions. Furthermore, it has been shown that the administration of vitamin D during therapy with PZA results in an increase of the release of anti-inflammatory cytokines and antimicrobial molecules, respectively IL-4 and LL-37. The latter would otherwise be reduced in patients taking PZA without vitamin D supplementation [23]. On the other hand, in some RCTs that have evaluated the infusion of high doses of vitamin D, beneficial effects were not observed, and the patients did not reach an earlier microscopic negativization compared to the patients who were given a placebo [24][25]. Of note, despite multiple studies of vitamin D supplementation in different doses, statistically significant benefits on sputum conversion have not been demonstrated [26]. It must be said, however, that few studies have been conducted on this topic, with numerically small samples and data inaccuracies. For these reasons, further clinical trials to evaluate the effective role of the vitamin D supplement in the treatment of TB are needed. One of the hypotheses to explain this difference between the in vivo and in vitro results is the possible interference between the drugs used for the treatment of TB and the metabolism of VD. In this regard, Chesdachai et al. have analyzed this relation through an in vitro study [27]. In the study, human monocytes were cultured with calcitriol and anti-tuberculosis drugs at different concentrations, and the activity of the hCAP18/cathelicidin system was analyzed. The study showed that the culture with lower concentration of INH led to a strong induction of hCAP18/cathelicidin system; Rifampicin at the same concentration resulted in a repression of its expression. The other cultures, at higher concentrations of isoniazid or rifampicin and all cultures with ethambutol or pyrazinamide alone, did not lead to any change in cathelicidin production. The culture with the four drugs, added together at maximum concentration (10 mcg/mL), showed strong inhibition of hCAP18/cathelicidin in presence of $1,25(OH)_2D_3$. They have demonstrated that the combination of the four drugs used in the first-line treatment of TB (PZA, INH, ETM, RIF) can inhibit the increase in hCAP18/LL-37 expression induced by the bioactive form of vitamin D in cultured human macrophages [27]. This result obtained in vitro, may explain why in subjects infected with drug-sensitive MT, who are administered full doses of the four anti-tuberculosis drugs, do not obtain rapid improvements with the administration of calcitriol in oral form; in fact, by analyzing both the expression of hCAP18 and the presence of LL-37 in the bloodstream, there are no increases after 8 weeks of administration, compared to the control subjects [25]. Several observational studies have shown the persistence of low levels of vitamin D in patients with active TB [28][29][30], and some have shown a statistically significant relationship with the intake of anti-tuberculosis drugs [28]. The level of vitamin D in the bloodstream depends above all on the hepatic metabolism and its level of impairment; an open question is to determine whether the conventional therapeutic regimen can influence the blood concentrations of the vitamin, because, as it is known, anti-TB antibiotics are characterized by a non-negligible liver toxicity. Some studies underline how the use of INH can change the levels of 25-hydroxylase and 1-hydroxylase, and consequently also of the active VD. This is because INH inhibits or induces the cytochrome P450 system which regulates enzymatic activation [29]. Another evidence concerns RIF, which appears to influence the metabolism, enhancing CYP3A4 (but not CYP24A1), which acts as a 24-hydroxylase for 25(OH)D, reducing the production of the active form of vitamin D [30]. Regarding PZA and ETB, there are no studies that demonstrate changes in vitamin D.

4. Synergism with Vitamin A in Prevention and Treatment of TB

A synergistic activity of vitamin D and vitamin A (VA) against MT has been hypothesized but the data regarding its role in treatment are discordant [31]. VA and vitamin D bind to their intracellular receptors, retinoic acid receptor (RAR) and VDR, respectively, and subsequently both bind to the retinoid X receptor (RXR) and mediate changes in gene expression within the cell. A study demonstrated in vitro the synergistic effect of these two vitamins in inhibiting the entry of MT and its survival in macrophages, through the downregulation of the expression of the TACO gene [32]. Some in vivo studies have not found a clear relationship between these two vitamins in the impact on the treatment of TB; however, they seem to suggest an influence of other micronutrients on the effects of vitamin D [21]. For this reason, further studies are needed.

5. Side Effects of Vitamin D Supplementation

Potential effects of vitamin D supplementation include hypercalcemia, hypercalciuria and potentially nephrocalcinosis, especially in patients with renal failure. However, a meta-analysis conducted on RCTs investigated the cumulative relative risk of any type of adverse event, as well as kidney stones, hypercalcemia and hypercalciuria following administration of at least 2800 IU/day of vitamin D_2 or D_3 for at least 1 year, concluding that high doses of vitamin D administered for a long time (at least one year) do not significantly increase the risk of adverse events, although there is a trend toward increased calcium and possibly hypercalciuria [33].

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