Clinical and Experimental Rheumatology 1999; 17: 453-456.

BRIEF PAPER

Disease modifying and immunomodulatory effects of high dose 1α(OH) D3 in rheumatoid arthritis patients

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This study was financially supported by Zdravlje-Leskovac, Yugoslavia.

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Received on June 23, 1998; accepted in revised form on December 22, 1998.

Key words:
Vitamin D3 analogues, rheumatoid arthritis, immunomodulation, therapy.

ABSTRACT

Objective

Vitamin D analogues such as 1α(OH) D3 (alphacalcidiol) have a possible physiological paracrine effect on cell proliferation and differentiation. Experimentally established possibilities to prevent autoimmune diseases suggest that alphacalcidiol may have therapeutic value as an immunomodulatory agent in patients with rheumatoid arthritis.

Methods

We organized a 3-month open-label trial on 19 patients being treated with standard DMARD therapy for acute RA. They were divided into 2 subgroups, those with highly active RA and those with moderately active RA. Their regular drug regimen was maintained during the trial and oral alphacalcidiol 2 μg/day was added. Therapy results were evaluated by ESR, CRP, morning stiffness, the Richie index, and the Lee index. Immunomodulatory effects were investigated by measuring lymphocyte proliferation and apoptosis both in the patients and in vitro in 10 nM alphacalcidiol-supplemented culture medium.

Results

After 3 months, high dose oral alphacalcidiol therapy showed a positive effect on disease activity in 89% of the patients (45% or 9 pts. with complete remission and 44% or 8 pts. with a satisfactory effect). Only two patients (11%) showed no improvement, but no new symptoms occurred. No side effects were observed.

Conclusion

These results suggest that alphacalcidiol is a powerful immunomodulatory agent with fairly low hypercalcemic activity. Clinical improvement was strongly correlated with the immunomodulating potential of this agent. We noticed dual effects on lymphocyte proliferation and apoptosis according to the prior cell activation state. Alphacalcidiol could therefore possibly be used as an adjunct therapy with DMARDs in patients with rheumatoid arthritis.

Introduction

The activated form of vitamin D, 1,25-dihydroxyvitamin D3, not only plays a central role in bone and calcium metabolism, but also has important general effects on cell proliferation and differentiation. Moreover, 1α 25-dihydroxyvitamin D3 behaves as a paracrine factor in the immune system as it can be produced by monocytes and has potent actions on all the cellular components of the immune system. $1,25(OH)_2D3$ exerts its effects via a specific vitamin D receptor (VDR) found in activated lymphocytes, thymocytes and other immunocompetent cells. Macrophage cell types are also capable of metabolizing $25(OH)_2D3$ to its further metabolites by a specific 1α-hydroxylase that is regulated differently from the renal enzyme type (1). The 1α (OH) group is thought to be the most important determinant for binding of the molecule to both vitamins D binding protein and vitamin D receptor (2).

The oral administration of alphacalcidiol significantly suppresses the incidence of arthritis and inhibited paw swelling in type II collagen induced arthritis (3). When there were an adequate number of activated macrophages in the synovial environment and when the concentration of substrate $25(OH)_2D3$ was sufficient, there was a consequent increase in $1,25(OH)_2D3$ in the synovial fluid (4). All of these data suggest that alphacalcidiol could have therapeutic value as an immunoregulatory agent in RA patients.

The aim of this study was to determine the possible therapeutic and immunomodulatory effects of alphacalcidiol, both *in vivo* in RA patients and *in vitro* in cell cultures of lymphocytes.

Patients and methods

We conducted a three-month open label trial on 19 RA patients diagnosed with RA according to the ARA criteria (5). They included 12 females and 7 males, age 23-71 years (mean 55 yrs.) who were being treated at the Department of Rheumatology, MMA Belgrade. The duration of disease was 3-13 years (mean 9.9 yrs.). Based on disease activity as measured by the Richie articular index (RAI) and ESR, the patients were divided into two subgroups: (i) the highly active RA subgroup composed of 9 patients (RAI

≥ 20 and ESR > 45 mm/hr) being treated with MTX (10 mg/week) plus steroids (10 mg/day); and (ii) the moderately active RA subgroup composed of 10 patients (RAI < 20 and ESR > 28 mm/hr) being treated with MTX only. After an observation period of 3 months with no improvement seen in the patients while on basic therapy, 2 μg of oral alphacalcidiol (Alpha D3; TEVA, Israel and Zdravlje, Leskovac, YU) was added to their daily drug regimens over the next 3 months.

Our regional ethics committee approved the study protocol. All patients were on a normal diet without calcium supplementation. The criteria for therapy efficiency were: (a) complete effect (remission): no swollen or painful joints, no morning stiffness, ESR < 17/hr; (b) satisfactory effect: pain or swelling in 8 or fewer joints, morning stiffness < 20 min, ESR < 28 mm/hr; and (c) no effect: same disease activity or new symptoms.

Disease activity was established by articular examination (RAI, Lee index, morning and night pain) and laboratory measurements (ESR, CRP, RF) as well as by standard metabolic measurements for drug toxicity and calcium metabolism.

We studied the spontaneous and mitogen-induced proliferation and apoptosis of lymphocytes in 6 healthy subjects and in all of the RA patients before and after high dose oral alphacalcidiol therapy. Parallel *in vitro* studies with or without 10 nM alphacalcidiol supplemented medium was carried out in all lymphocyte cultures and the respective results were compared. Spontaneous and phytohaemaglutinin- (PHA) (Sigma, Germany) induced lymphocyte proliferation assays were carried out following the modified method of Ferrante *et al*. (6). Lymphocyte apoptosis was investigated by the Pericle *et al.* (7) modified microscopic method. Briefly, previously separated lymphocytes were incubated for 24 hours in cell cultures and were stained by alaun-haematoxillin (Serva). Microscopic analysis was done on slides by counting the percentage of apoptotic cells in 10^6 cells/well.

Statistical analysis was carried out using the Student's t-test, and the Mann-Whitney or Wilcoxon rank signed test depending on the samples analyzed.

Results

After 3 months of alphacalcidiol therapy, 89% of the patients experienced a positive effect on disease activity (45% or 9 pts. with complete remission and 44% or 8 pts. with a satisfactory effect). Only two patients (11%) showed no improvement, but no new symptoms occurred. After 3 months of alphacalcidiol therapy a significant decrease in the number of painful joints was found in all RA patients (38.22 vs. 16.4; $p < 0.01$, t-test and Mann Whitney test). A significant decline in the number of swollen joints was seen as well (20.28 vs. 5.43 in the highly active group and 13.37 vs. 3.13 in the moderately active RA group). Thus, the RAI significantly decreased (52.17 versus 20.98) as did the Lee index (9.82 versus 3.71) in all RA patients after alphacalcidiol therapy. This clinical improvement was followed by a significant decline in the ESR (41.71 vs. 17.76; $p < 0.05$ Wilcoxon test). The CRP level also significantly decreased (11.07 vs. 5.8 mg%; $p < 0.05$), while the RF level was lowered (167.7 ± 82.2 vs. 126 ± 60 mg/L), although this difference was not statistically significant.

During the 3-month follow-up period no side effects were noticed. Serum calcium and ionized calcium levels remained in the physiological range. The increase in the urine calcium level was statistically significant (2.91 vs. 7.39 mmol/L) but remained in the physiological range, and only 6 patients (31.5%) experienced hypercalciuria (< 10.8 mmol/L) which was easily corrected by a dose reduction (0.25 μg). After 3 months of high dose oral therapy all the patients expressed full satisfaction with the therapy results; morning stiffness was reduced and joint mobility had improved (evaluation of therapy in the patient's opinion).

The percentage of apoptotic peripheral blood lymphocytes was significantly higher in all RA patients on DMARDs before high dose oral alphacalcidiol therapy compared to the controls (t-test, $p < 0.001$). After alphacalcidiol therapy a further significant increase in the percentage of apoptotic lymphocytes was noticed in all RA patients ($p < 0.01$, Student's t-test). *In vitro* alphacalcidiol significantly increased the percentage of apoptotic lymphocytes ($p < 0.01$, Student's t-test) from both healthy controls and RA patients on DMARD therapy.

The lymphocyte proliferation results are shown in Figure 1, but the other laboratory parameters measured are not shown or discussed because there were no important changes found.

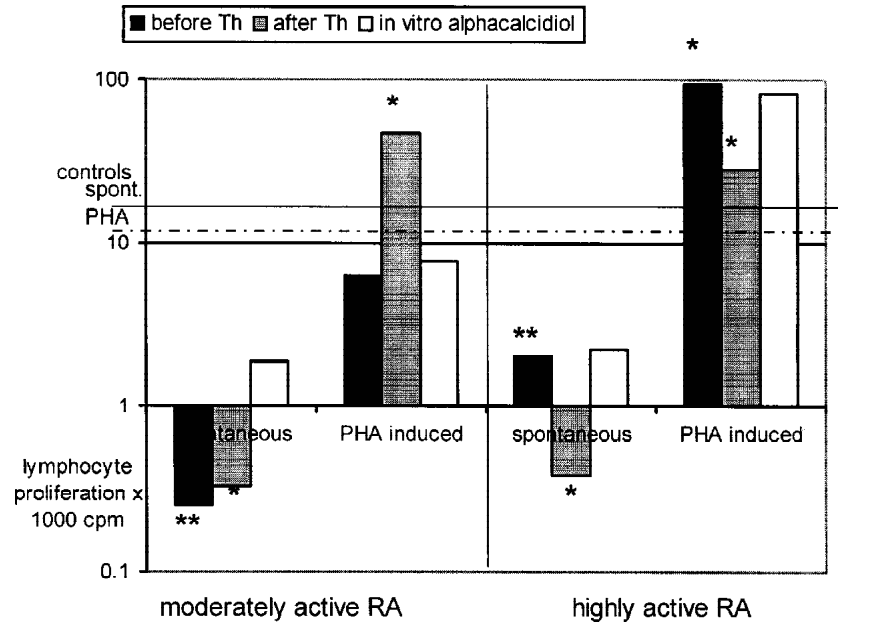

Fig. 1. PHA-induced lymphocyte proliferation after 3 months of high dose oral alphacalcidiol was significantly lower in the subgroup of patients with highly active RA, and significantly higher in patients with moderately active RA. When 10 nM alphacalcidiol was added to *in vitro* cultures, PHA-induced lymphocyte proliferation significantly decreased in the controls and in the patients with highly active RA ($p < 0.05$ compared to controls, Mann-Whitney test; **$p < 0.05$ compared before and after, Wilcoxon rank signed test).

Discussion

Our open-labeled study showed, as Huckins *et al*. reported (8), statistically significant improvement in both the physician's global assessment and in the acute phase response parameters evaluated. One of the principal drawbacks of this study is its open-label design, but this was only a pilot study with no earlier experience in practice.

Our study suggests that alphacalcidiol is a powerful immunomodulatory agent. Clinical improvement strongly correlated with the immunomodulating potential of alphacalcodiol. The dual modulatory effects we noticed should in particular be discussed.

First of all, we noticed dual effects on lymphocyte proliferation according to the prior cell activation state. Therefore, in the subgroup of patients with highly active RA and increased spontaneous and mitogen-induced lymphocyte proliferation before the trial, both high dose oral therapy and *in vitro* added alphacalcidiol significantly decreased the proliferation capacity. On the other hand, in the subgroup of patients with moderately active disease and in healthy persons there was no effect on lymphocyte proliferation.

It is interesting that in both subgroups of RA patients, high dose oral therapy had a positive clinical effect. What is the possible explanation for these results? It was initially demonstrated that 1,25 $(OH)_2$ D3 decreases IL-2 production and inhibits the proliferative response of lymphocytes to PHA (9). It is important that peripheral T-lymphocytes only express VDRs upon activation (10). Lemire *et al*. (11) discovered recently that 1,25 $(OH)_2$D3 inhibits the macrophage secretion of a key cytokine in lymphocyte activation, IL-12, which promotes the differentiation of precursor T-cells into T-helper (Th1) and directly activates these Th1 cells. In many autoimmune diseases, such as rheumatoid arthritis, Th1 cells are considered to be the main deleterious effector cells. By inhibiting IL-12 production, 1,25$(OH)_2$D3 drives the immune system toward Th2 functions and inhibits Th1 functions (12). The production of other lymphokines (IFN-γ, TNF-α) is also down-regulated by 1,25 $(OH)_2$D3 (13). Finally, 1,25$(OH)_2$ D3 inhibits B-lymphocyte proliferation as well as immunoglobulin production. When normal monocytes were incubated with 1,25$(OH)_2$D3, a decrease in accessory cell function, with down-regulation of the antigen presenting capacity, lower levels of HLA class II antigen expression and inhibition of monokine production (IL-1, IL-6 and TNF) were observed (13).

The relative potency of other vitamin D analogues corresponded closely with their relative affinity for the receptor protein, suggesting a receptor-mediated effect. Manolagas *et al*. (9) demonstrated biphasic effects of 1,25$(OH)_2$D3; thus it may either inhibit, have no influence, or stimulate lymphocyte proliferation depending upon the particular mode of lymphocyte activation. This fact should be further investigated, especially in correlation with corticosteroid effects and to confirm the hypothesis proposed by us that vitamin D analogues could represent more selective immunomodulatory agents. The possible mechanism inducing apoptosis is probably via the AP-1, jun and fos protooncogens and c-myc expression, which have been found to be inhibited by 1,25$(OH)_2$D3 (14).

Our results indicate that high oral doses of alphacalcidiol act as strongly as corticosteroids. The advantage of vitamin D would be its selective effect on activated cells, whereas steroids act unselectively on both healthy cells and cells affected by the disease process. Because of this possible point, we would hypothesise that high dose oral alphacalcidiol therapy could be used as a bridge therapy in the place of the steroids which are now commonly used in clinical practice.

The future of vitamin D therapy lies in finding its most favorable analogues for use in high doses with the lowest possible risk of adverse reactions. In the present study alphacaldiol showed promising therapeutic results with low hypercalcemic activity. Given orally, this vitamin D analogue is metabolized in both liver and bone to the active form 1α25 $(OH)_2$D3 by vitamin D 25-hydroxylase. This results in its higher concentrations in bone and at the sites of inflammation in spite of its lower concentration in the serum. The specific mechanism of this therapy could be connected with its positive immunomodulatory effect on disease progression and in the therapy of osteoporosis (both disease- and drug-induced) which is a well known complication linked to standard DMARD therapy.

Acknowledgements

This study was organized with help of Dr H. Milovanovic (marketing director) and Dr J. Jondzic (marketing manager) of Zdravlje-Leskovac, Yugoslavia. We own special thanks to Prof. Samuel Edelstein of the Weizmann Institute of Science, Biochemistry Department, for his professional help and consulting.

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