Growth inhibition and differentiation induction in human monoblastic leukaemia cells by \(1\alpha\)-hydroxyvitamin D derivatives and their enhancement by combination with hydroxyurea

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Summary The active form of vitamin D, \(1\alpha,25\)-dihydroxyvitamin \(D_3\) \((1,25(OH)_2D_3)\), is a potent inducer of differentiation in myeloid leukaemia cells, but its clinical use is limited because of its hypercalcaemic activity. We examined the ability of \(1,25(OH)_2D_3\) in combination with several anti-cancer drugs to inhibit the proliferation of, and induce differentiation in, human monoblastic leukaemia U937 cells. Hydroxyurea (HU), cytarabine and camptothecin showed effective synergism with \(1,25(OH)_2D_3\) with regard to growth inhibition, while daunorubicin and etoposide had only modest synergistic effects. HU and cytarabine effectively enhanced nitroblue tetrazolium-reducing activity induced by \(1,25(OH)_2D_3\). HU also enhanced the morphological maturation and expression of CD11b and CD14 in cells treated with \(1,25(OH)_2D_3\). Among the anti-cancer drugs examined, HU had the greatest synergistic effects with \(1,25(OH)_2D_3\) with regard to growth inhibition and differentiation induction in U937 cells. HU also enhanced the differentiation of other myeloid leukaemia cell lines: HL-60, ML-1, THP-1, P39/TSU, P31/FUJ and NB4 cells induced by \(1,25(OH)_2D_3\) and that of U937 cells induced by 24-epi-1,25(OH)_2D_3 and 1,25(OH)_2D_3. Interestingly, \(1\alpha(OH)D_3\) derivatives (1\(\alpha\)-hydroxyvitamin \(D_3\), \(D_2\), \(D_1\) and \(D_0\)) effectively induced the differentiation of monoblastic leukaemia U937, P39/TSU and P31/FUJ cells. HU also enhanced the growth inhibition and differentiation of U937 cells induced by \(1\alpha(OH)D_3\) derivatives. As \(1\alpha(OH)D_3\) derivatives preferentially act on monocytic cells, they may be useful in the treatment of acute monocytic leukaemia, both alone and in combination with HU.

Keywords: leukaemia; vitamin \(D_3\); vitamin \(D_2\); vitamin \(D_1\); vitamin \(D_0\); hydroxyurea

The prognosis of acute myeloid leukaemia has recently improved through the application of intensive chemotherapy and bone marrow transplantation. However, intensive chemotherapy is not used in elderly patients or in patients with hypoplastic leukaemia or myelodysplastic syndrome because of severe complications. The incidence of induction death among elderly patients in their initial induction therapy, even when supported with cytokines, is higher than 10% (Schiffer, 1996). Indications for bone marrow transplantation are limited to young patients with HLA-matched donors (Goldman, 1994). Differentiation therapy is one possible approach for surviving patients who cannot be treated with intensive chemotherapy or bone marrow transplantation.

Differentiation therapy has been successfully used to treat acute promyelocytic leukaemia (Degos et al., 1995). All-trans retinoic acid induces complete remission in more than 90% of patients with acute promyelocytic leukaemia with a t \((15;17)\) chromosomal translocation. However, the use of all-trans retinoic acid is limited to acute promyelocytic leukaemia. Vitamin D is another potential inducer for differentiation therapy. The active form of vitamin D, 1\(\alpha,25\)-dihydroxyvitamin \(D_3\) \((1,25(OH)_2D_3)\), induces differentiation in mouse and in human leukaemia cells ( Abe et al., 1981; Miyaura et al., 1981) and prolongs the survival of mice inoculated with myeloid leukaemia cells (Honma et al., 1983). However, clinical trials of \(1,25(OH)_2D_3\) in patients with myelodysplastic syndrome have not been successful because of hypercalcaemia (Koeffler et al., 1985). Several analogues of \(1,25(OH)_2D_3\) that show anti-cancer activity and only weak activity for inducing hypercalcaemia have been developed, but they are not yet available for the clinical treatment of cancer or leukaemia ( Abe et al., 1991; Pakkala et al., 1995). To overcome the adverse effects of vitamin D, we are investigating the effects of the combination of \(1,25(OH)_2D_3\) with other drugs (Makishima and Honma, 1996; Makishima et al., 1996). As the combination of all-trans retinoic acid with low doses of anti-cancer drugs produced better results than either drug alone for the treatment of acute myeloid leukaemia (Venditti et al., 1995), in this study, we investigated the effects of the combination of \(1,25(OH)_2D_3\) and its analogues with various anti-cancer drugs on growth inhibition and differentiation induction in myelomonocytic leukaemia cells.

MATERIALS AND METHODS

Materials

1\(\alpha,25\)-Dihydroxyvitamin \(D_3\) \((1,25(OH)_2D_3)\), 24-epi-1\(\alpha,25\)-dihydroxyvitamin \(D_3\) \((24\text{-}epi-1,25(OH)_2D_3)\), \(1\alpha\)-hydroxyvitamin \(D_1\) \((1\alpha(OH)D_1)\), \(1\alpha(OH)D_2\), \(1\alpha(OH)D_3\) and \(1\alpha(OH)D_4\) were synthesized (Figure 1) (Tachibana and Tsuji, 1992) and donated by the Fine Chemical Research Center, Nissin Flour Milling (Saitama, Japan). Chlorambucil, daunorubicin, actinomycin D, hydroxyurea
(HU), cytarabine (Ara-C) and camptothecin were purchased from Sigma (St Louis, MO, USA), and 1,25(OH)\textsubscript{2}D\textsubscript{3} was from Wako Pure Chemical Industry (Osaka, Japan). Etoposide was obtained from Nippon Kayaku (Tokyo, Japan).

**Cell lines and cell culture**

Human myeloid leukaemia U937, HL-60, ML-1, THP-1, P31/FUJ and NB4 cells (Lanotte et al, 1991) were cultured in suspension in RPMI 1640 medium containing 10% fetal bovine serum and 80 μg ml\textsuperscript{-1} gentamicin at 37°C in a humidified atmosphere of 5% carbon dioxide in air (Makishima et al, 1996).

**Cell growth and differentiation**

Suspensions of cells were cultured with or without the test compounds in multidishes. The cells were counted in a Model ZM Coulter Counter (Coulter Electronics, Luton, UK). Nitroblue tetrazolium (NBT) reduction was assayed colorimetrically (Makishima et al, 1996). Lysozyme activity in the conditioned medium was determined using a lysoplate (Makishima et al, 1996). One unit is equivalent to 1 μg ml\textsuperscript{-1} egg-white lysozyme. Cell morphology was examined in cell smears stained with May-Grünwald and Giemsa solutions (Merck, Darmstadt, Germany).

**Analysis of the effects of combinations of drugs**

The interaction of the two compounds was quantified by determining the combination index (CI) according to the classic isobologram equation:

$$CI = D_{2}/D_{1} + D_{2}/D_{3},$$

where $D_{x}$ is the concentration of one drug alone required to produce an effect and $D_{1}$ and $D_{2}$ are the doses of compounds 1 and 2, respectively, in combination that produce the same effect (Berenaum, 1989). Using this analysis, the combined effects of the two drugs can be assessed as being either additive (CI = 1), synergistic (CI < 1) or antagonistic (CI > 1). An isobologram was also used to determine the effect of combinations of drugs (Berenaum, 1989). Concentration-dependent effects were determined from isoeffective concentrations for each compound and for one compound with fixed concentrations of another. The additive lines were indicated as calculated by mode I and mode II systems (Steel and Peckham, 1979).

**Flow cytometry**

Expression of the granulocyte- and monocyte-specific antigens CD11b and CD14 on the cell surface was determined using indirect immunofluorescent staining and flow cytometry (Makishima and Honma, 1996). Mouse monoclonal antibodies to CD11b (2LPM19c), CD14 (TUK4), control mouse IgG1, IgG2a and FITC-conjugated F(ab\textsuperscript{\textprime})\textsubscript{2} fragment of goat antimouse IgG were obtained from Dako (Glostrup, Denmark). The stained cells were assayed using a flow cytometer (Epic XL; Coulter Electronics).

**Table 1 Effects of the combination of anticancer drugs and 1,25(OH)\textsubscript{2}D\textsubscript{3} on growth inhibition and NBT-reducing activity of human monoblastic leukaemia U937 cells**

| Drugs       | IC\textsubscript{50} for growth suppression | CI\textsuperscript{a} | NBT reduction\textsuperscript{b} (A\textsubscript{570} per 10\textsuperscript{\textprime} cells) | Ratio\textsuperscript{c} |
|-------------|------------------------------------------|------------------------|-----------------------------------------------------------------------------------------------|--------------------------|
|             | –VD\textsubscript{3}  | +VD\textsubscript{3}  |                                                                                               |                          |
| Chlorambucil| 5.19 μM          | 3.90 μM          | 0.90                                                                                         | 1.15 ± 0.13  | 2.14 ± 0.27  | 1.9          |
| Daunorubicin| 2.73 nm          | 1.53 nm          | 0.71                                                                                         | 0.93 ± 0.07  | 2.17 ± 0.14  | 2.3          |
| Actinomycin D| 88.1 pm         | 76.7 pm         | 1.02                                                                                         | 0.85 ± 0.07  | 2.06 ± 0.06  | 3.2          |
| HU          | 50.7 μm          | 15.8 μm         | 0.46                                                                                         | 1.26 ± 0.25  | 7.78 ± 0.34* | 6.2          |
| Ara-C       | 6.86 nm          | 2.41 nm         | 0.50                                                                                         | 1.02 ± 0.14  | 3.56 ± 0.46  | 3.5          |
| Camptothecin| 9.84 nm          | 4.14 nm         | 0.57                                                                                         | 1.84 ± 0.16  | 3.70 ± 0.38  | 2.0          |
| Etoposide   | 38.3 nm          | 25.3 nm         | 0.81                                                                                         | 1.20 ± 0.03  | 2.82 ± 0.10  | 2.4          |
| None        | 0.54 ± 0.04      | 1.42 ± 0.09     |                                                                                              |                          |

Cells (5 × 10\textsuperscript{4} cells ml\textsuperscript{-1}) were treated with anti-cancer drugs in the absence or presence of 1,25(OH)\textsubscript{2}D\textsubscript{3} (VD\textsubscript{3}) for 4 days. IC\textsubscript{50} values were determined from the means of triplicate data and values of NBT reduction represent the means ± SD of three separate experiments. IC\textsubscript{50} values for anti-cancer drugs in the presence of 3 × 10\textsuperscript{-8} M 1,25(OH)\textsubscript{2}D\textsubscript{3}. *Combination index (CI) at IC\textsubscript{50} for growth inhibition. CI values at a fixed concentration of 1,25(OH)\textsubscript{2}D\textsubscript{3} (3 × 10\textsuperscript{-8} M) were calculated as described in the Materials and methods. IC\textsubscript{50} of 1,25(OH)\textsubscript{2}D\textsubscript{3} was 2.01 × 10\textsuperscript{-4} M. In this assay, CI > 1 indicates an additive effect, CI < 1 indicates antagonism. NBT reduction in the cells treated with anti-cancer drugs at their IC\textsubscript{50} for growth inhibition in the absence or presence of 3 × 10\textsuperscript{-8} M 1,25(OH)\textsubscript{2}D\textsubscript{3}. 1,25(OH)\textsubscript{2}D\textsubscript{3} at the IC\textsubscript{50} induced the activity to 4.64 A\textsubscript{570} *Ratio of the NBT reduction: +1,25(OH)\textsubscript{2}D\textsubscript{3} / −1,25(OH)\textsubscript{2}D\textsubscript{3}. *P<0.0005 compared with other anti-cancer drugs plus 1,25(OH)\textsubscript{2}D\textsubscript{3}.
and the mean fluorescence intensity of fluorescence-positive cells was calculated using the Immuno-4 histogram analysis program (Coulter), with mouse immunoglobulin of the same isotype as a negative control. The Immuno-4 program subtracts a control histogram from a test histogram to calculate the mean fluorescence intensity in the test histogram (Overton, 1988).

Statistical evaluation

Statistical analyses were performed using an unpaired two-tailed Student’s t-test.

RESULTS

Effects of the combination of anti-cancer drugs with 1,25(OH)$_2$D$_3$ on the growth and differentiation of human monoblastic leukaemia U937 cells

We examined several anti-cancer drugs in combination with 1,25(OH)$_2$D$_3$ to determine the effects on growth inhibition in human monoblastic leukaemia U937 cells. Chlorambucil is an alkylating agent; daunorubicin and actinomycin D are antibiotics; HU and Ara-C are inhibitors of nucleotide metabolism; and camptothecin and etoposide are inhibitors of topoisomerases. These drugs all inhibited the proliferation of U937 cells concentration-dependently; their IC$_{50}$ values are indicated in Table 1. The effects of the combination of anti-cancer drugs and 1,25(OH)$_2$D$_3$ were determined using the CI calculated from the IC$_{50}$ values of anti-cancer drugs in the presence of 3 x 10$^{-4}$ M 1,25(OH)$_2$D$_3$. HU inhibited the proliferation of U937 cells at an IC$_{50}$ of 50.7 µM in the absence of 1,25(OH)$_2$D$_3$ and at an IC$_{50}$ of 15.8 µM in its presence (CI = 0.46, indicating synergism). The confidence intervals (CIs) for Ara-C, camptothecin, daunorubicin and etoposide were 0.50, 0.57, 0.71 and 0.81, respectively, also indicating synergism. The combinations of chlorambucil and actinomycin D with 1,25(OH)$_2$D$_3$ were additive.

We examined the effects of anti-cancer drugs in combination with 1,25(OH)$_2$D$_3$ on NBT-reducing activity, a typical marker of myelomonocytic differentiation, in U937 cells. The anti-cancer drugs showed only weak activity for inducing NBT reduction (Table 1). Next, the NBT-reducing activity induced by anti-cancer drugs in combination with 3 x 10$^{-4}$ M 1,25(OH)$_2$D$_3$ was examined. HU plus 1,25(OH)$_2$D$_3$ effectively increased the activity 6.2-fold from HU alone and 5.5-fold from 1,25(OH)$_2$D$_3$ alone (Table 1). Camptothecin and Ara-C in combination with 1,25(OH)$_2$D$_3$ modestly induced this activity. Among the anti-cancer drugs we examined, HU had the greatest synergistic effect with 1,25(OH)$_2$D$_3$ for growth inhibition and induction of NBT-reducing activity in U937 cells.

Effects of HU plus 1,25(OH)$_2$D$_3$ on growth inhibition and differentiation induction in human myelomonocytic leukaemia cells

The concentration-dependent effects of the combination of HU with 1,25(OH)$_2$D$_3$ on U937 cells were examined. Isoboles for growth inhibition show that their combination is synergistic and the presence of HU markedly reduced effective concentrations of 1,25(OH)$_2$D$_3$ (Figure 2A). HU up to 75 µM induced NBT-reducing activity of U937 cells only slightly (Figure 2B). While 1,25(OH)$_2$D$_3$ at 3 x 10$^{-10}$ M did not induce NBT-reducing activity of U937 cells, in the presence of 75 µM HU, it effectively induced this activity to 5.96 A$_{590}$ which is similar to the value (5.90 A$_{590}$) with a 100-fold greater concentration (3 x 10$^{-8}$ M) of 1,25(OH)$_2$D$_3$ (Figure 2B). Morphologically, monoblastic U937 cells were induced to differentiate into monocytic cells by 1,25(OH)$_2$D$_3$ and became more mature, having abundant and grey cytoplasm and a chromatin-condensed nucleus with the addition of HU (data not shown). HU also effectively enhanced the expression of CD11b in U937 cells induced by low concentrations of 1,25(OH)$_2$D$_3$ (Figure
(C), HU at 75 μM plus 1,25(OH)₂D₃ at 3 x 10⁻⁹ M increased this intensity to 8.00 units, which is greater than 6.41 units with 3 x 10⁻⁶ M 1,25(OH)₂D₃ alone. The enhancing effect of HU on CD14 expression induced by 1,25(OH)₂D₃ in U937 cells was weak (data not shown). Thus, HU effectively enhanced several differentiation markers in U937 cells treated with 1,25(OH)₂D₃.

Next, we examined the combination of HU and 1,25(OH)₂D₃ on the differentiation of human myeloid leukaemia HL-60, ML-1, THP-1, P39/TSU, P31/FUJ and NB4 cells. Cells (5 x 10⁶ cells ml⁻¹) were cultured with HU in combination with 0 (○), 3 x 10⁻⁹ M (▲) or 3 x 10⁻⁶ M (■) 1,25(OH)₂D₃ for 4 days. Values represent the means ± SD of three separate experiments.

**Effects of HU in combination with vitamin D derivatives on growth inhibition and differentiation induction in U937 cells**

24-Epi-1,25(OH)₂D₃ and 1,25(OH)₂D₃ have been reported to exhibit less hypercalcaemic activity than 1,25(OH)₂D₃ and to be able to induce the differentiation of HL-60 cells (Sato et al., 1991). They also induced the NBT-reducing activity of U937 cells, and HU effectively enhanced the activities induced by their suboptimal concentrations (Figure 4A). 24-Epi-1,25(OH)₂D₃ and 1,25-(OH)₂D₃ inhibited the proliferation of U937 cells concentration dependently, with IC₅₀ values of 2.25 x 10⁻² M and 2.28 x 10⁻² M respectively (data not shown). At a low concentration of 9 x 10⁻⁹ M, they slightly inhibited the proliferation of U937 cells and augmented the growth inhibition in combination with 50 μM HU (Table 2).

We have previously reported that 1α(OH)D₃ induces the differentiation of monoblastic leukaemia cells as well as 1,25(OH)₂D₃ and is less toxic than 1,25(OH)₂D₃ (Honma et al., 1983; Okabe-Kado et al., 1992). We examined the effects of several 1α(OH)D derivatives on
Table 2  Growth inhibition in human monoblastic leukaemia U937 cells by vitamin D derivatives in combination with HU

| Compounds                        | Growth (% of control) | Growth (%) of control | + HU Ratio* |
|----------------------------------|------------------------|------------------------|-------------|
| None                             | - HU 100               | + HU 54 ± 3)           | 100         |
| 1,25(OH)2D3 (3 × 10⁻⁶ M)         | 74 ± 1                 | 25 ± 3)                | 46          |
| 24-Epi-1,25(OH)2D3 (6 × 10⁻⁶ M)  | 87 ± 2                 | 33 ± 1)                | 61          |
| 1,25(OH)2D3 (9 × 10⁻⁶ M)         | 89 ± 2                 | 32 ± 1)                | 59          |
| 1α(OH)D3 (3 × 10⁻⁸ M)            | 72 ± 2                 | 27 ± 1)                | 50          |
| 1α(OH)D2 (6 × 10⁻⁸ M)            | 91 ± 2                 | 31 ± 1)                | 57          |
| 1α(OH)D1 (6 × 10⁻⁸ M)            | 89 ± 1                 | 28 ± 3)                | 52          |
| 1α(OH)D3 (6 × 10⁻⁸ M)            | 100 ± 4                | 27 ± 2)                | 50          |

Cells (5 × 10⁴ cells ml⁻¹) were cultured with vitamin D derivatives in the absence or presence of 50 μM HU for 4 days. *Ratio (%) represents the growth of cells in combination with HU compared with that of cells treated with 50 μM HU alone.

growth inhibition and differentiation induction in U937 cells in combination with HU. 1α(OH)D3 inhibited proliferation with an IC₅₀ value of 0.67 × 10⁻⁷ M and induced myelomonocytic differentiation markers, such as the NBT-reducing and lysozyme activities of U937 cells (Figure 4B, data not shown). HU effectively enhanced the NBT-reducing activity in U937 cells induced by 1α(OH)D3 (Figure 4B). For example, 1.2 × 10⁻⁴ M 1α(OH)D3 plus 50 μM HU induced this activity to 9.02 A₅₆₀, while this activity was 8.54 A₅₆₀ with 1.2 × 10⁻⁴ M 1α(OH)D3 alone, indicating that 1α(OH)D3 was more than 100 times as active in the presence of HU. 1α(OH)D3 at 3 × 10⁻⁸ M slightly inhibited the proliferation of U937 cells, but augmented the inhibition in combination with HU (Table 2). 1α(OH)D2, 1α(OH)D3, and 1α(OH)D7 also inhibited the proliferation with IC₅₀ values of 1.35 × 10⁻⁷, 1.47 × 10⁻⁷ and 3.25 × 10⁻⁷ M, respectively (data not shown), and induced the differentiation of monoblastic U937 (Figure 4C), P39/Tsu and P31/FUJ cells, but not of promyelocytic HL-60 cells (data not shown). Among these four 1α(OH)D derivatives, 1α(OH)D7 was the most effective in inhibiting the proliferation of U937 cells (P<0.005, compared at IC₅₀). At the IC₅₀ values for growth inhibition, 1α(OH)D7, 1α(OH)D3, 1α(OH)D2 and 1α(OH)D2, induced NBT-reducing activity of U937 cells from 0.83 A₅₆₀ to 5.80, 5.50, 6.08 and 5.84 A₅₆₀, respectively, and lysozyme activity from 1.99 units to 5.03, 5.11, 6.34 and 6.03 units, respectively (data not shown), indicating that 1α(OH)D7 was slightly more effective for inducing these activities than the others. The NBT-reducing activity induced by these 1α(OH)D derivatives in U937 cells was also effectively enhanced by HU (Figure 4C). HU enhanced the induction of NBT reduction by 1α(OH)D7 slightly more effectively than that by 1α(OH)D3 and 1α(OH)D2. At a concentration of 6 × 10⁻⁸ M, 1α(OH)D3 and 1α(OH)D2 inhibited the proliferation of U937 cells only slightly, while 1α(OH)D7 had no such effect, but they augmented the growth-inhibitory activity with HU (Table 2). Thus, the combination of HU with 1α(OH)D derivatives is effective for inhibiting the proliferation and inducing the differentiation of U937 cells.

**DISCUSSION**

Among the anti-cancer drugs we examined, HU showed the greatest synergistic effect with 1,25(OH)₂D₃ with regard to growth inhibition and differentiation induction in U937 cells. Ara-C and camptothecin showed modest synergism with regard to growth inhibition, and Ara-C enhanced the differentiation induced by 1,25(OH)₂D₃ second only to HU. In another study, treatment with

Figure 4  Effects of vitamin D derivatives in combination with HU with regard to the induction of NBT-reducing activity in human monoblastic leukaemia U937 cells. (A) NBT-reducing activities of cells treated with 24-epi-1,25(OH)₂D₃ (○) or 1,25(OH)₂D₃ (△) in the absence (○, △) or presence (●, ▲) of 50 μM HU. (B) NBT-reducing activities of cells treated with 1α(OH)D₃ (○, △), 1α(OH)D₃ (△) or 1α(OH)D₃ (●, ▲) in the absence (○, △, ▲) or presence (●, ▲) of 50 μM HU. (C) NBT-reducing activities of cells treated with 1α(OH)D₃ (○, ●), 1α(OH)D₃ (△, ▲) or 1α(OH)D₃ (●, ▲) in the absence (○, △, ▲) or presence (●, ▲) of 50 μM HU. Cells (5 × 10⁴ cells ml⁻¹) were cultured with the test compounds for 4 days. Values represent the means ± SD of three separate experiments.
1,25(OH)_{2}D_{3} increased the cytotoxicity of Ara-C and HU against HL-60 cells (Studzinski et al., 1986). HU inhibits nucleotide metabolism by inhibiting ribonucleotide reductase and Ara-C also inhibits nucleotide synthesis (Calabresi and Chadner, 1996). HU enhances the differentiation of HL-60 cells induced by all-trans retinoic acid (Yen et al., 1987). Ara-C induces the differentiation of some myeloid leukaemia cells and low doses of Ara-C have been used to treat acute myeloid leukaemia (Houssset et al., 1982). Other inhibitors of nucleotide metabolism also induce the differentiation of myeloid leukaemia cells (Bodner et al., 1981; Ishiguro and Sartorelli, 1985). These findings indicate that some inhibitors of nucleotide metabolism may induce leukaemia cells to differentiate and to enhance differentiation induced by other compounds more effectively than other types of anti-cancer drugs.

HU is useful for treating chronic myelogenous leukaemia and, when administered orally at daily doses from 500 to 3000 mg, for controlling blood cell counts within desirable ranges (Athens, 1993). Its major adverse effect is bone marrow suppression, but the bone marrow recovers promptly if the drug is discontinued for a few days. Thus, HU can be used safely in elderly patients. Pharmacokinetic studies have shown that serum concentrations of HU after a single oral administration of 1000 mg reach 20–30 μg ml^{-1} (263–394 μM) in 1–3 h, then gradually decrease and remain higher than 5 μg ml^{-1} (66 μM) for at least 10 h (Davidson and Winter, 1963; Bolton et al., 1965). These findings indicate that the concentrations of HU needed to enhance the anti-leukaemic activity of vitamin D derivatives can be achieved clinically.

In this study, we observed the differentiation-inducing activities of 1α(OH)D_{3} derivatives. After the administration of 1α(OH)D_{3}, it is converted to an active form, 1,25(OH)_{2}D_{3}, by liver 25-hydroxylase (Holick et al., 1975). 1α(OH)D_{3} was more potent than 1,25(OH)_{2}D_{3} in increasing the survival time of mice inoculated with mouse myeloid leukaemia M1 cells (Honma et al., 1983). The relatively stable concentrations of 1,25(OH)_{2}D_{3} after the administration of 1α(OH)D_{3} compared with 1,25(OH)_{2}D_{3} may contribute to the advantage offered by 1α(OH)D_{3}. Interestingly, 1α(OH)D_{3} induced the differentiation of monoblastic leukaemia cells in vitro and was converted to 1,25(OH)_{2}D_{3} in the cells (Okabe-Kado et al., 1992). As monocytes also have 24-hydroxylase activity (Kamimura et al., 1995) and 1α, 24-dihydroxyvitamin D_{3} can induce the differentiation of HL-60 cells to a similar extent as 1,25(OH)_{2}D_{3} (Tanaka et al., 1982), 1α(OH)D_{3} may act by being converted to 1,25(OH)_{2}D_{3} and 1α, 24-dihydroxyvitamin D_{3} in monocytic cells. Thus, 1α(OH)D_{3} affects leukaemia cells with monocytic characteristics both directly and indirectly. Acute monocytic leukaemia is more resistant to intensive chemotherapy than other types of acute myeloid leukaemia and its prognosis is poor (Fenaux et al., 1990). The use of 1α(OH)D_{3} derivatives may offer certain advantages in the treatment of monocytic leukaemia, as (a) 1,25(OH)_{2}D_{3} is physiologically catabolized to an inactive form in monocytic cells and such cells can activate 1α(OH)D_{3} and (b) drugs can be focused against leukaemia cells of monocytic lineage and their adverse effects against other organs can be diminished. Other 1α(OH)D_{3} derivatives, including 1α(OH)D_{2}, 1α(OH)D_{4} and 1α(OH)D_{5}, also induced the differentiation of myelomonocytic leukaemia cells. 1α(OH)D_{5} is 5–15 times less toxic than 1α(OH)D_{3} in rats (Sjöden et al., 1985). A clinical study in post-menopausal osteoporotic patients showed that 1α(OH)D_{3} at daily doses of less than 5.0 μg did not induce hypercalcaemia, whereas 1α(OH)D_{3} at daily doses above 1.0 μg had toxic effects (Gallagher et al., 1994). An active form of 1α(OH)D_{3}, 1,25(OH)_{2}D_{3}, has less hypercalcaemic activity (Sato et al., 1991). Therefore, the 1α(OH)D_{3} derivatives may be useful for treating monocytic leukaemia. Pharmacokinetics for serum concentrations of 1α(OH)D_{3} derivatives and their metabolites after administration should be further investigated. HU also effectively enhanced the differentiation induced by the 1α(OH)D_{3} derivatives. The combination of 1α(OH)D_{3} derivatives with HU may be a promising candidate for ‘chemo-differentiation therapy’ of acute monocytic leukaemia.

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