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Abstract

The Effects of 25-Hydroxyvitamin D3 and Ascorbate on Extracellular Cytokine Concentrations in THP-1 Monocytes and THP-1 Derived Macrophages †

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Abstract: Vitamins C and D are known to have immunomodulatory effects. Current recommendations state that plasma 25-hydroxyvitamin D3 should be maintained above 50 nmol/L, although concentrations of 100 nmol/L can enhance health benefits. Concentrations below 25 and 12.5 nmol/L are considered insufficient and deficient, respectively. The typical plasma ascorbate concentration is 50 µmol/L. Vitamin C supplementation can increase plasma concentration to 100–150 µmol/L. Vitamin C insufficiency and deficiency occur at 25 µmol/L and <10 µmol/L, respectively. This study investigates cytokine production by THP-1 monocytes and macrophages, following vitamin C and D treatment at concentrations representing deficiency, insufficiency, sufficiency and following supplementation. Macrophages were differentiated from THP-1 monocytes using PMA. THP-1 cells (monocytes or macrophages) were pre-treated with ascorbate or 25-hydroxyvitamin D3 for 24 h at the aforementioned concentrations, then challenged with lipopolysaccharide for 6 and 24 h. Extracellular concentrations of IL-1β, IL-6, IL-10 and TNF-α were measured using Luminex assays. In THP-1 monocytes, 25-hydroxvitamin D3 and ascorbate, at concentrations representing sufficiency and supplementation, decreased TNF-α, IL-1β and IL-6 at 6 and 24 h. Ascorbate at concentrations of >50 µmol/L also increased IL-10 at both time points. At supplemented concentrations, 25-hydroxvitamin D3 and ascorbate lowered the TNF-α/IL-10 ratio from 39:1 to 31:1 and 17:1, respectively, at 6 h. At 24 h, TNF-α/IL-10 was lowered from 88:1 to 31:1, following 150 µmol/L ascorbate treatment, and from 185:1 to 108:1 following 100 nmol/L 25-hydroxyvitamin D3 treatment. In THP-1 macrophages, pro-inflammatory cytokines were unaffected by 25-hydroxvitamin D3 at 6 h. However, IL-10 concentration increased at concentrations > 50 nmol/L. At 24 h, the inflammatory cytokines decreased as the 25-hydroxyvitamin D3 concentration increased. 25-hydroxvitamin D3 (100 nmol/L) reduced the TNF-α/IL-10 ratio from 88:1 to 64:1 at 6 h and from 105:1 to 35:1 at 24 h. Ascorbate, at concentrations representing sufficiency and supplementation, decreased the inflammatory cytokines at 6 and 24 h. Ascorbate at 150 µmol/L decreased TNF-α/IL-10 from 116:1 to 35:1 at 6 h and from 102:1 to 21:1 at 24 h. These data demonstrate that both 25-hydroxyvitamin D3 and ascorbate decrease the inflammatory burden in THP-1 monocytes and THP-1 derived macrophages. Future work will investigate vitamin interactions and underlying mechanisms.

Keywords: monocyte; macrophage; inflammation; cytokine; vitamin D; vitamin C; ascorbate

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