Enhancement of Activity of 1α,25-Dihydroxyvitamin D3 for Growth Inhibition and Differentiation Induction of Human Myelomonocytic Leukemia Cells by Tretinoin Tocoferil, an α-Tocopherol Ester of All-trans Retinoic Acid

By Makoto Makishima, Yasuhiro Kanatani, Yuri Yamamoto-Yamaguchi, and Yoshio Honma

Tretinoin tocoferil is an α-tocopherol ester of all-trans retinoic acid (RA) and safely used in the treatment of skin ulcer. Tretinoin tocoferil inhibited proliferation of human promyelocytic leukemia HL-60 cells and induced granulocytic differentiation of the cells, but less than RA. α-Tocopherol did not affect differentiation of HL-60 cells, but at high concentrations enhanced its nitroblue tetrazolium (NBT)-reducing activity and expression of surface antigen CD11b, which are markers of myelomonocytic differentiation induced by RA. Tretinoin tocoferil increased NBT reduction in HL-60 cells treated with RA. It also enhanced the differentiation of HL-60 cells induced by dimethyl sulfoxide, phorbol-12-myristate 13-acetate or 1α,25-dihydroxyvitamin D3 (VD3). In combination with a low concentration of VD3, it induced the NBT-reducing activity of human monoblastic U937 cells very effectively. Moreover, it enhanced the differentiation of human myelomonocytic ML-1, THP-1, P39/TSU, and P31/PUJ cells induced by VD3. In combination with VD3, it synergistically inhibited the proliferation of HL-60, U937, ML-1, THP-1, P39/TSU, and P31/PUJ cells and decreased the effective concentration of VD3 to a 10^-10 mol/L level. Because tretinoin tocoferil was reported to induce neither retinoid-related toxicity nor teratogenicity, the therapeutic advantage of the use of it in treatment of myelomonocytic leukemia is suggested.

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Materials and Methods

Materials. Tretinoin tocoferil (cocoretinate(+)3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyldodec-2H-1-benzopyran-6-yl)(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,8-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-tetraenonaetanoate) (Fig 1) was synthesized by Pharmaceutical Research Center, Nicho Milling Co (Saitama, Japan) and gifted from Lederle (Saitama, Japan). Tretinoin tocoferil was dissolved in liquid paraffin at 0.352 mol/L and diluted with ethanol to 4 × 10^-2 mol/L. Dimethyl sulfoxide (DMSO) and VD3 were purchased from Wako Pure Chemical Industry (Osaka, Japan), and nitroblue tetrazolium (NBT), RA, α-tocopherol, and phorbol-12-my-
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A

B

C

Fig 1. Chemical structures of tretinoin tocoferol (A), all-trans retinoic acid (RA) (B), and α-tocopherol (C). RA is also named tretinoin.

riatate 13-acetate (TPA) were from Sigma (St Louis, MO). The concentrations of RA, α-tocopherol, and VD₃ in the ethanol stock solution were 4 x 10⁻³ mol/L, 6 x 10⁻² mol/L, and 1.2 x 10⁻³ mol/L, respectively.

Cell lines and cell cultures. Human myeloid leukemia HL-60, U937, ML-1, THP-1, P39/TSU, and P31/FUJ cells were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal bovine serum and 88 μg/mL gentamicin at 37°C in a humidified atmosphere of 5% CO₂ in air. P39/TSU cells were established from a patient with overt leukemia following the myelodysplastic syndrome, and P31/FUJ cells were from a patient with acute monocytic leukemia. Both cell lines gave positive reactions for NaF-sensitive α-naphthyl butyrate esterase activity, Fcy-receptors, C3-receptors, and showed phagocytic activity and reactivity with monoclonal antibodies characteristic of monocytic cells. P39/TSU and P31/FUJ cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan).

Cell growth and differentiation. Suspensions of cells were cultured with or without compounds in multidishes. Cell numbers were counted in a Model ZM Coulter Counter (Coulter Electronics, Luton, England). Cell viability was determined by exclusion of trypan blue. Cell morphology was examined in cell smears stained with May-Grünwald and Giemsa solutions by examination of more than 200 cells. NBT reduction was assayed microscopically and colorimetrically as reported previously. In the microscopic assay, the percentage of cells staining intracellular blue-black formazan deposits was determined by examination of more than 200 cells. In the colorimetric assay, the reaction was stopped by adding HCl. The formazan deposits were solubilized by adding DMSO, and the absorption of the formazan solution at 560 nm was measured in a spectrophotometer (U-2000; Hitachi, Tokyo, Japan). Lysozyme activity in conditioned medium was determined by a method with lysoplates containing 1% agar, 1/15 mol/L sodium phosphate buffer (pH 5.6), 50 mmol/L NaCl, and heat-killed Micrococcus lysodeikticus (0.5 mg/mL). One unit is equivalent to 1 µg/mL egg-white lysozyme. Expression of the granulocyte- and macrophage-specific antigen CD11b on the cell surface was determined by indirect immunofluorescent staining and flow cytometry. The cells were incubated with mouse anti-CD11b antibody (Nichirei, Tokyo) and stained with fluorescein isothiocyanate (FITC) conjugated antimouse IgG (Tago, Burlingame, CA). The CD11b-positive cells were counted with a flow cytometer (Epics XL; Coulter Electronics).

Analysis of the effects of combinations of drugs. Isobologram analysis was used to determine the effects of combinations of drugs on leukemia cells. Dose-dependent effects were determined for each compound and for one compound with fixed concentrations of another. The interaction of the two compounds was quantified by determining the combination index (CI) according to the classic isobologram equation,

\[ CI = \frac{(D_1/Dx_1) + (D_2/Dx_2)}{1} \]

where Dx is the dose of one drug alone required to produce an effect and (D₁) and (D₂) are the doses of compounds 1 and 2, respectively, in combination that produce the same effect. From this analysis the combined effects of the two drugs can be assessed as: summation (additive or zero interaction) indicated as CI = 1, synergism indicated as CI < 1, and antagonism indicated as CI > 1.

Statistical evaluation. Statistical analyses in the experiments were performed using Student’s t-test.

RESULTS

Effects of tretinoin tocoferol on growth and differentiation of human promyelocytic leukemia HL-60 cells. Human promyelocytic leukemia HL-60 cells are known to be induced to differentiate by several compounds including RA. We examined the effect of tretinoin tocoferol on growth and differentiation of HL-60 cells. Tretinoin tocoferol inhibited proliferation of these cells concentration-dependently, its concentration for 50% growth inhibition (IC₅₀) being 1.3 x 10⁻⁴ mol/L (Fig 2A). However, the effective concentrations for tretinoin tocoferol for inhibiting cell growth were higher than those of RA (Fig 2B). Both RA and tretinoin tocoferol induced NBT-reducing activity, a typical marker of myelomonocytic differentiation, of the HL-60 cells concentration-dependently (Table 1, Fig 2). In the cells treated with tretinoin tocoferol, however, the intensity of NBT-reducing activity was weak, and the colorimetric assay of NBT reduction, which was measured after dissolving the formazan deposits in the cells, showed low absorbance values as compared with those in the RA-treated cells (Fig 2C). Tretinoin tocoferol, like RA, induced morphological change of HL-60 cells into granulocytic lineage and increased the percentage of cells positive for CD11b antigen, which is a myeloid differentiation marker (Table 1). Although the culture medium treated with tretinoin tocoferol contains liquid paraffin and ethanol at the maximum concentrations of 0.0426% and 0.5%, respectively, the combination of liquid paraffin (0.0426%) and ethanol (0.5%) did not induce either NBT-reducing activity (Fig 2A) or morphological change of HL-
Fig 2. Effects of tretinoin tocoferil (A) and RA (B) on growth and NBT reducing activity of HL-60 cells. Cells (2 × 10⁶/mL) were treated with RA or tretinoin tocoferil for 4 days. Growth is indicated as (□) and NBT reduction as (▲). Tretinoin tocoferil is dissolved in liquid paraffin and diluted with ethanol. Maximum final concentrations of liquid paraffin and ethanol were 0.0426% and 0.5%, respectively, and did not increase NBT-reducing activity of HL-60 cells (▲). (C) Relationship between absorbance values and percentage values of NBT reduction in HL-60 cells treated with RA (□) or tretinoin tocoferil (▲). Values are the mean ± SD for three separate experiments.

60 cells (data not shown). Thus tretinoin tocoferil was a weak inducer of granulocytic differentiation of HL-60 cells. We found previously that α-tocopherol inhibited the differentiation of mouse myeloid leukemia M1 cells induced by dexamethasone.α Because tretinoin tocoferil is an α-tocopherol ester of RA, we examined whether α-tocopherol affected the differentiation of HL-60 cells induced by RA. α-Tocopherol at concentrations of up to 3 × 10⁻⁴ mol/L neither inhibited proliferation nor induced NBT-reducing activity of the HL-60 cells, and it only slightly affected the growth inhibition induced by RA (Fig 3). On the other hand, it significantly enhanced the induction of NBT-reducing ac-
Differentialiation by Tretinoin Tocopheril

Table 1. Effects of Tretinoin Tocopheril on Differentialiation of HL-60 Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NBT-Reducing Cells (%)</th>
<th>Pro</th>
<th>Mye</th>
<th>Gr</th>
<th>CD11b-Positive Cells (%)</th>
<th>Viable Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 ± 0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
<td>96 ± 0</td>
</tr>
<tr>
<td>TT 1.35 x 10^-4 mol/L</td>
<td>77 ± 4</td>
<td>33</td>
<td>41</td>
<td>26</td>
<td>19 ± 1</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>2 x 10^-4 mol/L</td>
<td>69 ± 3</td>
<td>12</td>
<td>36</td>
<td>52</td>
<td>41 ± 11</td>
<td>98 ± 0</td>
</tr>
<tr>
<td>RA 9 x 10^-4 mol/L</td>
<td>92 ± 2</td>
<td>1</td>
<td>25</td>
<td>74</td>
<td>23 ± 2</td>
<td>97 ± 1</td>
</tr>
</tbody>
</table>

Cells (1 to 2 x 10^5 cells/mL) were cultured with TT or RA for 4 days (for assay of NBT reduction, CD11b expression and viability) and 6 days (for assay of morphological changes). Values are means ± SD for three separate experiments.

Abbreviations: TT, tretinoin tocoferil; Pro, promyelocytes; Mye, myelocytes; Gr, granulocytes.

Next we examined the effect of tretinoin tocoferil on the induction of NBT-reducing activity of HL-60 cells by RA. As shown in Fig 4, the addition of tretinoin tocoferil with RA significantly enhanced the NBT-reducing activity of HL-60 cells: tretinoin tocoferil at 9 x 10^-4 mol/L increased NBT reduction in HL-60 cells treated with 9 x 10^-7 mol/L and 3 x 10^-6 mol/L RA 2.9-fold (P < .0001) and 2.0-fold (P < .001); respectively. Tretinoin tocoferil and RA additionally increased CD11b-positive HL-60 cells (Table 2). Thus the effect of RA on differentialiation was enhanced by tretinoin tocoferil.

Fig 3. Effects of α-tocopherol on growth inhibition (A) and NBT-reducing activity (B) of HL-60 cells in combination with RA. Cells (2 x 10^5/mL) were treated with α-tocopherol in combination with 0 (○), 3 x 10^-7 (△), 9 x 10^-7 (■), and 3 x 10^-6 mol/L RA (●) for 4 days. Values are the mean ± SD for three separate experiments.
Table 2. Effect of α-Tocopherol and Tretinoin Tocoferil on Differentiation of HL-60 Cells Induced by RA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NBT-Reducing Cells (%)</th>
<th>CD11b-Positive Cells (%)</th>
<th>Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−RA</td>
<td>+RA</td>
<td>−RA</td>
</tr>
<tr>
<td>None</td>
<td>0 ± 0</td>
<td>42 ± 1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>α-Tocopherol 3 × 10⁻⁴ mol/L</td>
<td>0 ± 0</td>
<td>58 ± 5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>TT 3 × 10⁻⁶ mol/L</td>
<td>2 ± 1</td>
<td>69 ± 1</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>

Cells (2 × 10⁶ cells/mL) were cultured with α-tocopherol or TT without or with 9 × 10⁻⁷ mol/L RA for 4 days. Values are the mean ± SD for three separate experiments.

< .005) and 5.4 ± 0.2 A₅₆₀/10⁷ cells (P < .01), respectively. TPA less than 1 nmol/L induced NBT-reducing activity of HL-60 cells. Tretinoin tocoferil also enhanced differentiation of HL-60 cells treated with TPA: TPA at 0.32 nmol/L increased NBT reduction to 3.4 ± 0.4 A₅₆₀/10⁷ cells and tretinoin tocoferil further increased this activity to 6.5 ± 1.0 A₅₆₀/10⁷ cells (P < .01; Fig 5B). The NBT-reducing activity of HL-60 cells induced by VD₃ was increased on combined treatment with tretinoin tocoferil: VD₃ (3 × 10⁻⁸ mol/L) plus tretinoin tocoferil (3 × 10⁻⁷ mol/L) increased the activity to 5.6 ± 1.1 A₅₆₀/10⁷ cells, which was more than twice that with VD₃ alone (2.6 ± 0.3 A₅₆₀/10⁷ cells) (P < .02) (Fig 5C). Lysozyme activity, another marker of myelomonocytic differentiation, of HL-60 cells was induced concentration-dependently by TPA and VD₃, and tretinoin tocoferil also enhanced this activity (Fig 5). The combination of liquid paraffin at 0.00006% and ethanol at 0.1%, which were solvents for 3 × 10⁻⁷ mol/L tretinoin tocoferil, did not increase either NBT-reducing (Fig 5 legend) or lysozyme activities of the cells treated with DMSO, TPA, or VD₃ (data not shown). Thus, tretinoin tocoferil enhanced the differentiation of HL-60 cells induced by DMSO, TPA, or VD₃.

Synergistic effects of the combination of VD₃ and tretinoin tocoferil in inhibiting proliferation and inducing differentiation of monocytic U937 cells. VD₃ is a promising inducer of differentiation for the treatment of some types of leukemia and cancer. We next examined the effects of VD₃ plus tretinoin tocoferil on proliferation and differentiation of human monocytic U937 cells. VD₃ inhibited proliferation of U937 cells concentration-dependently, its IC₅₀ value after 6 days of treatment being 2.29 × 10⁻⁹ mol/L (Table 3). In combination with tretinoin tocoferil at a concentration that did not affect proliferation, VD₃ inhibited cell growth more effectively (Fig 6A). As shown in Table 3, 9 × 10⁻⁸ mol/L tretinoin tocoferil decreased the IC₅₀ concentration of VD₃ to 4.26 × 10⁻¹⁵ mol/L (18.6% of that without tretinoin tocoferil). Figure 6B shows isoboles for the combination of VD₃ with tretinoin tocoferil that were isoeffective for inhibition of proliferation of U937 cells. These isoboles and the CI indices in Table 3 indicated that the combination of these drugs had synergistic effects. Tretinoin tocoferil at 4 × 10⁻⁹ mol/L to 4 × 10⁻⁷ mol/L did not induce NBT-reducing activity in U937 cells, but in combination with a low concentration of VD₃, this concentration range effectively induced the activity: VD₃ at 3 × 10⁻⁹ mol/L did not induce appreciable activity (0.5 ± 0.1 ± 0.6 ± 0.0 A₅₆₀/10⁷ cells for control cells), but in combination with 4 × 10⁻⁷ mol/L tretinoin tocoferil, it induced activity of 4.7 ± 0.5 A₅₆₀/10⁷ cells (P < .0005; Fig 6C). Thus VD₃ and tretinoin tocoferil in combination showed synergistic effects in inhibiting proliferation and inducing differentiation of U937 cells.

Effects of VD₃ plus tretinoin tocoferil on proliferation and differentiation of several myelomonocytic leukemia cells. As shown in Fig 7, VD₃ induced NBT-reducing activity in human myelomonoblastic ML-1, monocytic THP-1, P39/TSU, and P3I/FSU cells concentration-dependently, and tretinoin tocoferil enhanced these activities: at 9 × 10⁻⁷ mol/L, it increased the activities of ML-1, THP-1, P39/TSU, and...
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Fig 5. Effects of the combination of tretinoin tocoferil with DMSO (A), TPA (B), or VD3 (C) on NBT-reducing and lysozyme activities of HL-60 cells. Cells (2 x 10^6 cells/mL) were treated with DMSO, TPA, or VD3 in the absence (○) or presence of 3 x 10^-7 mol/L tretinoin tocoferil (△) for 4 days. The final concentrations of liquid paraffin and ethanol in the medium with tretinoin tocoferil were 0.00006% and 0.1%, respectively. The values of NBT reduction of the cells treated with the solvents (0.00006% liquid paraffin plus 0.1% ethanol) in combination with 1.3% DMSO, 0.32 mol/L TPA, and 3 x 10^-7 mol/L VD3 were 4.2 ± 0.3 versus 4.4 ± 0.3 AOD/10^7 cells for cells without the solvents, 3.8 ± 0.4 versus 3.4 ± 0.1 AOD/10^7 cells, respectively. The solvents did not effectively increase the lysozyme activities in the cells treated with TPA or VD3 (data not shown). These findings indicated that the effect of tretinoin tocoferil was not due to either liquid paraffin or ethanol. Values are the mean ± SD for three separate experiments.

P31/FUJ cells induced by 3 x 10^-8 mol/L VD3 alone 1.9-fold (P < .0001), 2.1-fold (P < .0005), 1.7-fold (P < .002), and 1.3-fold (P < .01), respectively (Fig 7). Lysozyme activity was induced in P39/TSU and P31/FUJ cells by 3 x 10^-4 mol/L VD3 and tretinoin tocoferil at 9 x 10^-6 mol/L slightly increased these activities (data not shown). Next, we examined the proliferations of these myelomonocytic leukemia cells on treatment with VD3 plus tretinoin tocoferil for 6 days (Table 3). The IC50 concentrations of VD3 for the myelomonocytic leukemia cells were decreased by combined treatment with VD3 and tretinoin tocoferil at 9 x 10^-6 mol/L slightly increased these activities (data not shown). Next, we examined indicated synergism between VD3 and tretinoin tocoferil in inhibiting proliferation. Addition of 9 x 10^-5 mol/L tretinoin tocoferil decreased the IC50 concentration of VD3 for inhibiting proliferation to 10^-10 mol/L level in all the cells examined. Thus, tretinoin tocoferil and VD3 enhanced differentiation and inhibited the proliferation of human myelomonocytic cells synergistically.

DISCUSSION

Tretinoin tocoferil is an α-tocopherol ester of RA, but its activity was different from those of RA and α-tocopherol. Tretinoin tocoferil at 1 x 10^-8 mol/L and 1 x 10^-7 mol/L enhanced migration of guinea pig peritoneal macrophage 1.4-fold and 1.8-fold, respectively, but RA or α-tocopherol at the same concentrations did not. Because colchicine inhibited the induction of migration, tretinoin tocoferil was suggested to stimulate chemotaxis of macrophage. Growth of human skin fibroblasts was enhanced 77% by treatment with 1 x 10^-8 mol/L tretinoin tocoferil, but inhibited 61% by 5 x 10^-10 mol/L RA, and not affected by 5 x 10^-10 mol/L α-tocopherol. Tretinoin tocoferil enhanced the differenti-
The pharmacologic study in patients with acute promyelocytic leukemia showed that the peak plasma concentration of RA following a single oral dose of RA at 45 mg/m² was 346 ± 266 ng/mL (1.15 × 10⁻⁹ mol/L), but the plasma concentrations declined to the physiologic level within 12 hours. The short half-life of RA requires repeated administrations for achievement of effective concentrations. The continued administration of RA, however, increases catalytic enzyme activity and cellular binding protein such as CRABPII, and the resulting decrease of the plasma concentrations contributes to clinical resistance to RA. On the other hand, when rats were treated with nontoxic 5 mg/kg tretinoin tocoferil intravenously and orally, its plasma concentrations were 10⁻⁷ mol/L and 10⁻⁹ mol/L, respectively, for 24 hours and 10⁻⁸ mol/L and 10⁻⁹ mol/L, respectively, even 7 days after administrations. These concentrations of tretinoin tocoferil were effective for inhibition of growth and induction of differentiation of myelomonocytic leukemia cells in combination with other inducers such as LD₅₀. Thus, addition of α-tocopheryl group to RA may contribute to the maintenance of its plasma concentrations in effective range.

### Table 3. Effects of Combination Treatment With Tretinoin Tocoferil and VD₃ on Proliferation of Human Myelomonocytic Leukemia Cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC₅₀ of TT (mol/L)</th>
<th>IC₅₀ of VD₃ (mol/L)</th>
<th>CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U937</td>
<td>1.05 × 10⁻⁶</td>
<td>2.29 × 10⁻⁸</td>
<td>0.27</td>
</tr>
<tr>
<td>HL-60</td>
<td>6.36 × 10⁻⁶</td>
<td>3.35 × 10⁻⁹</td>
<td>0.17</td>
</tr>
<tr>
<td>ML-1</td>
<td>6.61 × 10⁻⁷</td>
<td>5.48 × 10⁻¹⁰</td>
<td>0.58</td>
</tr>
<tr>
<td>THP-1</td>
<td>9.22 × 10⁻⁷</td>
<td>3.38 × 10⁻⁶</td>
<td>0.35</td>
</tr>
<tr>
<td>P38/TSU</td>
<td>1.17 × 10⁻⁰</td>
<td>2.36 × 10⁻⁴</td>
<td>0.38</td>
</tr>
<tr>
<td>P31/FUJ</td>
<td>1.44 × 10⁻⁰</td>
<td>1.48 × 10⁻⁴</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* Combination index at IC₅₀. CI values with a fixed concentration of TT (9 × 10⁻⁶ mol/L) were calculated as described in Materials and Methods. In the assay, CI = 1 indicates summation (additive or zero interaction), CI < 1 synergism, and CI > 1 antagonism.
VD₃ is a promising inducer for differentiation. Although VD₃ is reported to induce differentiation of cell lines of leukemia, colon, and breast cancer, its adverse effects, mainly hypercalcemia, limit its clinical use in cancer treatment when its serum concentration exceeds 10⁻⁹ mol/L. Several reports showed RA acted synergistically with VD₃ to induce differentiation of leukemia cells. Similar to VD₃, however, RA was reported to cause hypercalcemia in the treatment of acute promyelocytic leukemia. Hypercalcemia was also reported in clinical trials of another active
Fig 7. Effects of combination of tretinoin tocoferil with VD₃ on NBT-reducing activity of ML-1 (A), THP-1 (B), P39/TSU (C), and P31/FUJ cells (D). Cells (2 × 10⁶ cells/mL) were treated with VD₃ and 0 (●), 9 × 10⁻⁹ (○), or 9 × 10⁻⁸ mol/L tretinoin tocoferil (■) for 4 days. Values are the mean ± SD for three separate experiments.
retinoid, 13-cis retinoic acid. Retinoids are known to induce skeletal abnormalities in hypervitaminosis A syndrome. Therefore, the combination of a strong retinoid with VD₃ still has a risk of inducing hypercalcemia. On the contrary, tretinoin tocoferil in the long-term administration in rats did not induce hypercalcemia or any pathological changes of bone, and its clinical toxicity in bone metabolism has not been reported. Thus, tretinoin tocoferil did not induce retinoid-related toxicity and teratogenicity. Therefore, as tretinoin tocoferil at the 10⁻⁸ mol/L level and VD₃ at the 10⁻¹⁰ mol/L level synergistically inhibited proliferation of human myelomonocytic leukemia cells, the combined treatment with VD₃ and tretinoin tocoferil may be useful in therapy of myelomonocytic leukemia.

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Enhancement of activity of 1alpha, 25-dihydroxyvitamin D3 for growth inhibition and differentiation induction of human myelomonocytic leukemia cells by tretinoin tocoferil, an alpha-tocopherol ester of all-trans retinoic acid

M Makishima, Y Kanatani, Y Yamamoto-Yamaguchi and Y Honma