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1α,25-Dihydroxyvitamin D_3-26,23-lactone analogs antagonize differentiation of human leukemia cells (HL-60 cells) but not of human acute promyelocytic leukemia cells (NB4 cells)

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Abstract **We examined the effects of two novel 1α,25-dihydroxyvitamin D_3-26,23-lactone (1α,25-$(OH)_2D_3$-26,23-lactone) analogs on 1α,25$(OH)_2D_3$-induced differentiation of human leukemia HL-60 cells thought to be mediated by the genomic action of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) and of acute promyelocytic leukemia NB4 cells thought to be mediated by non-genomic actions of 1α,25-$(OH)_2D_3$. We found that the 1α,25-$(OH)_2D_3$-26,23-lactone analogs, (23*S*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9647) and (23*R*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9648), inhibited differentiation of HL-60 cells induced by 1α,25-$(OH)_2D_3$. However, 1β-hydroxyl diastereomers of these analogs, i.e. (23*S*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone (1β-TEI-9647) and (23*R*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone (1β-TEI-9648), did not inhibit differentiation of HL-60 cells caused by 1α,25-$(OH)_2D_3$. A separate study showed that the nuclear vitamin D receptor (VDR) binding affinities of the 1-hydroxyl diastereomers were about 200 and 90 times weaker than that of 1α-hydroxyl diastereomers, respectively. Moreover, none of these lactone analogs inhibited NB4 cell differentiation induced by 1α,25-$(OH)_2D_3$. In contrast, 1β,25-dihydroxyvitamin D_3 (1β,25-$(OH)_2D_3$) and 1β,24*R*-dihydroxyvitamin D_3 (1β,24*R*-$(OH)_2D_3$) inhibited NB4 cell differentiation but not HL-60 cell differentiation. Collectively, the results suggested that 1-hydroxyl lactone analogs, i.e. TEI-9647 and TEI-9648, are antagonists of 1α,25-$(OH)_2D_3$, specifically for the nuclear VDR-mediated genomic actions, but not for non-genomic actions.**

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Key words: 1α,25-Dihydroxyvitamin D_3; (23*S*,25*R*)-1α,25-Dihydroxyvitamin D_3-26,23-lactone analog; 1β,25-Dihydroxyvitamin D_3; Antagonist; HL-60 cell; NB4 cell

1. Introduction

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) regulates a wide variety of biological functions. It is widely accepted that the fundamental activities of 1α,25-$(OH)_2D_3$ are to stimulate intestinal calcium absorption and to increase bone calcium mobilization [1,2]. In recent years, many other new biological functions different from those mentioned above have been reported [3]. These include inhibition of cell proliferation and induction of cell differentiation [4], modulation of immunological responses [5], stimulation of insulin secretion [6,7] and neurobiological functions [8,9]. 1α,25-$(OH)_2D_3$ is believed to mediate biological responses as a consequence of its interaction with both a nuclear vitamin D receptor (VDR) to regulate gene transcription [10,11] and with a putative membrane receptor to generate rapid non-genomic effects [12], including opening voltage-gated calcium and chloride channels [13] and activation of mitogen-activated protein kinase [14].

A considerable number of vitamin D analogs has been synthesized and the use thereof has prompted the clarification of the mode of action of vitamin D and the finding of new therapeutically useful compounds [3]. To date, however, antagonists of vitamin D that oppose the VDR/vitamin D-responsive element (VDRE)-mediated genomic actions of 1α,25-$(OH)_2D_3$ have not been reported. Very recently, we found that the two novel 1α,25-dihydroxyvitamin D_3-26,23-lactone (1α,25-$(OH)_2D_3$-26,23-lactone) analogs, (23*S*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9647) and (23*R*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9648), inhibit human leukemia cell (HL-60) differentiation induced by 1α,25-$(OH)_2D_3$ [15]. These results strongly suggested that TEI-9647 and TEI-9648 might be antagonists of VDR/VDRE-mediated genomic actions of 1α,25-$(OH)_2D_3$, because HL-60 cell differentiation initiated by 1α,25-$(OH)_2D_3$ is believed to occur through a VDR/VDRE-mediated pathway [16]. On the other hand, it was not clear whether these two analogs could also antagonize 1α,25-$(OH)_2D_3$-mediated rapid non-genomic actions.

Bhatia et al. reported that 1α,25-$(OH)_2D_3$ and a 6-s-*cis*-locked analog (1α,25-7-dehydrocholesture), thought to be a selective activator of non-genomic actions, primed human acute promyelocytic leukemia cells (NB4 cells) for 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced monocytic differentiation [17,18]. In these reports, 1α,25-$(OH)_2D_3$ action was considered to be independent of 1α,25-$(OH)_2D_3$-VDR binding and mediated via a VDR/VDRE-independent pathway [17,18,19]. They also reported that 1β,25-$(OH)_2D_3$ could suppress 1α,25-$(OH)_2D_3$ action in NB4 cells. Norman et al.

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Abbreviations: 1α,25-$(OH)_2D_3$-26,23-lactone, (23*S*,25*R*)-1α,25-dihydroxyvitamin D_3-26,23-lactone; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; NB4 cells, human acute promyelocytic leukemia cells; HL-60 cells, human leukemia cells; TEI-9647, (23*S*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone; TEI-9648, (23*R*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone; 1β-TEI-9647, (23*S*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone; 1β-TEI-9648, (23*R*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone; 1β,25-$(OH)_2D_3$, 1β,25-dihydroxyvitamin D_3; 1β,24*R*-$(OH)_2D_3$, 1β,24*R*-dihydroxyvitamin D_3; VDR, nuclear receptor for 1α,25-$(OH)_2D_3$; VDRE, vitamin D-responsive element; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NBT, nitro blue tetrazolium; FBS, fetal bovine serum; PBS, phosphate-buffered saline

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reported that 1β,25-$(OH)_2D_3$ acted as an antagonist of vitamin D_3-mediated rapid non-genomic actions [20]. From these data, the NB4 cell differentiation system is regarded as a model for characterization of non-genomic actions or their signaling pathway(s) [17].

In this paper, we report the effect of the novel 1α,25-$(OH)_2D_3$-26,23-lactone analogs, TEI-9647 and TEI-9648, on 1α,25-$(OH)_2D_3$-mediated non-genomic actions using the NB4 cell differentiation system. These analogs were found to suppress 1α,25-$(OH)_2D_3$-mediated HL-60 cell differentiation but not NB4 cell differentiation. On the contrary, 1β,25-$(OH)_2D_3$ and 1β,24*R*-dihydroxyvitamin D_3 (1β,24*R*-$(OH)_2D_3$) suppressed NB4 cell differentiation caused by 1α,25-$(OH)_2D_3$. These results suggest that TEI-9647 and TEI-9648 might be the first antagonists specific for 1α,25-$(OH)_2D_3$-mediated genomic actions but not for non-genomic actions.

2. Materials and methods

2.1. Chemicals

1α,25-$(OH)_2D_3$, 1β,25-$(OH)_2D_3$, 1β,24*R*-$(OH)_2D_3$, TEI-9647, TEI-9648, (23*S*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone (1β-TEI-9647) and (23*R*)-25-dehydro-1β-hydroxyvitamin D_3-26,23-lactone (1β-TEI-9648) were synthesized in our laboratory as described previously [21,22]. The chemical structures of these compounds are shown in Fig. 1. TPA was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nitro blue tetrazolium (NBT) was purchased from Tokyo Kasei kogyo (Tokyo, Japan). [26,27-methyl-^{3}H]1α,25-$(OH)_2D_3$ (specific activity, 180 Ci/mmol) was purchased from Amersham International (Little Chalfont, Buckinghamshire, UK).

2.2. Cell and cell culture

HL-60 cells were obtained from the Japanese Cancer Research Resources Bank. NB4 cells were obtained from K.A. Meckinling-Gill (University of Guelph, Ont., Canada). Cells were passaged twice a week to maintain the exponential proliferating phase. RPMI 1640 (Gibco BRL, Life Technologies, Rockville, MO, USA) containing 10% heat-inactivated fetal bovine serum (FBS) (Bioserum, Lot No. 01307-01) was used as culture medium.

2.3. Binding affinity to VDR

A competitive receptor binding assay for 1α,25-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs was performed using chick intestinal VDR as described previously [23,24]. [26,27-Methyl-^{3}H]1α,25-$(OH)_2D_3$ (specific activity, 180 Ci/mmol) and various amounts of the 1α,25-$(OH)_2D_3$-26,23-lactone analogs to be tested were dissolved in 50 μl of absolute ethanol in 12×75 mm polypropylene tubes (Sarstedt, Nümbrecht, Germany). One ml of the chick intestinal cytosol receptor protein diluted to 0.2 mg protein/ml in phosphate buffer (25 mM KH_2PO_4, 0.1 M KCl, 1 mM dithiothreitol, pH 7.4) and 1 mg of gelatin were added to each tube in an ice bath. The assay tubes were incubated in a shaking water bath for 1 h at 25°C and then chilled in an ice bath. One ml of 40% (w/v) polyethylene glycol 6000 in distilled water was added to each tube, which was then mixed vigorously and centrifuged at 2260×*g* for 60 min at 4°C. After the supernatant was decanted, the bottom of the tube containing the pellet was cut off into a scintillation vial containing 10 ml of dioxane-based scintillation fluid consisting of 10% naphthalene and 0.5% Omnifluor (DuPont, Boston, MA, USA) in 1,4-dioxane. The radioactivity was measured in a Beckman liquid scintillation counter (Model LS6500) using an external standard.

2.4. Cell differentiation assay (NBT reducing activity)

NBT reducing activity was used as a cell differentiation marker. HL-60 cells and NB4 cells were cultured in RPMI 1640 medium supplemented with 10% FBS. Exponentially proliferating cells were collected, suspended in fresh medium, seeded in culture vessels and treated with various amounts of 1α,25-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs. Twenty-four well culture plates (Falcon, Becton Dickinson and Company, Franklin Lakes, NJ, USA) were used. In the HL-60 cell differentiation assay, cells were treated with the compounds for 96 h at 37°C in a humidified atmosphere of 5% CO_2/air without medium change. In the NB4 cell differentiation assay, cells were treated with 1α,25-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs for 8 h, then washed and re-suspended in media containing 3×10^{-10} M TPA for up to 72 h. In both cell lines, the cell concentration at seeding was adjusted to 2×10^4 cells/ml and the seeded volume was 1 ml/well. 1α,25-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs were dissolved in ethanol and added to the culture medium at 0.1% volume. The same amount of vehicle was added to the control culture. The NBT reduction assay was performed according to the method of S.J. Collins et al. [25]. Briefly, cells were collected and washed with phosphate-buffered saline (PBS). After washing, cells were suspended in serum-free medium and NBT/TPA

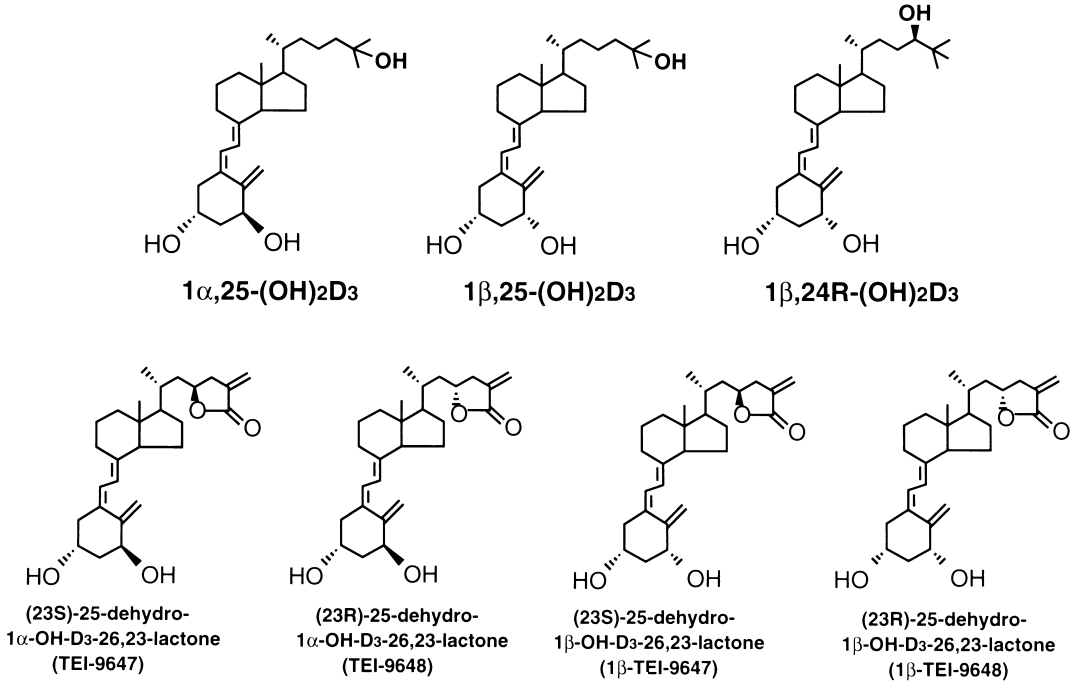

Fig. 1. Structures of 1β,25-$(OH)_2D_3$, 1β,24*R*-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs.

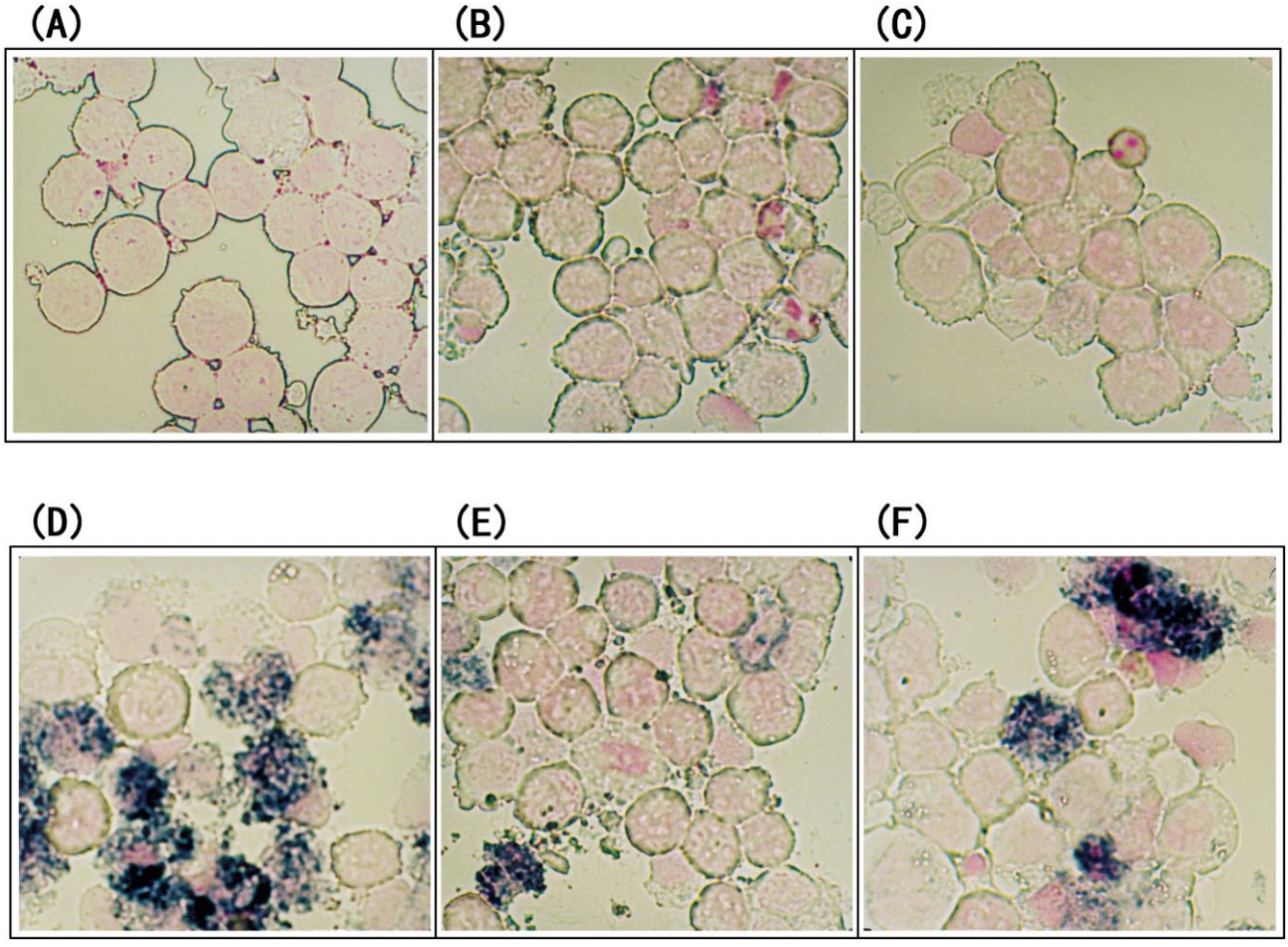

Fig. 2. NB4 cell differentiation induced by 1α,25-$(OH)_2D_3$ and TPA. NB4 cells were treated with 10^{-8} M 1α,25-$(OH)_2D_3$ or vehicle (ethanol 0.1% v/v) for 8 h, were then washed and re-suspended in media containing 3×10^{-10} M TPA or vehicle (ethanol 0.1% v/v) for up to 72 h. NBT reducing activity was examined as described in Section 2. (A) Vehicle control. (B) 1α,25-$(OH)_2D_3$ for 8 h followed by vehicle for up to 72 h. (C) Vehicle for 8 h followed by TPA for up to 72 h. (D) 1α,25-$(OH)_2D_3$ for 8 h followed by TPA for up to 72 h. (E) 10^{-6} M 1β,25-$(OH)_2D_3$ was added to media during 1α,25-$(OH)_2D_3$ treatment. (F) 10^{-6} M 1β,24*R*-$(OH)_2D_3$ was added to media during 1α,25-$(OH)_2D_3$ treatment.

solution (dissolved in PBS) was added. Final concentrations of NBT and TPA were 0.1% and 100 ng/ml, respectively. Then, the cell suspension was incubated at 37°C for 25 min. After incubation, cells were collected by centrifugation and re-suspended in PBS. Cytospin smears were prepared and the nuclei were counterstained with Kernechtrot solution. At least 500 cells per preparation were observed.

3. Results

Fig. 1 indicates the structure of 1α,25-$(OH)_2D_3$, 1β,25-$(OH)_2D_3$, 1β,24*R*-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs. TEI-9647 and TEI-9648 are both 25-dehydrated lactones of the (23*S*,25*R*)- and (23*R*,25*R*)-1α,25-$(OH)_2D_3$-26,23-lactone, respectively. Formally, TEI-9647 and TEI-9648 are 23-diastereoisomers of one another. 1β-TEI-9647 and 1β-TEI-9648 are 1β-hydroxyl diastereomers of TEI-9647 and TEI-9648, respectively.

The receptor binding of 1β,25$(OH)_2D_3$, 1β,24*R*-$(OH)_2D_3$ and 1α,25-$(OH)_2D_3$-26,23-lactone analogs is shown in Table 1. The chick intestinal VDR binding affinities of TEI-9647 and TEI-9648 were about 10 and 7%, respectively, as compared with 1α,25-$(OH)_2D_3$. In contrast, the VDR binding affinities of 1β-TEI-9647 and 1β-TEI-9648 were about 200 and 90 times weaker than their 1α-hydroxyl diastereomers. The binding affinities of 1β-25-$(OH)_2D_3$ and 1β,24*R*-$(OH)_2D_3$ were 0.3 and 0.4%, respectively, as compared with 1α-25-$(OH)_2D_3$.

Bhatia et al. showed that treatment with 1α,25-$(OH)_2D_3$ or TPA alone could not induce NB4 cell differentiation. However, combined treatment with both compounds induced NB4 cell differentiation [17,18]. Also, they reported that 1β,25-$(OH)_2D_3$ inhibited cell differentiation induced by 1α,25-$(OH)_2D_3$. In agreement with their results, we confirmed that treatment with 10^{-8} M 1α,25-$(OH)_2D_3$ or 10^{-10} M TPA alone could not induce cell differentiation. However, treatment with 10^{-8} M 1α,25-$(OH)_2D_3$ followed by 3×10^{-10} M TPA did induce differentiation (Fig. 2). In addition, we also confirmed that 10^{-6} M 1β,25-$(OH)_2D_3$ potently inhibited cell differentiation induced by 1α,25-$(OH)_2D_3$ (Fig. 2). Interestingly, NB4 cell differentiation caused by 1α,25-$(OH)_2D_3$ was also inhibited by the addition of the 24-hydroxyl analog of 1β,24*R*-$(OH)_2D_3$ (Fig. 2). Both 1β,25- and 1β,24*R*-$(OH)_2D_3$ showed similar dose-dependent response curves, but the inhibition effect of 1β,24*R*-$(OH)_2D_3$ was weaker than that of 1β,25-$(OH)_2D_3$ (Fig. 3A). On the contrary, HL-60 cell differentiation induced by 1α,25-$(OH)_2D_3$ was not inhibited by either 1β,25-$(OH)_2D_3$ or 1β,24*R*-$(OH)_2D_3$ (Fig. 3B).

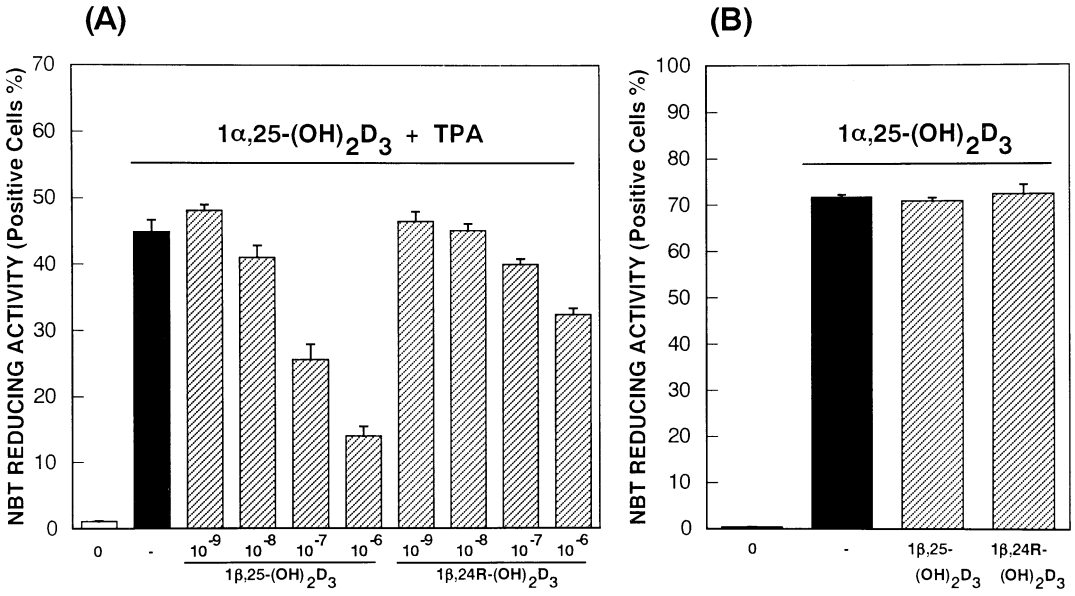

Fig. 3. Effects of 1β,25-$(OH)_2D_3$ and 1β,24*R*-$(OH)_2D_3$ on NB4 cell differentiation (A) or HL-60 cell differentiation (B). (A) NB4 cells were treated with 10^{-8} M 1α,25-$(OH)_2D_3$ in the absence (−) or presence (10^{-9}–10^{-6} M) of 1β,25-$(OH)_2D_3$ or 1β,24*R*-$(OH)_2D_3$ for 8 h and re-suspended in media containing 3×10^{-10} M TPA for up to 72 h and NBT reducing activity was examined. (B) HL-60 cells were treated with 10^{-8} M 1α,25-$(OH)_2D_3$ in the absence (−) or presence of 1β,25-$(OH)_2D_3$ or 1β,24*R*-$(OH)_2D_3$ (10^{-6} M) for 96 h and NBT reducing activity was examined. Vehicle control cultures (0) were treated with ethanol (added 0.1% v/v). Rectangles and bars show mean ± S.D. of triplicates, respectively.

Next, we examined the effect of lactone analogs on HL-60 or NB4 cell differentiation induced by 1α,25-$(OH)_2D_3$. Neither TEI-9647 nor TEI-9648 inhibited NB4 cell differentiation (Fig. 4A). 1β-TEI-9647 and 1β-TEI-9648, 1β-hydroxyl diastereomers of TEI-9647 and TEI-9648, respectively, also did not inhibit (Fig. 4A). We also examined agonistic effects of the four lactone analogs, but none of them induced NB4 cell differentiation (data not shown). Both TEI-9647 and TEI-9648 inhibited 1α,25-$(OH)_2D_3$-induced HL-60 cell differentiation dose-dependently (Fig. 4B). However, the 1β-hydroxyl diastereomers, 1β-TEI-9647 and 1β-TEI-9648, did not inhibit differentiation even after treatment with 10^{-6} M. The agonist effect of the four lactone analogs was also examined, but none of them induced HL-60 cell differentiation (data not shown).

4. Discussion

We recently reported that the novel lactone analogs, TEI-9647 and TEI-9648, could inhibit the differentiation of HL-60 cells induced by 1α,25-$(OH)_2D_3$ [15]. Because HL-60 cell differentiation is believed to be mediated through a VDR/VDRE-dependent pathway [16], we considered that TEI-9647 and TEI-9648 might be antagonists of VDR-mediated genomic actions of 1α,25-$(OH)_2D_3$. Ozono et al. have presented direct evidence that TEI-9647 could antagonize the transactivation function of VDR elicited by 1α,25-$(OH)_2D_3$ ([26], in preparation). However, it was not clear whether TEI-9647 and TEI-9648 also function as antagonists of 1α,25-$(OH)_2D_3$-mediated non-genomic actions.

The differentiation of NB4 cells is considered to be a good model for 1α,25-$(OH)_2D_3$-mediated non-genomic actions [17,18]. Therefore, we examined the effects of TEI-9647 and TEI-9648 on NB4 cell differentiation induced by 1α,25-$(OH)_2D_3$ to evaluate their antagonistic activity to non-genomic actions. As shown in Fig. 4A, neither analogs could inhibit NB4 cell differentiation. In this study, we also examined their antagonist activity for 1α,25$(OH)_2D_3$-mediated HL-60 cell differentiation and confirmed our previous results (Fig. 4B). Moreover, in agreement with a previous report [20], we found that 1β,25-$(OH)_2D_3$, the specific antagonist of 1α,25-

Table 1
Binding affinities of vitamin D_3 analogs to VDR in various cells

Vitamin D_3 analogs	Relative activity		
	Chick intestinal VDR	MG-63 cells VDR	HL-60 cells VDR
1α,25$(OH)_2D_3$	100.0	100.0	100.0
1β,25$(OH)_2D_3$	0.4	0.1	n.e.
1β,24*R*$(OH)_2D_3$	0.3	0.3	n.e.
TEI-9647	10.2	7.9	9.9
TEI-9648	7.2	6.0	8.5
1β-TEI-9647	0.05	0.03	n.e.
1β-TEI-9648	0.08	0.03	n.e.
TEI-9616	0.5	0.6	0.4
(23*S*,25*R*)-1α,25$(OH)_2D_3$-26,23-lactone	0.1	0.07	0.07

n.e.: not examined. The relative activity for each analog was calculated from their respective EC_{50} results and then normalized to the result obtained for 1α,25$(OH)_2D_3$, which was set to 100%.

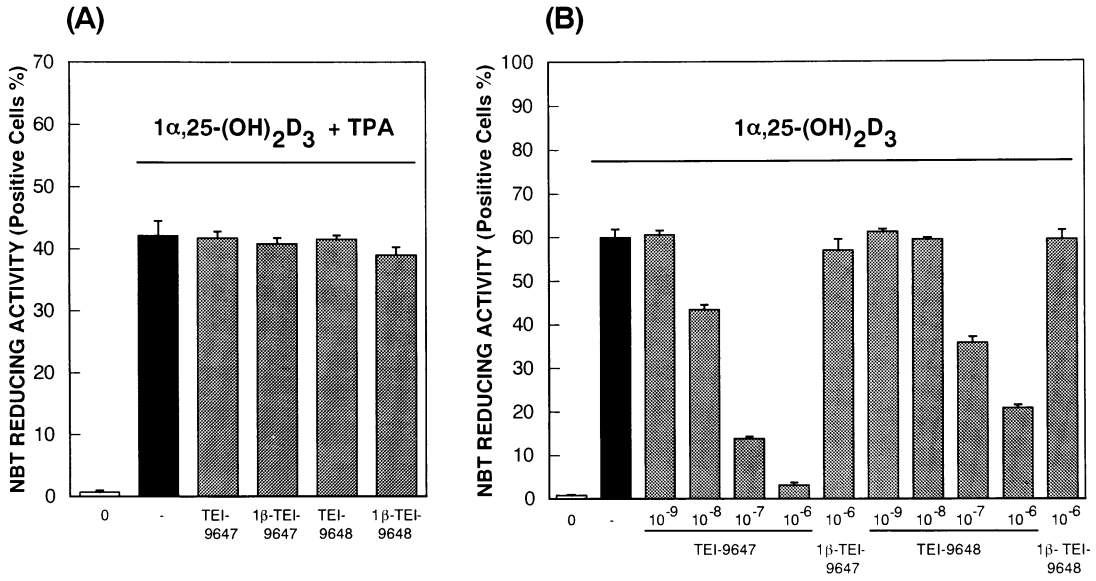

Fig. 4. Effects of 1α,25-$(OH)_2D_3$-26,23-lactone analogs (TEI-9647, TEI-9648, 1β-TEI-9647 and 1β-TEI-9648) on NB4 cell differentiation (A) or HL-60 cell differentiation (B). (A) NB4 cells were treated with 10^{-8} M 1α,25-$(OH)_2D_3$ in the absence (−) or presence of 1α,25-$(OH)_2D_3$-26,23-lactone analogs (10^{-6} M) for 8 h and re-suspended in media containing 3×10^{-10} M TPA for up to 72 h before NBT reducing activity was examined. (B) HL-60 cells were treated with 10^{-8} M 1α,25-$(OH)_2D_3$ in the absence (−) or presence (10^{-9}–10^{-6} M) of 1α,25-$(OH)_2D_3$-26,23-lactone analogs for 96 h and NBT reducing activity was examined. Vehicle control cultures (0) were treated with ethanol (added 0.1% v/v). Rectangles and bars show mean ± S.D. of triplicates, respectively.

$(OH)_2D_3$-mediated non-genomic actions, clearly inhibits NB4 cell differentiation (Fig. 3A). The results described above suggested that TEI-9647 and TEI-9648 are not antagonists of 1α,25-$(OH)_2D_3$-mediated non-genomic actions, but of VDR/VDRE-mediated genomic actions.

1β-TEI-9647 and 1β-TEI-9648, 1α-hydroxyl diastereomers of TEI-9647 and TEI-9648, respectively, did not antagonize HL-60 cell differentiation (Fig. 4B). We also found that inversion of the 1α-hydroxyl groups to the 1β-orientation resulted in a dramatic change in their receptor binding affinities (Table 1). Affinities of 1β-TEI-9647 and 1β-TEI-9647 for VDR were about 1/100–1/200 of TEI-9647 or TEI-9648. The detailed mechanism of the antagonistic action of the lactone analogs is not yet completely elucidated, but Norman et al. have suggested that TEI-9647 binding to the VDR would not elicit the conformational change of helix 12 required for transactivation [27]. Conformational change of helix 12 after ligand (1α,25-$(OH)_2D_3$) binding to VDR is perceived to be important for interaction with co-activators, such as steroid receptor co-activator-1, and elicitation of transactivation [27,28]. Collectively, these results suggest that receptor binding affinity may be important for the antagonistic effect of TEI-9647 and TEI-9648 on VDR/VDRE-mediated genomic action. Therefore, the fact that 1β-TEI-9647 and 1β-TEI-9648 could not inhibit HL-60 cell differentiation may be mainly due to their weak binding affinity to receptors.

Norman et al. synthesized various A-ring diastereomers of 1α,25-$(OH)_2D_3$ and reported that the orientation of carbon-1 and -3 hydroxyl groups would be important for genomic or non-genomic actions [20]. Interestingly, they reported that 1β,25-$(OH)_2D_3$ was a potent antagonist of 1α,25-$(OH)_2D_3$-mediated non-genomic responses. In this study, we found that 1β,24*R*-$(OH)_2D_3$ also showed antagonistic activity to non-genomic action (Fig. 3A) but not to genomic action (Fig. 3B). These results indicated that the β-orientation of the carbon-1 hydroxyl group may play an important role for antagonism of non-genomic actions. However, 1β-TEI-9647 and 1β-TEI-9648, which possess a 1β-hydroxyl group but have a side-chain structure different from 1β,25-$(OH)_2D_3$, did not show antagonistic activity to non-genomic actions (Fig. 4A). As yet, the specific factors or receptors which mediate the non-genomic actions of 1α,25-$(OH)_2D_3$ are not yet clear. However, these results suggest that both the orientation of carbon-1 and the side-chain structure may be important in antagonism of non-genomic actions and both would affect affinity for the putative receptors or factors of the non-genomic actions.

In conclusion, our data demonstrate that the novel lactone analogs, TEI-9647 and TEI-9648, may be antagonists of VDR/VDRE-mediated genomic actions but not of non-genomic actions. They are the first specific antagonists of 1α,25-$(OH)_2D_3$-mediated genomic action. Comparison of the antagonistic activity of 1β,25-$(OH)_2D_3$ and combination studies of these antagonists can be expected to advance our understanding of structure-function relationships in ligands and receptors interaction and function.

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