The Effect of 1,25 Dihydroxyvitamin D_3 on Lymphocyte Transformation in Patients with Chronic Renal Failure

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The changes in mitogen-induced lymphocyte response occurring in nine patients undergoing chronic hemodialysis and treated with 1,25 dihydroxyvitamin D_3 (0.5 μg/day) were investigated. Prior to treatment the stimulation indices (SI) in patients with 15.4±4.2 for phytohemagglutinin (PHA) and 7.2±0.7 for conconavalin A (Con A). In the controls, stimulation indices were 44.4±13.5 and 20.2±5.3 for PHA and Con A, respectively. Following treatment, the stimulation indices increased to 36.9±6.2 for PHA ($p<0.05$) and 18.6±3.9 for Con A ($p>0.05$) indicating the beneficial effect of oral 1,25 dihydroxyvitamin D_3 treatment on lymphocyte function in patients with chronic renal failure.
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Key words: vitamin D, lymphocyte function, conconavalin A, phytohemagglutinin

Introduction

It has been shown that cellular immunity and mitogen-induced lymphocyte transformation are defective in patients with chronic renal failure (CRF) (1). In recent studies the defective cellular immunity has been further investigated and decreased numbers of CD4 (+) lymphocytes and Ia (+) cells, a decreased CD4/CD8 ratio, and defective interleukin-2 release and IL-2-induced cell activation have been demonstrated. In contrast, the numbers of IL-2 receptors on lymphocytes were found to be increased (2–4).

Secondary hyperparathyroidism is one of the most common metabolic disturbances in patients with chronic renal failure. Increased parathyroid hormone (PTH) levels in these patients consistently augments intracellular ionized calcium levels; it has been suggested that this causes failure of calcium signal transduction in T cell activation (5).

It has also been shown that vitamin D has an immunoregulatory role in lymphocyte function. Recently receptors for 1,25 dihydroxyvitamin D_3 have been found on activated T lymphocytes, malignant T and B cell clones and EBV infected B cell clones (6). The decreased serum concentration of dihydroxyvitamin D_3 found in CRF has been postulated to be one of the causes of defective cellular immunity in these patients (7).

If activated T lymphocytes are reacted with vitamin D in vitro, IL-2 release, gamma interferon and GM-CSF production are suppressed (8–10). In contrast, vitamin D has a stimulatory effect on monocyte-macrophage differentiation (11). A number of clinical studies have demonstrated improved T lymphocyte response in CRF patients following treatment with oral vitamin D (7, 12).

Here, we investigated the changes in lymphocyte mitogen response in chronic hemodialysis patients following administration of oral 1,25 dihydroxyvitamin D_3.

Materials and Methods

Nine patients (five male and four female) aged 35.7±4.8 years (range 14 to 58 years) with end stage renal failure receiving chronic hemodialysis treatment were studied. The mean time on hemodialysis was 18.8±4.6 months (range 2 to 48 months). Patients underwent hemodialysis three times a week for 4 hours using cuprophane dialysers and acetate buffer at a blood flow rate of 300 ml/min. Full blood counts and biochemical testing were routinely performed for all patients. Three patients had a history of chronic pyelonephritis, two of hypertensive nephropathy, two of hypertension and preeclampsia, one of chronic glomerulonephritis, and one of nephrosclerosis. A control group of seven healthy subjects (three male and four female) aged 27±0.8 years (range 24 to 30 years) was also examined. Control and treatment groups were not statistically different in terms of male/female ratio or age.

Patients were administered 0.5 μg 1-alpha, 25 dihydroxyvitamin D_3 (Rocatrol, Roche Pharmaceuticals) once a day p.o. for 4 weeks. During the period of the study patients continued

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to take their regular medication; no changes in medication were made during the trial. Medications prescribed to patients included antihypertensives, erythropoietin and phosphate binders. Anticoagulation during hemodialysis was with low molecular weight heparin. Mitogen-induced lymphocyte transformation was studied in blood samples which were collected before and after vitamin D treatment. Blood samples were obtained from patients before routine hemodialysis and from healthy controls before breakfast.

Mononuclear cells were separated by ficoll-hypaque (Sigma Chemicals, St. Louis, MO, USA) density gradient and 1×10^6 cells in 1 ml of medium were placed into culture tubes. The culture medium employed was RPMI-1640 containing 10% FCS, 0.225% $NaHCO_3$, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 mcg/ml penicillin and 100 μg/ml streptomycin. Phytohemagglutinin (Difco Laboratories, Detroit, MI, USA) diluted 1 in 100 at final concentration or concanavalin A (Phamacia Fine Chemicals, Uppsala, Sweden) at a final concentration of 0.1 μg/ml were added to tubes as mitogen. Following incubation at 37°C for 48 hours in a humidified atmosphere containing 7% carbon dioxide, 1 μCi ^{3}H-thymidine (Sigma Chemicals) was added to each tube and incubation was continued for a further 16 hours. Following incubation tubes were centrifuged, supernatants removed, and cells absorbed onto Whatman filter paper discs. The filter discs were next washed sequentially with 30% TCA for 30 minutes, 5% TCA for 15 minutes, 1:1 alcohol-acetone for 15 minutes, and finally pure acetone for 15 minutes. Discs were allowed to dry at room temperature before being placed in scintillation fluid and counted in a beta counter (Betaman 1206, LKB, Bromma, Sweden). All incubations were performed and counted in triplicate. The stimulation index was calculated from the following formula:

SI = cpm following exposure to mitogen/cpm without mitogen

Statistical analysis was performed using the nonparametric Wilcoxon and Mann-Whitney U tests and results were expressed as the mean±SEM.

Results

Administration of oral 1,25 dihydroxyvitamin D_3 at a dose of 0.5 μg/day did not affect routinely estimated hematological or biochemical factors including serum calcium and phosphate levels. Pre- and post-treatment calcium levels were 8.05±0.88 mg/dl and 7.95±0.77 m/dl, white phosphate levels were 6.79±1.6 mg/dl and 6.83±1.59 mg/dl, respectively. Stimulation indices measured in patients and controls are shown in Table 1 with mean values given in Table 2. When compared with controls, the mean SIs in patients prior to treatment were significantly depressed [15.4±4.2 vs 44.4±13.5 for PHA ($p<0.01$) and 7.2±0.7 vs 20.2±5.3 for Con A ($p<0.05$)]. Following treatment with vitamin D_3 SIs increased to levels not significantly different than those of the healthy controls (36.9±6.2 for PHA and 18.6±3.9 for Con A). The pre and post treatment SIs were statistically different ($p<0.05$).

Table 1. Stimulation Indices in Patients and Controls

	Patients					Controls	
	Pretreatment		Posttreatment				
	PHA	Con A	PHA	Con A		PHA	Con A
1.	17.7	8.8	55.7	18.4	1.	11.8	20.0
2.	11.2	7.0	27.8	4.4	2.	14.2	12.2
3.	4.3	5.6	18.8	10.2	3.	85.0	14.3
4.	20.0	6.2	25.4	20.4	4.	42.8	10.0
5.	7.3	9.2	14.5	12.1	5.	13.3	11.7
6.	18.3	5.5	69.3	40.0	6.	100.2	50.0
7.	7.6	3.4	33.3	25.8	7.	44.0	23.0
8.	45.0	10.0	54.5	29.7			
9.	7.5	9.3	32.5	6.2			

Table 2. Mean Stimulation Indices in Patients and Controls (±SEM)

	Pre-Treatment	Post-Treatment	Control
PHA	15.4±4.2	36.9±6.2*	44.4±13.5**
Con A	7.2±0.7	18.6±3.9*	20.2±5.3*

*$p<0.05$ compared with pre-treatment values, **$p<0.01$ compared with pre-treatment values.

Discussion

In this study mitogen-induced lymphocyte transformation in patients with CRF was found to be impaired as compared with normal controls; this impairment improved following administration of oral 1,25 dihydroxyvitamin D_3 at a dose of 0.5 μg/day.

Since active vitamin D_3 receptors have been shown to be present on the surface of white blood cells, various in vitro experiments demonstrating the immunological effects of vitamin D have been reported. It has been shown that 1,25 dihydroxyvitamin D_3 suppresses T lymphocyte proliferation and cytokine release (IL-2, GM-CSF, gamma interferon) in different cell clones (6, 8–10). In contrast, it has been reported that when cloned T cells are used 1,25 dihydroxyvitamin D_3 augments the mitogen-induced proliferation (13).

There are however, only a few clinical reports concerning the immunological effects of vitamin D. Tabata et al showed that treatment of patients with 1 hydroxyvitamin D_3 improves mitogen-induced lymphocyte transformation (7) and significantly increases IL-2 release (12). They suggested that the conflicting results obtained in the in vivo and in vitro studies were the result of differences in experimental methods.

It has been suggested that the effect of vitamin D_3 on calcium metabolism in patients with CRF includes modulation of defective lymphocyte metabolism. It is known that the intracytoplasmic free calcium concentration plays an important role in T cell activation. Induced intracellular calcium elevation actives protein kinase-C which binds to phospholipids. This activation also stimulates T cell receptors (TCR). In patients with CRF

increased PTH levels lead to elevated intracellular calcium levels and it has been suggested that this continuously elevated intracellular calcium causes a type of metabolic paralysis leading to mitogen-induced proliferation (5). Active vitamin D reverses the effects of secondary hyperparathyroidism by acting to normalize the serum calcium concentration with subsequent restoration of intracellular calcium which becomes available as a messenger.

Although we were unable to measure intracellular calcium levels in the present patients' lymphocytes, we postulate that this mechanism may play a role in the elevation of SIs observed following vitamin D_3 treatment. Further work is necessary to elucidate the role of vitamin D_3 in improving lymphocyte function in patients with CRF.

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