ABSTRACT

Effect of cholecalciferol and retinyl palmitate supplementation separately and in combination on the second-line anti-tuberculosis therapy in the mouse model of tuberculosis.

Objectives: Micronutrients deficiencies increase the risk of TB. The vitamin D receptor (VDR) bind as heterodimers with the retinoic acid receptor (RAR) in target genes. Our goal was to investigate wether cholecalciferol supplementation alone or in combination with retinyl palmitate increases the efficacy of second line anti-TB drugs (SLDs) as shown by decreasing the colony forming units (CFU) of bacteria and interstitial collagenase MMP1, due to their implication in cell death signaling.

Material and Methods: C3HeB/FeJ mice (n=8) were randomized to 6 groups. The first group (G1), consisting of mice that were intratracheally infected with MDR-TB strain of *M. tuberculosis* and sacrificed on 2 weeks post infection to confirm successful infection. (G2) pulmonary TB (PTB) without therapy. (G3) PTB with the SLDs recomended by the Indonesian National TB Control Program. (G4) PTB receiving not only SLDs but also cholecalciferol for 6 months. (G5) PTB with SLDs supplemented with retinyl palmitate for 6 months. (G6) PTB with SLDs supplemented with cholecalciferol and retinyl palmitate for 6 months. We used immunohistochemistry to assess VDR and RARγ2; apoptosis marker caspase-3; autophagy markers CRAMP and LC3B; necrosis marker RIPK3; and MMP1. Quantitative culture technique was used to assess the CFU. Statistical differences between multiple groups were evaluated using One Way Anova or Kruskal-Wallis test. The statistical differences between two individual group scores were analyzed using Tukey's test or Mann-Whitney test. Partial least square structural equation modeling (PLS-SEM) was utilized to explore relationships among the variables.

Results: VDR and RARγ2 were higher in the supplement group than in SLDs treated goup (p=0.026, p=0.019). Cholecalciferol and retinyl palmitate in combination contribute to suppressing necrosis of the infected cells (p=0.002). Cholecalciferol supplementation responsible for increasing CRAMP and LC3B expression (p=0.000, p=0.001) and reducing MMP1 expression (p=0.010). Cholecalciferol and retinyl palmitate supplementation are equally effective for casp3-inducing apoptosis (p=0.035, p=0.027 respectively), and suppressing CFU (p=0.000, p=0.000 respectively). PLS-SEM demonstrated that cholecalciferol and retinyl palmitate in combination suppressed necrosis. Reduced necrosis was accompanied by increased autophagy (β= -0.413). Induction of autophagy was associated with apoptosis (β= 0.644).

Conclusions: A comprehensive analysis demonstrated that cholecalciferol and retinyl palmitate in combination suppressed necrosis concomitant with up regulation of autophagy and apoptosis resulted in decreasing CFU and MMP1.

***Keyword**: cholecalciferol, retinyl palmitate, MDR-TB, cell death pathway.*