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# Synthesis of active metabolite(s) from 1α-hydroxyvitamin $D_3$ by human monocytic leukemia cells

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Synthesis of the biologically active metabolite(s) from 1α-hydroxyvitamin $D_3$ (1α(OH)$D_3$) was examined in various types of human leukemia cell lines. Untreated monocytoid leukemia cells (U937 and HEL/S) metabolized 1α(OH)$D_3$ to the active metabolite(s), possibly 1α,24- and/or 1α,25-dihydroxyvitamin $D_3$, and these cells were efficiently induced to differentiate by treatment with 1α(OH)$D_3$. However, the other types of leukemia cells did not efficiently metabolize it and were not induced to differentiate by 1α(OH)$D_3$. The possible therapeutic advantage of 1α(OH)$D_3$ in the treatment of monocytic leukemia is discussed.

Monocytic leukemia cell; Vitamin $D_3$ activation; Differentiation

## 1. INTRODUCTION

The biologically active metabolite of vitamin $D_3$, 1α,25-dihydroxyvitamin $D_3$ (1α,25(OH)$_2D_3$), is a calcium-regulating hormone secreted by the kidney [1]. The hormone exerts its effect of inducing intestinal calcium transport and bone mineral mobilization activities by binding to a specific cytosol receptor in these organs. Some tumor cells also possess similar cytosol receptors to which 1α,25(OH)$_2D_3$ binds specifically [2]. 1α,25(OH)$_2D_3$ inhibits cell growth and induces differentiation of some tumor cells, including myelomonocytic leukemia cells [2,3].

Although 1α(OH)$D_3$ was only 0.01 as active 1α,25(OH)$_2D_3$ in inducing differentiation and inhibiting growth of mouse myeloid leukemia M1 cells in vitro, 1α(OH)$D_3$ was more potent in enhancing the survival time of mice inoculated with the M1 cells [4]. It has been reported that liver is a major site of 25-hydroxylation of vitamin $D_3$, but tissues other than the liver may also possess 25-hydroxylating ability [1]. Activated macrophages or 1α,25(OH)$_2D_3$-treated promyelocytic leukemia HL-60 cells have some vitamin D-metabolizing enzymes, including the 24-hydroxylase [5–7]. These results suggest that some myeloid leukemia cells may have the 24- and/or 25-hydroxylating activities, and that 1α(OH)$D_3$, may be efficiently metabolized to active metabolite(s) in the leukemia cells. In the present experiment we examined the possibility of metabolic conversion of 1α(OH)$D_3$ to the active metabolite(s) in the leukemia cells.

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## 2. MATERIALS AND METHODS

### 2.1. *Cells and cell culture*

Human leukemia cell lines were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% $CO_2$ in air [8]. The human leukemia cell lines used in the present experiment were as follows: U937 and HEL/S (monocytoid), K562 and HEL/O (erythroid), HL-60 and ML-1 (myeloid), and BALL-1 and MOLT4 (lymphoid).

### 2.2. *Assay of cell growth and properties of differentiated cells*

Cell numbers were counted in a Model ZM Coulter Counter (Coulter Electronics, Luton, UK). Nitro blue tetrazolium (NBT) reduction was assayed as reported previously [9]. 1α(OH)$D_3$ and 1α,25(OH)$_2D_3$ were obtained from Chugai Pharmaceutical Co. (Tokyo).

### 2.3. *Binding assay of active metabolites*

The assay was performed by competition between unlabeled samples and $^3$H-labeled 1α,25(OH)$_2D_3$ for binding a protein which has a high specificity and affinity for 1α,25(OH)$_2D_3$, using an Amersham assay kit.

## 3. RESULTS

1α,25(OH)$_2D_3$ dose-dependently induced NBT reducing activity (a typical differentiation marker) of HL-60 cells and HEL/S cells, and 1α(OH)$D_3$ did not induce NBT reduction of HL-60 cells, as previously reported [2], however, HEL/S cells were induced to differentiate by 1α(OH)$D_3$, and more efficiently than 1α,25(OH)$_2D_3$ (Fig.1). To examine the metabolic activation of 1α(OH)$D_3$ in leukemia cells, several types of leukemia cells ($5 \times 10^5$/ml) were cultured with or without 100 ng/ml of 1α(OH)$D_3$ for 24 h and the conditioned medium was harvested. Conversion of 1α(OH)$D_3$ to an active metabolite was examined by inducing NBT reduction of HL-60 cells (Fig. 2). The conditioned medium from U937 or HEL/S cells significantly induced

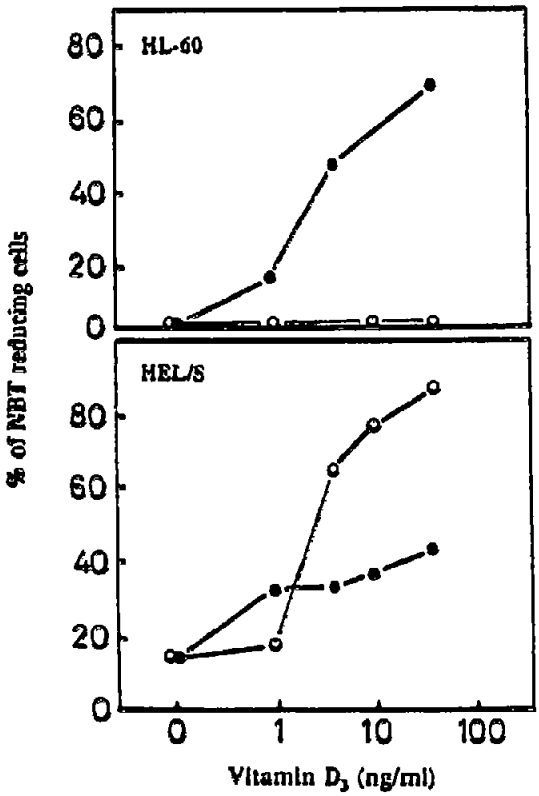


Fig. 1. Induction of NBT reduction of HEL/S cells by $1\alpha(OH)D_3$. Cells were treated with various concentrations of $1\alpha(OH)D_3$ (○) or $1\alpha,25(OH)_2D_3$ (●) for 4 days. NBT reduction was expressed as the % of NBT-positive cells.

NBT reduction of HL-60 cells, but not those from the other cells. Although untreated HL-60 cells are promyelocytic, monocytic differentiation of the HL-60 cells can be induced by several compounds. The HL-60 cells which were induced to monocytic differentiation by $1\alpha,25(OH)_2D_3$ could metabolize $1\alpha(OH)D_3$ to an active metabolite(s), but the differentiated cells by the other inducers could not metabolize the $1\alpha(OH)D_3$ (Table I).

Conversion of $1\alpha(OH)D_3$ to an active metabolite, and metabolic inactivation of $1\alpha,25(OH)_2D_3$, increased with higher densities of cultured cells and longer incubation times. In the culture of U937 cells ($5 \times 10^5$ cells/ml) with 100 ng/ml of $1\alpha(OH)D_3$, the NBT-inducing activity was not detected within 5 h, but thereafter increased to a maximal level at 2 days (Fig. 3). Metabolic inactivation of $1\alpha,25(OH)_2D_3$ was also observed in the U937 cells, but it was later than the activation of $1\alpha(OH)D_3$. The amount of $1\alpha,25(OH)_2D_3$ in the conditioned medium of $1\alpha(OH)D_3$-treated culture was determined by the radioreceptor assay. A greater than 100 molar excess of $1\alpha(OH)D_3$ was required to displace the bound $1\alpha,25(OH)_2[^3H]D_3$ [2]. The result of the radioreceptor assay was consistent with that of induction of NBT reduction (Fig. 3).

Table I

Activation of $1\alpha(OH)D_3$ by differentiated HL-60 cells induced by $1\alpha,25(OH)_2D_3$.

| Treatment | Differentiation (NBT reduction, $A_{560}/10^3$ cells | Metabolic activation of $1\alpha(OH)D_3$ (NBT reduction, $A_{560}/10^3$ cells) |
|---|---|---|
| None | 1.3 ± 0.6 | 1.1 ± 0.8 |
| $1\alpha,25(OH)_2D_3$ | 5.9 ± 1.4 | 2.7 ± 1.2 |
| TNFα | 5.2 ± 0.9 | 1.3 ± 0.6 |
| TPA | 6.1 ± 0.7 | 1.1 ± 0.5 |
| Retinoic acid | 5.3 ± 0.6 | 1.2 ± 0.6 |

HL-60 cells were cultured with 100 ng/ml of $1\alpha,25(OH)_2D_3$, 2 ng/ml of tumor necrosis factor α (TNFα), 0.5 ng/ml of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), or $4 \times 10^{-7}$ M retinoic acid for 4 days. NBT reducing activity in an aliquot of the treated cells was determined (Differentiation), and the remaining cells were washed with phosphate buffered saline, and then were cultured with 100 ng/ml of $1\alpha(OH)D_3$ for 24 h. The resulting conditioned medium was collected and the metabolic activation of $1\alpha(OH)D_3$ was assayed by measuring NBT reduction of HL-60 cells after treatment of the cells with 10% (v/v) of the conditioned medium for 4 days (Metabolic activation of $1\alpha(OH)D_3$). The conditioned medium from culture without $1\alpha(OH)D_3$ of the $1\alpha,25(OH)_2D_3$-treated cells was unable to induce NBT reduction of HL-60 cells.

## 4. DISCUSSION

Normal pulmonary macrophages are known to metabolize $25(OH)D_3$ by treatment of the cells with interferon-γ or lipopolysaccharide [5,6]. The polar metabolites were identified as $1\alpha,25(OH)_2D_3$ and $24,25(OH)_2D_3$. It is of interest to examine metabolism of vitamin $D_3$ in monocytic leukemia cells. Although some myelomonocytic leukemia cells can metabolize $25(OH)D_3$, the metabolite isolated is not a biologically active metabolite such as $1\alpha,25(OH)_2D_3$ [7,10].

The present results clearly demonstrate that the untreated monocytic leukemia cells preferentially convert $1\alpha(OH)D_3$ to a biologically active metabolite(s). The metabolite(s) comigrated with authentic $1\alpha,25(OH)_2D_3$ on a Sephadex LH-20 chromatography column. The metabolite(s) had the same affinity for the $1\alpha,25(OH)_2D_3$-specific receptor as authentic $1\alpha,25(OH)_2D_3$. However, we can not eliminate the possibility that the untreated monocytic leukemia cells can synthesize $1\alpha,24(OH)_2D_3$ as well as $1\alpha,25(OH)_2D_3$. The 24-hydroxylase was induced in interferon-γ-stimulated macrophages and $1\alpha,25(OH)_2D_3$-treated HL-60 cells, but not in the untreated cells [5,7]. $1\alpha,24(OH)_2D_3$ has a similar potency of inducing NBT reduction of HL-60 cells and the same affinity for the specific receptor as $1\alpha,25(OH)_2D_3$ [2].

Untreated monocytic leukemia cells can metabolize $1\alpha(OH)D_3$ to a biologically active metabolite(s) and are induced to differentiate into mature macrophages by their own metabolite(s). It is tempting to speculate on the therapeutic significance of the active metabolite(s).

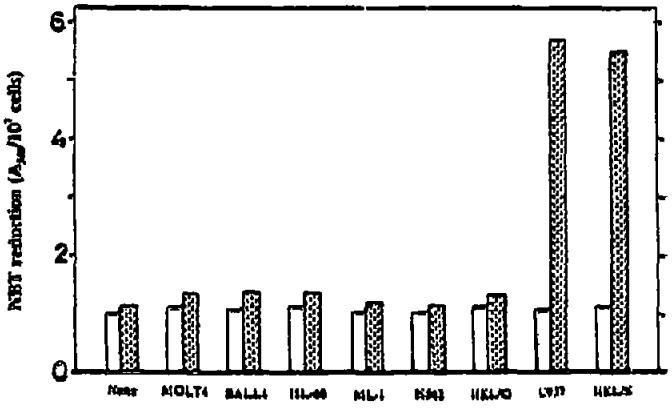

Fig. 2. Activation of $1\alpha(OH)D_3$ in monocytoid leukemia cell lines. Cells ($5 \times 10^5$/ml) were cultured with (stippled bars) or without (open bars) 100 ng/ml of $1\alpha(OH)D_3$ for 24 h. The conditioned medium was collected and added (final concentration, 10%) to the culture of HL-60 cells for 4 days. Conversion of the active metabolite(s) was examined by induction of NBT reduction.

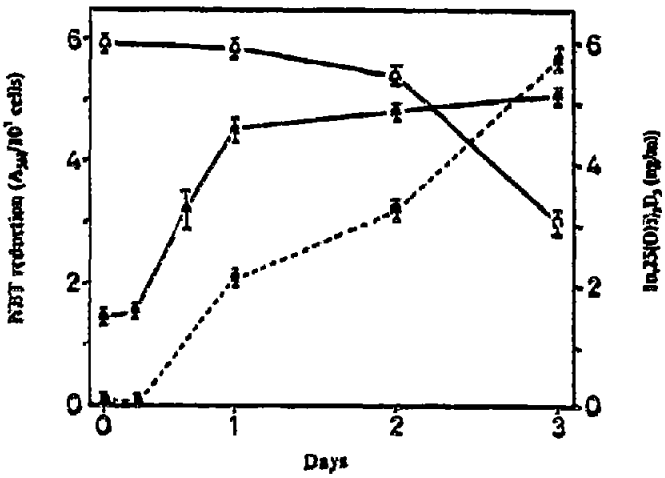

Fig. 3. Time-courses of activation and inactivation of vitamin $D_3$ in U937 cells. U937 cells ($3 \times 10^5$/ml) were treated with 100 ng/ml of $1\alpha(OH)D_3$ (●) or $1\alpha,25(OH)_2D_3$ (○) for the indicated times. The resulting conditioned medium (final concentration 10%) was added to HL-60 cells. NBT reduction of HL-60 cells was examined after 4-day culture. ■, amounts of $1\alpha,25(OH)_2D_3$-competitive compounds. Bars are means ± S.D. for three different experiments.

Our previous results indicate that $1\alpha(OH)D_3$ is more effective than $1\alpha,25(OH)_2D_3$ for enhancing the survival time of mice inoculated with mouse myeloid leukemia M1 cells [4]. Endogenous cytokines, such as interferon-$\gamma$, may induce 24- or 25-hydroxylase activity in the inoculated M1 cells and then the cells may convert $1\alpha(OH)D_3$ to $1\alpha,25(OH)_2D_3$ or $1\alpha,24(OH)_2D_3$. The metabolite(s) may be effective in inhibiting proliferation of their own cells and in prolonging the survival time of the leukemic mice without any serious side effects. Because in vivo administration of $1\alpha(OH)D_3$ to mice resulted in no appreciable hypercalcemia [4], it might be useful for treatment of some types of leukemias, including monocytic leukemia and vitamin $D_3$-responsive myeloid leukemia.

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## REFERENCES

[1] DeLuca, H.F. and Schnoes, H.K. (1976) Annu. Rev. Biochem. 45, 631–666.
[2] Tanaka, H., Abe, E., Miyaura, C., Kuribayashi, T., Konno, K., Nishii, Y. and Suda, T. (1982) Biochem. J. 204, 713–719.
[3] Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshiki, S. and Suda, T. (1981) Proc. Natl. Acad. Sci. USA 78, 4990–4994.
[4] Honma, Y., Hozumi, M., Abe, E., Konno, K., Fukushima, M., Hata, S., Nishii, Y., DeLuca, H.F. and Suda, T. (1983) Proc. Natl. Acad. Sci. USA 80, 201–204.
[5] Reichel, H., Koeffler, H.P. and Norman, A.W. (1987) J. Biol. Chem. 262, 10931–10937.
[6] Reichel, H., Koeffler, H.P., Bishop, J.E. and Norman, A.W. (1987) J. Clin. Endocrinol. Metab. 64, 1–9.
[7] Inaba, M., Burgos-Trinidad, M. and DeLuca, H.F. (1991) Arch. Biochem. Biophys. 284, 257–263.
[8] Okabe-Kado, J., Honma, Y., Hayashi, M. and Hozumi, M. (1991) Anticancer Res. 11, 181–186.
[9] Kasukabe, T., Honma, Y., Hozumi, M. and Nomura, H. (1990) Jpn. J. Cancer Res. 81, 807–812.
[10] Gray, T.K., Millington, D.S., Maltby, D.A., Williams, M.E., Cohen, M.S. and Dodd, R.C. (1985) Proc. Natl. Acad. Sci. USA 82, 8218–8221.