The effect of 1α -hydroxyvitamin D_3 on cellular and humoral immunity in hemodialyzed patients

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<Abstract>

The immunoregulatory effect of 1α OH D_3 , a precursor form of active vitamin D_3 ($1,25(\text{OH})_2D_3$), was examined in hemodialyzed patients. Four weeks of oral administration of $0.5\mu\text{g/day}$ of 1α OH D_3 markedly enhanced the lymphoproliferative responses to mitogens, without influencing lymphocyte counts or the ratios of lymphocyte subpopulations. In fact, these responses were nearly normal after 1α OH D_3 treatment. Furthermore, immunoglobulin (IgG, IgM) levels were significantly increased after the same treatment. These results suggest that deficiency of $1,25(\text{OH})_2D_3$ may play a role in the impairment of immunity in hemodialyzed patients, and that administration of 1α OH D_3 may have therapeutic immunologic benefits.

慢性腎不全血液透析患者の免疫能に及ぼす 1α OH D_3 の影響

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慢性腎不全血液透析患者（透析患者）7 名に 1α OH D_3 $0.5\mu\text{g/日}$ を 4 週間経口投与し、その前後で、末梢血白血球数、リンパ球数、リンパ球 subpopulation, Mitogen (PHA, ConA, PWM) 刺激に対するリンパ球幼若化能、血清 IgG, IgM 値を測定し、 1α OH D_3 の透析患者の免疫能に及ぼす影響を検討した。

透析患者では、健常者に比しリンパ球数は有意な減少がみられたが、白血球数、リンパ球 subpopulation の比率では有意な差はみられず、 1α OH D_3 投与後も有意な変化はみられなかった。リンパ球幼若化能に関し、 1α OH D_3 投与前の透析患者では、健常者に比し、PHA, ConA, PWM 刺激に対するリンパ球幼若化能はともに、有意に低下していたが、 1α OH D_3 投与後 PHA, ConA, PWM 反応ともに有意に上昇し健常者のレベルにまで改善した。透析患者において血清 IgG 値は健常者のそれと有意な差はなく、血清 IgM 値は、健常者に比し有意な低下がみられたが、血清 IgG, IgM 値ともに 1α OH D_3 投与後有意な上昇がみられた。

透析患者では健常者に比し、細胞性免疫能が低下しており、 1α OH D_3 投与は、透析患者の免疫能を改善させることが示唆された。

Introduction

Serum $1,25$ -dihydroxyvitamin D_3 ($1,25(\text{OH})_2D_3$) concentrations are decreased in patients with chronic

renal failure, including those who are treated by hemodialysis^{1~4)}. Renal osteodystrophy is one of the most hazardous complications in hemodialyzed patients, and impaired synthesis of $1,25(\text{OH})_2D_3$ is a major cause for this disorder. 1α -hydroxyvitamin D_3

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($1\alpha\text{OHD}_3$), a vitamin D analog which is hydroxylated and converted to $1,25(\text{OH})_2\text{D}_3$ in the liver, has been reported to be of therapeutic value in the management of patients with renal osteodystrophy.

Recently, receptors for $1,25(\text{OH})_2\text{D}_3$ were found on immunologically competent cells, such as monocytes and activated T and B lymphocytes^{5,6}. Tsoukas et al. reported that a physiological concentration of $1,25(\text{OH})_2\text{D}_3$ suppressed interleukin 2 release and mitogen-induced lymphocyte proliferation *in vitro*⁷. These observations suggest that $1,25(\text{OH})_2\text{D}_3$ might be involved in the regulation of immune response.

Cellular immunity is impaired in hemodialyzed patients, but the exact mechanism causing the impairment is unknown¹¹⁻¹⁵. While the action of $1\alpha\text{OHD}_3$ on calcium metabolism in hemodialyzed patients is known, it is not known whether $1\alpha\text{OHD}_3$ has an effect on the immune system as well. To elucidate this question, we studied the effect of $1\alpha\text{OHD}_3$ on mitogen-induced lymphoproliferative responses in hemodialyzed patients.

Materials and Methods

1. Subjects

Seven patients with end-stage chronic renal failure were studied. They were treated by hemodialysis three times a week for two to five months (mean 3.43 ± 0.37 (SEM) months). They were five men and two women, their ages ranged from 51 to 70 years (mean 59.1 ± 2.6 (SEM)). None had a clinically evident intercurrent viral or bacterial infection during the study. We also studied six normal men and three normal women, ranging in age from 36 to 77 years (mean 53.6 ± 5.7).

2. Procedures

The patients were treated daily with $0.5 \mu\text{g}$ of $1\alpha\text{OHD}_3$ for four weeks. Peripheral blood lymphocyte counts, lymphocyte subpopulations, mitogen-induced lymphoproliferative responses, and immunoglobulin (IgG, IgM) levels were determined before and after the treatment.

Blood (20 ml) was obtained from the patients before regular hemodialysis and from the normal subjects before breakfast. The blood was overlaid on ficoll-sodium metrizoate, and centrifuged at 1500 rpm for 30 min. The resulting mononuclear cell layer was collected. These cells were washed three times with

phosphate-buffered saline (pH 7.4) without divalent cations (PBS) then resuspended in PBS.

T lymphocytes in blood were identified by their capacity to form spontaneous rosettes with sheep red blood cells. B lymphocytes were identified by the capacity to form rosettes with antibody-coated polystyrene beads (Immunobeads®, Bio-Rad Laboratories, CA)¹⁶.

To measure cellular responses to mitogen, mononuclear cells were resuspended in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (GIBCO) at a concentration of 1×10^6 cells/ml. One hundred microliters of the cell suspension was poured in triplicate into each well of round-bottom microplates. Then, one of the following mitogens was added: phytohemagglutinin (PHA, $2 \mu\text{l}$ /well, Difco Laboratories Inc., Detroit, MI), concanavalin A (Con A, $2 \mu\text{l}$ NaCl containing $1 \mu\text{g}$ Con A/well, Pharmacia Fine Chemicals, Uppsala, Sweden), or pokeweed mitogen (PWM, $2 \mu\text{l}$ /well, GIBCO). The mitogen doses used had been previously established as optimal. After 48 h of incubation at 37°C in a humidified atmosphere containing 5% CO_2 , 95% air, the cultures were pulsed with $0.5 \mu\text{Ci}$ /well ^3H -thymidine (specific activity, 5.0 Ci/mmol, Amersham, England). The cells were collected on glass microfiber filters 24 hr later and washed twice sequentially with saline, 5 percent trichloroacetic acid and cold absolute methanol. The radioactivity was counted in a Beckman LS 1800 liquid scintillation counter (Beckman Instruments Inc., California) and the stimulation index (SI) was calculated by the formula.

$$\text{SI} = \frac{\text{cpm of cultures with mitogen}}{\text{cpm of cultures without mitogen}}$$

Statistical analysis was performed by Student's *t*-test.

Results

The levels of blood urea nitrogen, serum creatinine, serum calcium, and serum phosphorus are shown in Fig. 1. Treatment of $0.5 \mu\text{g/day}$ $1\alpha\text{OHD}_3$ for 4 weeks did not cause a significant change in blood urea nitrogen, creatinine, calcium, or phosphorus levels in the hemodialyzed patients. The white blood cell counts, lymphocyte counts, and the percentages of T and B lymphocytes are shown in Fig. 2 and 3. The white

B : Before treatment with $1\alpha\text{OHD}_3$ A : After treatment with $1\alpha\text{OHD}_3$
 n=7 ; mean $\pm$ SEM

Fig. 1 Blood urea nitrogen, serum creatinine, serum Ca, and serum P levels in hemodialyzed patients before (open bars) and after (striped bars) treatment with $1\alpha\text{OHD}_3$.

C : control subjects (n=9)
 B : HD patients (Before $1\alpha\text{OHD}_3$) (n=7)
 A : HD patients (After $1\alpha\text{OHD}_3$) (n=7)
 mean $\pm$ SEM

Fig. 2 White blood cell counts and lymphocyte counts in normal subjects (open bars) and hemodialyzed patients before (striped bars) and after treatment with $1\alpha\text{OHD}_3$ (shaded bars).

C : Control subjects (n=9)
 B : HD patients (Before $1\alpha\text{OHD}_3$) (n=7)
 A : HD patients (After $1\alpha\text{OHD}_3$) (n=7)
 mean $\pm$ SEM

Fig. 3 The percentages of lymphocyte subpopulations in normal subjects (open bars) and hemodialyzed patients before (striped bars) and after treatment with $1\alpha\text{OHD}_3$ (shaded bars).

blood cells were not significantly different in the hemodialyzed patients and the normal subjects. Peripheral blood lymphocytes were significantly decreased in number in the hemodialyzed patients compared to the normal subjects. White blood cells

and lymphocytes did not increase after administration of $1\alpha\text{OHD}_3$. The numbers of either T or B cells relative to the total cell population was not different in the two groups. $1\alpha\text{OHD}_3$ had no significant effect on the percentages of T or B lymphocytes in the hemodial-

Fig. 4 Mitogen-induced proliferative responses of PBM from normal subjects (open bars) and hemodialyzed patients before (striped bars) and after treatment with 1 α OHD₃ (shaded bars).

Fig. 5 Immunoglobulin (IgG, IgM) levels in normal subjects (open bars) and hemodialyzed patients before (striped bars) and after treatment with 1 α OHD₃ (shaded bars).

ized patients.

The proliferative responses of peripheral blood mononuclear cells (PBM) to PHA, Con A, and PWM, as measured by ³H-thymidine uptake, are shown for both groups in Fig. 4. The mononuclear cells from the hemodialyzed patients before treatment with 1 α OHD₃ had significantly lower responses than did those of the normal subjects to stimulation with PHA (56.6 ± 36.1 (SD) vs 216.0 ± 79.9 , $p < 0.001$), Con A (62.3 ± 43.0 vs 183.1 ± 71.6 , $p < 0.01$), and PWM (48.5 ± 22.9 vs 147.5 ± 44.4 , $p < 0.001$). In the hemodialyzed patients, the responses to PHA, Con A, and PWM after four weeks of treatment with 1 α OHD₃ were increased almost to the same level as the normal subjects.

Immunoglobulin (IgG, IgM) levels are shown in Fig. 5. The IgG levels were normal but the IgM levels were decreased in the hemodialyzed patients. In the hemodialyzed patients, both the IgG and IgM levels were increased after treatment with 1 α OHD₃.

Discussion

1,25 (OH)₂D₃, the most biologically active form of vitamin D, induces differentiation of both human and murine myeloid leukemia cell lines¹⁷⁾ and the U937 monoblast cell line¹⁸⁾. Receptors for vitamin D have been found in monocytes and also in lymphocytes activated in vitro by mitogenic lectin^{5,6)}. More recently, 1,25(OH)₂D₃ was found to be a potent inhibitor of mitogen-induced lymphocyte proliferation⁷⁻¹⁰⁾. These data raise the possibility that 1 α OHD₃, a precursor form of active vitamin D₃, might play a role in the regulation of the immune response. In this study peripheral blood mononuclear cells (PBM) from hemodialyzed patients responded significantly less to T cell mitogens than did cells from normal subjects. Similar results were reported previously¹¹⁻¹⁵⁾. 1 α OHD₃, given for four weeks in a dose of 0.5 μ g/day, had no appreciable effect on the number of PBM or the ratio of either T or B subpopulation in hemodialyzed patients. In spite of this, 1 α OHD₃ nearly normalized the depressed lymphoproliferative responses to mitogens in our patients. Furthermore, 1 α OHD₃ increased the IgG levels and IgM levels in hemodialyzed patients. Lund et al. reported that in hypoparathyroidism patients given 0.5-1.0 μ g/day 1 α OHD₃ po, the precursor was efficiently metabolized to 1,25(OH)₂D₃, and their serum levels of 1,25(OH)₂D₃ were increased

within a month¹⁹⁾.

The $1\alpha\text{OHD}_3$ treatment caused no notable change in serum calcium concentration in our patients. Thus, the effect of $1\alpha\text{OHD}_3$ and/or $1,25(\text{OH})_2\text{D}_3$ on mitogen-induced PBM proliferation in the hemodialyzed patients was probably direct rather being mediated by increased serum calcium levels.

Kauffman et al. reported that the depressed responsiveness of lymphocytes to mitogen was not corrected by long-term hemodialysis in patients with chronic renal failure¹³⁾. It is unlikely, therefore, that mitogen-induced lymphoproliferative response was enhanced by hemodialysis. Although the mechanism of $1\alpha\text{OHD}_3$ -induced enhancement of lymphoproliferative response to mitogen in hemodialyzed patients is not known, one possible mechanism can be proposed : $1,25(\text{OH})_2\text{D}_3$, a metabolite of $1\alpha\text{OHD}_3$, may induce maturation and/or differentiation of immature thymic lymphocytes. Maturation impairment of thymic lymphocytes and thymic atrophy has been reported in chronic renal failure^{20,21)}. Reinhardt et al.²²⁾ and Ravid et al.²³⁾ detected receptors for $1,25(\text{OH})_2\text{D}_3$ on bovine and mouse immature thymic lymphocytes. In addition, Ohsugi et al. reported that orally administered $1\alpha\text{OHD}_3$ prevented thymic involution in rats with renal osteodystrophy²⁴⁾.

Tsoukas et al.⁷⁾ and Lemire et al.¹⁰⁾ reported that $1,25(\text{OH})_2\text{D}_3$ suppressed proliferation of normal human PBM activated in vitro by mitogens. The discrepancies between their observations and ours may be due to the differences in experimental design (in vitro vs in vivo ; normal subjects vs hemodialyzed patients).

In conclusion, $1\alpha\text{OHD}_3$ enhanced depressed non-specific immune responses in hemodialyzed patients, restoring them nearly to the normal level without altering total lymphocyte count and the percentage of T and B lymphocytes.

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