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Biochemical effects of KH 1060 and anti-TNF monoclonal antibody on human peripheral blood mononuclear cells

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Abstract

The aim of this study was to investigate whether the vitamin D analogue KH 1060 could exert a suppressive action on Tumor necrosis factor-alpha (TNF-α). The chimeric anti-TNF-α monoclonal antibody (anti-TNF), alone or in combination with KH 1060, was also used. KH 1060 (0.01, 0.1, 1 nM) significantly inhibited cell proliferation, determined after 5 days by [^{3}H]thymidine incorporation, when peripheral blood mononuclear cells (PBMC), obtained from healthy subjects, were stimulated with phytohaemagglutinin (PHA) and incubated for 24 h in the absence and in the presence of lipopolysaccharide (LPS). In the same experimental conditions, anti-TNF exerted a significant inhibition on PBMC proliferation, at the lowest doses (0.001, 0.01 μg/ml) in the absence of LPS, and at 0.001, 1, 10 μg/ml in its presence. A synergistic inhibition was registered combining KH 1060 and anti-TNF, at well-defined concentrations. 0.1 nM KH 1060 produced a significant decrease in TNF-α levels, determined by ELISA, although less remarkable than in the presence of anti-TNF. This decrease was synergistic, associating 0.1 nM KH 1060 and 0.1 μg/ml anti-TNF. VDR protein levels were increased by 0.1 nM KH 1060, 0.1 μg/ml anti-TNF or their combination. The protein levels of two oncogenes, Bax and Bcl-2, remained unchanged, when PBMC were incubated with KH 1060, anti-TNF or their combination in the absence of LPS, while, in its presence, an increase was registered. The demonstrated anti-TNF-α effect of KH 1060 may suggest for this compound an immunosuppressive action and the possibility to synergistically act with other drugs.

Keywords: Vitamin D analogue KH 1060; Anti-TNF-α monoclonal antibody; PBMC proliferation; TNF-α; Vitamin D receptor; Oncogenes Bax and Bcl-2

Abbreviations: 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D3; CD, Crohn's disease; CsA, cyclosporin A; IFN-γ, interferon-gamma; IL-1, interleukin 1; IL-6, interleukin 6; IL-8, interleukin 8; LPS, lipopolysaccharide; PBMC, human peripheral blood mononuclear cells; PHA, phytohaemagglutinin; TNF-α, tumor necrosis factor-alpha; UC, ulcerative colitis; VDR, vitamin D receptor; VDRE, vitamin D response element.

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1. Introduction

Tumor necrosis factor-alpha (TNF-α) is a cytokine produced by monocytes/macrophages, T and B lymphocytes [1,2], which elicits a wide spectrum of physiologic and pathologic functions and cellular responses, including shock, tissue injury, tumor necrosis, anorexia, induction of immunoregulatory molecules and proinflammatory cytokines, such as interleukin 1 (IL-1), IL-6 and IL-8. In addition, TNF-α affects cell proliferation, differentiation and apoptosis [3]. TNF-α has been implicated in the pathogenesis of many human diseases as chronic processes such autoimmunity, rheumatoid arthritis, Crohn's disease (CD) and acquired immunodeficiency syndrome [4]. In recent years, an important role for TNF-α as a pivotal proinflammatory mediator in CD has emerged, and this has resulted in the development of several therapeutic strategies that target TNF-α.

Infliximab is a chimeric monoclonal antibody to TNF-α, which has been shown to reduce inflammatory cell migration and TNF-α production in areas of intestinal inflammation in CD patients. Infliximab was recently shown to be efficacious in controlled trials in patients with moderately to severely active CD and fistulizing CD [5,6].

Vitamin D metabolites, as 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], influence the expression of various genes, whose products are involved in calcium homeostasis, cell differentiation, and regulation of the immune response [7]. The expression of these genes is mediated by the nuclear vitamin D receptor (VDR), which belongs to the same family of the steroid and retinoid receptors. The binding of vitamin D to the VDR initiates a sequence of events resulting in the activation or repression of transcription. VDR is constitutively present in normal human monocytes and in malignant lymphocytes, while T and B lymphocytes, obtained from normal subjects, express VDR after in vitro activation with mytogenic lectins and Epstein–Barr virus, respectively [8,9]. The mytogenic lectin phytohemagglutinin (PHA) stimulates T lymphocytes proliferation and the production of interleukin-2 (IL-2), which plays a critical role in T cell growth [10,11]. Many studies have shown that $1,25(OH)_2D_3$ inhibits IL-2 production and IFN-γ secretion by T cells [12,13].

In previous studies, we have demonstrated that $1,25(OH)_2D_3$ and two vitamin D analogues with fewer hypercalcemic effects, EB 1089 and KH 1060, significantly inhibited T cell proliferation in healthy controls and in patients with active ulcerative colitis (UC) [14]. This inhibitory effect on cell proliferation was synergistic when vitamin D derivatives were associated with cyclosporin A (CsA), a well-known immunosuppressor agent used in UC therapy, so demonstrating the critical role exerted by $1,25(OH)_2D_3$ in the immune system [15]. Moreover, we have studied the proliferative response of peripheral blood mononuclear cells (PBMC) obtained from CD patients during the infliximab therapy and treated in vitro with vitamin D derivatives, and we have suggested that PBMC proliferation, determined in well established experimental conditions, and VDR expression could be useful indicators to predict the response of CD patients to the infliximab therapy (paper in press). KH 1060, used for our studies, is a vitamin D derivative which belongs to a family of 20-epi-vitamin D_3 analogues, and is more potent than $1,25(OH)_2D_3$ as in vitro inhibitor of clonal cell growth [16,17].

The aim of the present study was to investigate whether KH 1060 could be an agent with suppressive properties on TNF-α. For this purpose, we have also used the murine chimeric anti-TNF-α monoclonal antibody, alone or in combination with KH 1060, in order to investigate the in vitro effect on PBMC proliferation, on TNF-α levels, and on the protein levels of either VDR or two oncogenes related with apoptosis (Bcl-2 and Bax). PBMC were obtained from healthy subjects.

2. Materials and methods

2.1. Reagents

RPMI 1640 medium Hepes modification, PBS, heat-inactivated fetal bovine serum, L-glutamine, antibiotics, PHA, LPS from *Escherichia coli* (Serotype 0127:B8) and Whatman glass-microfiber filters were obtained from Sigma. Ficoll-Paque, research grade, was purchased from Amersham Pharmacia Biotech. Anti-TNF-α monoclonal antibody (Infliximab) was from Schering-Plough. KH 1060 stock