

Evaluation of C-2-substituted 19-nor-1 α ,25-dihydroxyvitamin D₃ analogs as therapeutic agents for prostate cancer

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Abstract

1 α ,25-Dihydroxyvitamin D₃ (1 α ,25(OH)₂D₃) is known to inhibit the proliferation and invasiveness of prostate cancer cells. However, 1 α ,25(OH)₂D₃ can cause hypercalcemia and is not suitable as a therapeutic agent. 19-Nor-vitamin D derivatives are known to be less calcemic when administered systemically. In order to develop more potent anti-cancer agents with less calcemic side effect, we therefore utilized ³H-thymidine incorporation as an index for cell proliferation and examined the antiproliferative activities of nine C-2-substituted 19-nor-1 α ,25(OH)₂D₃ analogs in the immortalized PZ-HPV-7 normal prostate cell line. Among the nine analogs we observed that the substitution with 2 α - or 2 β -hydroxypropyl group produced two analogs having antiproliferative potency that is approximately 500- to 1000-fold higher than 1 α ,25(OH)₂D₃. The ³H-thymidine incorporation data were supported by the cell counting data after cells were treated with 1 α ,25(OH)₂D₃, 19-nor-2 α -(3-hydroxypropyl)-1 α ,25(OH)₂D₃ or 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH)₂D₃ for 7 days. 19-Nor-2 α -(3-hydroxypropyl)-1 α ,25(OH)₂D₃ and 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH)₂D₃ were also shown to be about 10-fold more active than 1 α ,25(OH)₂D₃ in cell invasion studies using prostate cancer cells. In conclusion, a substitution at the C-2 position of 19-nor-1 α ,25(OH)₂D₃ molecule with a hydroxypropyl group greatly increased the antiproliferative and anti-invasion potencies. Thus, these two analogs could be developed to be effective therapeutic agents for treating early and late stages of prostate cancer.

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1. Introduction

Prostate cancer is the second leading cause of male cancer deaths in the US [1]. Approximately, 234,460 new cases of prostate cancer and 27,350 deaths are projected in 2006. For tumors that fail to respond to prostatectomy or radiation, androgen deprivation has been the mainline of prostate cancer therapy. Although 3/4 of men respond to androgen deprivation, the median duration of the response is only about 2 years. At the present time no effective therapies are available for prostate cancers that have become refractory to androgen deprivation. Besides androgens, prostate

cells are also responsive to the active form of vitamin D, 1 α ,25-dihydroxyvitamin D (1 α ,25(OH)₂D). Numerous studies have shown that 1 α ,25(OH)₂D₃ is a potent inducer of prostate cancer cell differentiation and an inhibitor of prostate cancer cell proliferation, invasiveness, and metastasis. These data strongly indicate that vitamin D-based therapies could be a second line of therapy when androgen deprivation has failed. However, the use of 1 α ,25(OH)₂D-based therapies is limited by the risk of hypercalcemia and hypercalciuria [2,3]. Thus, less- or non-calcemic analogues of 1 α ,25(OH)₂D₃ with potent antiproliferative activity would be attractive therapeutic agents [4]. One example is the 19-nor vitamin D analogs synthesized by DeLuca and colleagues [5]. Both 19-nor-1 α ,25(OH)₂D₂ and 19-nor-1 α ,25(OH)₂D₃ have potency similar to 1 α ,25(OH)₂D₃ in inducing 24-hydroxylase promoter activity in transcription assay, and in suppressing parathyroid hormone secretion in

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hemodialysis patients with secondary hyperparathyroidism, without inducing hypercalcemia or hyperphosphatemia [6]. Along this line, we examined the antiproliferative activity of 19-nor-1 α ,25(OH) $_2$ D $_2$ in LNCaP prostate cancer cells and in primary cultures of prostate cancer cells [7]. We observed that 19-nor-1 α ,25(OH) $_2$ D $_2$ had antiproliferative activity comparable to 1 α ,25(OH) $_2$ D $_3$, as determined by 3 H-thymidine incorporation and cell counting. These findings led to further modification of A-ring, including the synthesis of C-2 modified 19-nor vitamin D compounds by several groups [8,9]. In this report, we investigated the effect of a series of 19-nor-1 α ,25(OH) $_2$ D $_3$ analogs modified at C-2 position on the proliferation of prostate cells and on the invasiveness of prostate cancer cells.

2. Materials and methods

2.1. Vitamin D compounds

1 α ,25(OH) $_2$ D $_3$ was a generous gift from Dr. M. Uskokovic. C-2 substituted 19-nor-1 α ,25(OH) $_2$ D $_3$ analogs were synthesized in the laboratory of Dr. A. Kittaka [9].

2.2. Cell cultures

The PC-3 prostate cancer cell line and an immortalized normal prostate epithelial cell line, PZ-HPV-7, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). PC-3 cells were grown on DMEM (GIBCOBRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS) (GIBCOBRL, Gaithersburg, MD, USA). Cells were fed three times per week. PZ-HPV-7 cells were cultured in a serum-free defined growth medium used for keratinocyte primary culture [10].

2.3. Cellular proliferation assay

Two assays were performed to compare the antiproliferative activity of C-2 modified 19-nor-1 α ,25(OH) $_2$ D $_3$ against 1 α ,25(OH) $_2$ D $_3$; 3 H-thymidine incorporation method and cell counting method using a hemocytometer as described previously [7,10]. The results were expressed as percent of controls.

2.4. Invasion assay

The invasion assay was performed as described by Bao et al. [11]. Briefly, PC-3 cells, which were pretreated with ethanol vehicle, or different concentrations of 1 α ,25(OH) $_2$ D $_3$, or 19-nor-2 α -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ or 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ for 72 h in FBS-supplemented medium, were harvested and counted. To each Matrigel coated insert (Becton Dickinson Labware, Bedford, MA), 5×10^4

cells were added in serum-free media containing ethanol vehicle or different concentrations of 1 α ,25(OH) $_2$ D $_3$, 19-nor-2 α -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ or 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ as indicated. The lower chambers contained medium with 10% FBS and ethanol vehicle or same concentration of 1 α ,25(OH) $_2$ D $_3$, 19-nor-2 α -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ or 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ as the upper chambers. The chambers were incubated for 22 h at 37 °C. The cells that had invaded to the lower surface of the membranes were fixed and stained with 1% Toluidine Blue, and total invading cell number in five random fields was counted under a light microscope.

2.5. Statistical analysis

Comparisons of the antiproliferative and anti-invasion activities between controls and treated groups, and between two different treated groups were performed using one-way ANOVA. Differences between groups were considered statistically significant when p values were ≤ 0.05 .

3. Results and discussion

The effects of 1 α ,25(OH) $_2$ D $_3$ and C-2-substituted 19-nor-1 α ,25(OH) $_2$ D $_3$ analogs on the 3 H-thymidine incorporation into DNA of cultured PZ-HPV-7 prostate cells are shown in Fig. 1. Fig. 1 demonstrates that 19-nor-2-phenyl-carbamoyl-1 α ,25(OH) $_2$ D $_3$ was not active, and 19-nor-2 α ,25(OH) $_2$ D $_3$ (ED $_{50}$ = 4.1 ± 0.5 μ M, $N = 2$) and 19-nor-1 α ,2 β ,25(OH) $_3$ D $_3$ (ED $_{50}$ = 1.1 ± 0.3 μ M, $N = 2$) were about 100-fold less active than 1 α ,25(OH) $_2$ D $_3$ (ED $_{50}$ = 61 ± 25 nM, $N = 12$), whereas,

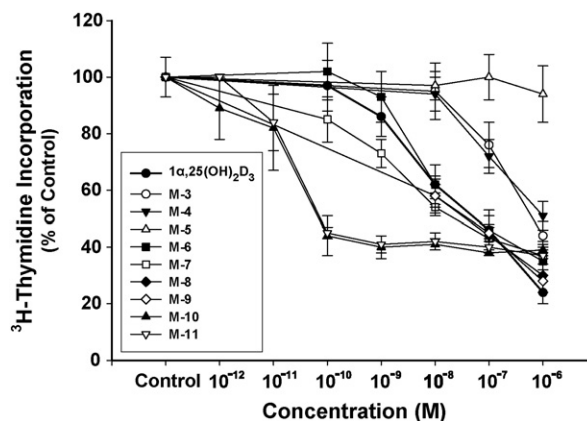


Fig. 1. Effect of 1 α ,25(OH) $_2$ D $_3$ and C-2 substituted 19-nor-1 α ,25(OH) $_2$ D $_3$ analogs, including 19-nor-2 α ,25(OH) $_2$ D $_3$ (M-3), 19-nor-1 α ,2 β ,25(OH) $_3$ D $_3$ (M-4), 19-nor-2-phenyl-carbamoyl-25(OH) $_2$ D $_3$ (M-5), 19-nor-2 α -allyl-1 α ,25(OH) $_2$ D $_3$ (M-6), 19-nor-2 β -allyl-1 α ,25(OH) $_2$ D $_3$ (M-7), 19-nor-2 α -propyl-1 α ,25(OH) $_2$ D $_3$ (M-8), 19-nor-2 β -propyl-1 α ,25(OH) $_2$ D $_3$ (M-9), 19-nor-2 α -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ (M-10) and 19-nor-2 β -(3-hydroxypropyl)-1 α ,25(OH) $_2$ D $_3$ (M-11) on the 3 H-thymidine incorporation into DNA of PZ-HPV-7 cells. The results are presented as the means \pm S.D. of eight determinations.

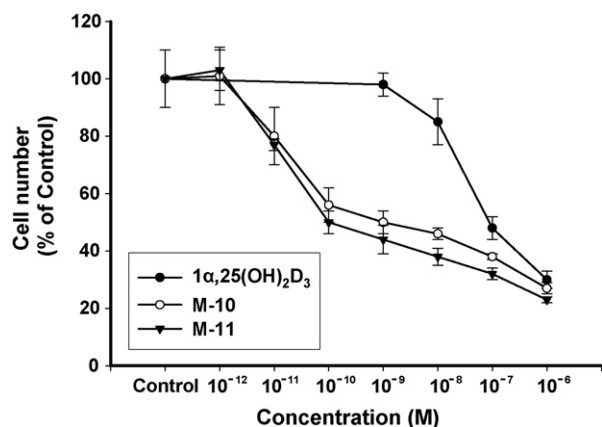


Fig. 2. Effect of $1\alpha,25(\text{OH})_2\text{D}_3$, 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ (M-10) and 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ (M-11) on PZ-HPV-7 cell proliferation. The results are presented as the means \pm S.D. of six determinations.

19-nor-2 α -allyl- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 60 \pm 27$ nM, $N = 4$), 19-nor-2 β -allyl- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 18 \pm 7$ nM, $N = 4$), 19-nor-2 α -propyl- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 37 \pm 4$ nM, $N = 2$), 19-nor-2 β -propyl- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 37 \pm 4$ nM, $N = 2$) were approximately as active as $1\alpha,25(\text{OH})_2\text{D}_3$. Interestingly, 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 61 \pm 21$ pM, $N = 2$) and 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ ($\text{ED}_{50} = 87 \pm 18$ pM, $N = 2$) were about 500- to 1000-fold more active than $1\alpha,25(\text{OH})_2\text{D}_3$. The ^3H -thymidine incorporation data for 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ were supported by the cell counting data after cells were treated with $1\alpha,25(\text{OH})_2\text{D}_3$, 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ or 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ for 7 days (Fig. 2). In addition to the proliferation assay, we also performed an invasion assay using PC-3 prostate cancer cells. We found that 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ was about 10-fold more active than $1\alpha,25(\text{OH})_2\text{D}_3$ in inhibiting PC-3 cell invasion (Fig. 3). Similar results were obtained

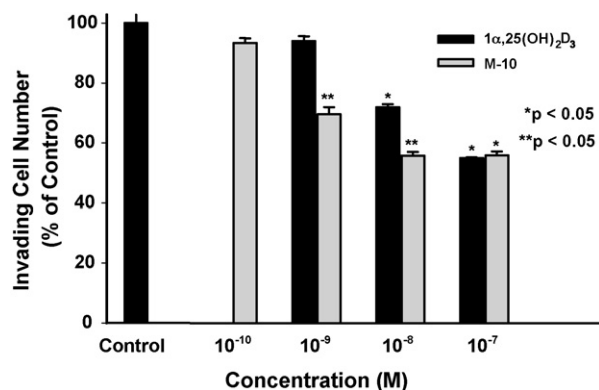


Fig. 3. Effect of $1\alpha,25(\text{OH})_2\text{D}_3$, and 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ (M-10) on the invasion of PC-3 cells. The results are presented as the means \pm S.D. of three determinations. *Comparison between control and $1\alpha,25(\text{OH})_2\text{D}_3$ or M-10; **Comparison between $1\alpha,25(\text{OH})_2\text{D}_3$ and M-10.

with 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ (data not shown).

In summary, substitution at C-2 position may decrease, increase, or cause no significant effect on the antiproliferative activity of 19-nor compounds. The substitution with a bulky phenyl-carbamoyl group essentially abolished the antiproliferative activity of 19-nor vitamin D compounds. Substitution with just a hydroxyl group decreased the activity about 100-fold, whereas, substitution with a 3-carbon allyl or propyl group, had no significant effect. Interestingly, the addition of a hydroxyl group at the C-3 of the propyl chain generated two stereo isomers, 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ and 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$, which are much more active than 19-nor-2 α -propyl- $1\alpha,25(\text{OH})_2\text{D}_3$ and 19-nor-2 β -propyl- $1\alpha,25(\text{OH})_2\text{D}_3$ in inhibiting prostate cell proliferation. It is possible that the additional hydroxyl group may increase the docking of 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ or 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ ligand to the VDR binding pocket through additional hydrogen bonding. Alternately, it may contribute to the increased binding of co-activators to the transcription complexes through unknown mechanisms and lead to enhanced anti-proliferative activity specifically.

It is known that 19-nor- $1\alpha,25(\text{OH})_2\text{D}_2$ is safe within a wide dosing range (6) and is approved for human use (for other indications). Our demonstration that 19-nor-2 α -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ and 19-nor-2 β -(3-hydroxypropyl)- $1\alpha,25(\text{OH})_2\text{D}_3$ have higher antiproliferative and anti-invasion activities than $1\alpha,25(\text{OH})_2\text{D}_3$ suggests that these two C-2 substituted 19-nor- $1\alpha,25(\text{OH})_2\text{D}_3$ compounds may be excellent candidates for human clinical trials in prostate cancer, especially for prostate cancers that have failed conventional therapies such as androgen deprivation.

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