The in Vitro Evaluation of 25-Hydroxyvitamin D₃ and 19-nor-1α,25-Dihydroxyvitamin D₂ as Therapeutic Agents for Prostate Cancer

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ABSTRACT

Prostate cancer cells contain specific receptors [vitamin D receptors (VDRs)] for 1α,25-dihydroxyvitamin D₃ (1α,25(OH)₂D₃), which is known to inhibit the proliferation and invasiveness of these cells. These findings support the use of 1α,25(OH)₂D₃ for prostate cancer therapy. However, because 1α,25(OH)₂D₃ can cause hypercalcemia, analogues of 1α,25(OH)₂D₃ that are less calcemic but that exhibit potent antiproliferative activity would be attractive as therapeutic agents. We investigated the effects of two different types of less calcemic vitamin D compounds, 25-hydroxyvitamin D₃ [25(OH)D₃] and 19-nor-1α,25-dihydroxyvitamin D₃ [19-nor-1α,25(OH)₂D₃], and compared their activity to 1α,25(OH)₂D₃ on (a) the proliferation of primary cultures and cell lines of human prostate cancer cells; and (b) the transactivation of the VDRs in the androgen-insensitive PC-3 cancer cell line stably transfected with VDR (PC-3/VDR). 19-nor-1α,25(OH)₂D₃, an analogue of 1α,25(OH)₂D₃ that was originally developed for the treatment of parathyroid disease, has been shown to be less calcemic than 1α,25(OH)₂D₃ in clinical trials. Additionally, we recently showed that human prostate cells in primary culture possess 25(OH)D₃-1α-hydroxylase, an enzyme that hydroxylates the inactive prohormone, 25(OH)D₃, to the active hormone, 1α,25(OH)₂D₃ intracellularly. We reasoned that the hormone that is formed intracellularly would inhibit prostate cell proliferation in an autocrine fashion. We found that 1α,25(OH)₂D₃ and 19-nor-1α,25(OH)₂D₃ caused similar dose-dependent inhibition in the cell lines and primary cultures in the [³H]thymidine incorporation assay and that both compounds were significantly more active in the primary cultures than in LNCaP cells. Likewise, 25(OH)D₃ had inhibitory effects comparable to those of 1α,25(OH)₂D₃ in the primary cultures. In the chloramphenicol acetyltransferase (CAT) reporter gene transactivation assay in PC-3/VDR cells, 1α,25(OH)₂D₃ and 19-nor-1α,25(OH)₂D₃ caused similar increases in CAT activity between 10⁻¹¹ and 10⁻⁹ M. Incubation of PC-3/VDR cells with 5 x 10⁻⁸ M 25(OH)D₃ induced a 29-fold increase in CAT activity, similar to that induced by 10⁻⁹ M 1α,25(OH)₂D₃. In conclusion, our data indicate that 25(OH)D₃ and 19-nor-1α,25(OH)₂D₃ represent two different solutions to the problem of hypercalcemia associated with vitamin D-based therapies: 25(OH)D₃ requires the presence of 1α-hydroxylase, whereas 19-nor-1α,25(OH)₂D₃ does not. Both drugs are approved for human use and may be good candidates for human clinical trials in prostate cancer.

INTRODUCTION

Prostate cancer is the second leading cause of cancer deaths among U.S. men (after lung cancer), with 37,000 deaths projected in 1999 (1). For tumors that are ineligible for, or that fail to respond to, surgery or radiation, the mainstay of prostate cancer therapy is androgen deprivation. About 75% of men respond to androgen deprivation, but the median duration of response is only about 2 years (2). There are no effective therapies for prostate cancers that fail to respond to androgen deprivation.

In addition to androgens, it is now clear that prostatic cells are responsive to another class of steroid hormones, namely, vitamin D (3). Most human prostate cells contain specific intracellular receptors (commonly called VDRs) for 1α,25(OH)₂D₃, the active hormonal form of vitamin D (4, 5). Numerous studies have shown that in response to 1α,25(OH)₂D₃, prostate cancer cells show an increase in differentiation and a decrease in proliferation, invasiveness, and metastasis (6-9). These findings strongly support the use of vitamin D-based therapies for prostate cancer, e.g., as differentiation therapy and/or as a second-line therapy once androgen deprivation has failed. However, the use of 1α,25(OH)₂D₃-based therapies for prostate cancer is limi-

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3 The abbreviations used are: VDR, vitamin D receptor; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 19-nor-1α,25(OH)₂D₃, 19-nor-1α,25-dihydroxyvitamin D₃; 1α-OHase, 1α-hydroxylase; FBS, fetal bovine serum; CAT, chloramphenicol acetyltransferase; DPPD, 1,2-dianilinoethane; VDRE, VDR element; MOP, mouse osteopontin; tk, thymidine kinase; CMV, cytomegalovirus; β-gal, β-galactosidase.
vitamin D for prostate cancer therapy

by the risk of hypercalcemia and hypercalciuria (10, 11). Thus, less calcemic or noncalcemic analogues of 1α,25(OH)2D3, with potent antiproliferative activity would be attractive therapeutic agents.

Recently, our group has shown that human prostate cancer cells in primary culture and several prostate cancer cell lines possess 1α-OHase, the enzyme that converts the major circulating, prohormonal form of vitamin D, 25(OH)D, to 1α,25(OH)2D (12, 13). Because the conversion from prohormone to active hormone occurs within the cell, the problem of systemic hypercalcemia should be avoided. 25(OH)D would be an attractive candidate for human clinical trials in prostate cancer because this drug has been approved by the FDA for human use (e.g., for treating vitamin D deficiency due to liver disease; Ref. 14).

Similarly, 19-nor-1α,25(OH)2D2, a synthetic analogue of 1α,25(OH)2D2, has recently been approved by the FDA for the treatment of secondary hyperparathyroidism. Several randomized controlled clinical trials have shown that 19-nor-1α,25(OH)2D2 is noncalcemic (15, 16). The structural similarity between 1α,25(OH)2D2 and 19-nor-1α,25(OH)2D2 suggested to us that the behavior of 19-nor-1α,25(OH)2D2 in prostatic cells might be similar to that of 1α,25(OH)2D3.

In this report, we investigated the effect of 25(OH)D3, 1α,25(OH)2D3, and 19-nor-1α,25(OH)2D3 on the proliferation of primary cultures and cell lines of human prostate cancer. In addition, we evaluated the abilities of these three vitamin D compounds to transactivate the VDR in a prostate cancer cell line, PC-3, that was stably transfected with VDR.

MATERIALS AND METHODS

Vitamin D Compounds. 25(OH)D3 and 1α,25(OH)2D3 were a generous gift from Dr. M. Uskokovic (Hoffman-La Roche, Nutley, NJ). 19-nor-1α,25(OH)2D2 was a gift from Tetrionics (Madison, WI).

Cell Cultures. Prostate cancer cell lines, LNCaP and PC-3 cells, were obtained from the American Type Culture Collection (Rockville, MD) and were grown on 24-well culture dishes with DMEM (Life Technologies, Inc.) supplemented with 5% FBS (Life Technologies, Inc.). Cells were fed three times per week. Primary cultures of human prostate epithelial cells were prepared as described previously (17). Prostate epithelial cells were cultured in a serum-free defined-growth medium (Prostate Epithelial Growth Medium BulletKit, Clonetics, San Diego, CA). Prostate cells used for this study were at their second passage.

[^3]HThymidine Incorporation. [^3]Hthymidine incorporation into DNA was used as an index of cell proliferation as described previously (18). Briefly, when LNCaP cells or the second passage primary culture cells reached about 50% confluence, FBS (in the case of LNCaP) or growth factors (in the case of primary cultures) were removed from the media, and cells were grown for an additional 24 h in the absence of FBS or growth factors. Cells were then treated with and without different concentrations of 25(OH)D3, 1α,25(OH)2D3, or 19-nor-1α,25(OH)2D3 as indicated in the figure legends. Eighteen h later, the dosing medium was replaced with 0.5 ml of fresh basal medium containing [methyl-[^3]H]thymidine (New England Nuclear, Boston, MA) and incubated for 3 h at 37°C. [^3]HThymidine incorporation into DNA was stopped by placing the 24-well plates on ice. Unincorporated[^3]HTymidine was then removed, and the cells were washed three times with ice-cold PBS. DNA labeled with[^3]HTymidine and other macromolecules were first precipitated with ice-cold 5% perchloric acid for 20 min and then extracted with 0.5 ml of 5% perchloric acid at 70°C for 20 min as described previously (19). The radioactivity in the extracts was determined by a liquid scintillation counter. The results were expressed as percent of control.

Morphological Studies during Cellular Proliferation. The second-passage primary culture cells were subcultured in the complete medium into 35-mm dishes for the morphological studies (18). Two days after the initial plating, triplicate plates of cells were incubated with complete media without insulin but containing 25(OH)D3, 1α,25(OH)2D3, or vehicle. Cells were dosed again with 25(OH)D3 or 1α,25(OH)2D3 2 and 4 days later. Three days after the last dosing, the media were removed from cultures. The attached cells were then trypsinized for 30 min with 0.1% EDTA and 0.1% trypsin at 37°C and then neutralized with basal medium. The detached cells were spun down and resuspended in a known volume of basal medium. Triplicate aliquots were applied to a hemocytometer for cell counting.

Recombinant Plasmids, Transfection, and CAT Assay. The reporter plasmid MOPVDREthCAt was constructed as described previously (19) and consisted of two copies of the VDRE of the MOP gene linked 5′ to the tk promoter and CAT gene of the vector pBLCAT2 (20). MOPVDREthCAt was transfected into a PC-3/VDR clone (clone 3B2) using the calcium phosphate method. CMV-β-gal, which encodes the β-gal gene driven by the CMV promoter, was included in all of the transfections to normalize for differences in transfection efficiency. Clone 3B2 (PC-3/VDR) was generated by transfecting PC-3 cells with the VDR cDNA expression vector pRc CMV-VDR followed by the selection and expansion of stable clonal isolates as described previously (21). The PC-3/VDR and LNCaP cell line expressed comparable levels of VDR (approximately 25 fmol/mg protein). The PC-3/VDR cells transfected with the reporter gene were then cultured in the presence or absence of 10^-8 M 25(OH)D3, 1α,25(OH)2D3, or 19-nor-1α,25(OH)2D3 in RPMI containing 10% FBS. Cells were harvested about 40 h after transfection, and cell extracts were prepared for analyzing β-gal and CAT activity. Cell extracts containing equivalent amounts of β-gal activities were used for an analysis of CAT using an adaptation of the method of Gorman et al. (22). The percentage of conversion of[^14C]chlormephalone to acetylated forms on thin-layer chromatograms was quantified using a Molecular Dynamics Phosphorimager and Image Quant software (Sunnyvale, CA).

Statistical Analysis. Comparisons of the antiproliferative and transactivation activities between controls and drug-treated groups, and between two different drugs, were performed using one-way ANOVA. Differences between groups were considered statistically significant when P values were ≤ 0.05.

RESULTS

The effects of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D3 on the[^3]HThymidine incorporation into cultured LNCaP pros-


**Fig. 1** Effect of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 on the [3H]thymidine incorporation into DNA at the same concentration. Significant inhibition was detected in the presence of 10⁻⁶ M, 25(OH)D3 and 19-nor-1α,25(OH)2D2, and 19-nor-1α,25(OH)2D2 was observed among the dosages studied.

**Fig. 2** Effect of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 on [3H]thymidine incorporation in primary cultures of prostate cancer cells. Results are presented as the means ± SD of five to eight determinations. *P < 0.05 and **P < 0.001, respectively, versus controls. No statistical difference between 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 was observed among the dosages studied.

Fig. 2 shows a dose-dependent antiproliferative effect of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 in the primary cultures of human prostate cancer cells. 1α,25(OH)2D3 caused a 18 ± 3%, 41 ± 1%, 77 ± 1%, and 86 ± 1% inhibition of [3H]thymidine incorporation into DNA at 10⁻⁹, 10⁻⁸, 10⁻⁷, and 10⁻⁶ M, respectively; and 19-nor-1α,25(OH)2D2 caused a 20 ± 5%, 39 ± 2%, 80 ± 1%, and 88 ± 1% inhibition of [3H]thymidine incorporation into DNA at the same concentrations, respectively. The data show that the activities of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 in the primary prostate cultures are very similar. Both compounds were more effective in inhibiting [3H]thymidine incorporation in the primary cultures than in LNCaP cells.

We have previously established the presence of 1α-OHase mRNA and its enzyme activity in cultured prostate cancer cell lines and in primary cultures derived from normal, benign prostatic hyperplasia and prostate cancer cells (PC-3 and DU145; 12, 13). Unlike the primary cultures, DU145 and PC-3 cells, very little 1α-OHase activity was detected in LNCaP cells. We reasoned that 25(OH)D3 should be converted to 1α,25(OH)2D3 by prostatic cancer cells that possess 1α-OHase and that the 1α,25(OH)2D3 would cause an inhibition in the proliferation of these cells. Fig. 3 demonstrates that when primary cultures of prostatic cancer cells were treated with 25(OH)D3 or 1α,25(OH)2D3 for 7 days, the two vitamin D3 metabolites caused a similar inhibition of cell proliferation. At 10⁻⁶ M, 25(OH)D3 and 1α,25(OH)2D3 inhibited cell proliferation by decreasing cell number 60 ± 3% and 60 ± 1%, respectively, and at 10⁻⁷ M, 25(OH)D3 and 1α,25(OH)2D3 inhibited cell proliferation by decreasing cell number 24 ± 1% and 25 ± 5% in primary cultures of prostate cancer cells, respectively. At 10⁻⁸ M, neither compound exhibited significant activity. Using the [3H]thymidine incorporation assay for cell proliferation, we also demonstrated that 25(OH)D3 was highly active in inhibiting primary prostate cell proliferation (Fig. 4). 25(OH)D3 caused 33 ± 7%, 46 ± 4%, and 70 ± 3% inhibition at 10⁻⁹, 10⁻⁸, and 10⁻⁷ M, respectively, as compared with 55 ± 4%, 60 ± 4%, and 73 ± 4% inhibition by 1α,25(OH)2D3. Thus, in these assays, 25(OH)D3 and 1α,25(OH)2D3 were equipotent in inhibiting the proliferation of prostate cancer cells at 10⁻⁷ M.

Fig. 3 Effect of 1α,25(OH)2D3 and 25(OH)D3 on cell proliferation in prostate primary cultures. Results are presented as the means ± SD of nine determinations. *, P < 0.05 and **, P < 0.001, respectively, versus controls. No statistical difference between 1α,25(OH)2D3 and 25(OH)2D3 was observed among the dosages studied.
controls; the means ± SD of five to eight determinations. $P < 0.05$ versus controls; * $P < 0.05$; ** $P > 0.1$, respectively, between the two compounds.

cDNA (PC-3/VDR). To assess the ability of VDR to be activated by endogenously synthesized $1\alpha,25(OH)_2 D_3$, we performed CAT reporter gene transactivation assays in PC-3/VDR cells cultured in $25(OH)D_3$. DPPD was added to inhibit the auto-oxidation of $25(OH)D_3$ to $1\alpha,25(OH)_2 D_3$. The CAT reporter gene plasmid contains two tandem copies of the VDRE found in the MOP promoter. $1\alpha,25(OH)_2 D_3$ caused a 2-, 16-, 37-, and 15-fold increase, and 19-nor-$1\alpha,25(OH)_2 D_3$ caused a 3-, 21-, 40-, and 32-fold increase over the controls in CAT activity at $10^{-11}$, $10^{-10}$, $10^{-9}$, and $10^{-8}$ M, respectively (Fig. 5). Incubation of PC-3/VDR cells with $5 \times 10^{-8}$ M $25(OH)D_3$ induced a 29-fold increase over the controls in CAT activity, which was similar to that induced by $10^{-8}$ M $1\alpha,25(OH)_2 D_3$ (Fig. 6).

**DISCUSSION**

During the past two decades, the actions of $1\alpha,25(OH)_2 D_3$ have extended far beyond its classical role on intestine, bone, kidney, and parathyroid glands to regulate serum calcium levels. $1\alpha,25(OH)_2 D_3$ has been shown to have important antiproliferative and prodifferentiating activities in a variety of tissues or cells that possess VDRs, including prostatic cells (4–9). The epidemiological similarities between prostate cancer and vitamin D deficiency (23–25) and the impressive anticancer effects of $1\alpha,25(OH)_2 D_3$ on prostatic cells has led to great interest in the use of this hormone as a therapeutic agent for prostate cancer (10, 11). Many different types of non- or less-calcemic vitamin D analogues have been investigated for their effects on prostate cancer cell proliferation in vitro. Potent inhibitors of prostate cancer cell proliferation include 19-nor-hexahydroflavine vitamin D$_3$ and 20-cyclopropyl-vitamin D$_3$ analogues (26, 27). Hisatake et al. (28) recently reported that 5,6-trans-16-ene-Vitamin D$_3$ analogues were more potent than 1$\alpha,25(OH)_2 D_3$ in inhibiting LNCAp cells in vitro and were about 40-fold less calcemic than 1$\alpha,25(OH)_2 D_3$ in normal mice in vivo. However, the antiproliferative effects of these analogues has yet to be demonstrated in vivo.

Recently, it was demonstrated that EB1089, an analogue of 1$\alpha,25(OH)_2 D_3$, was as effective as 1$\alpha,25(OH)_2 D_3$ in inhibiting metastasis in an in vivo model of androgen-insensitive prostate cancer, the rat Dunning MAT LyLu prostate cancer model (9). Although EB1089 was significantly less calcemic than 1$\alpha,25(OH)_2 D_3$, it still caused an 18% increase in serum calcium level (versus a 34% increase by 1$\alpha,25(OH)_2 D_3$). Thus, less calcemic or noncalcemic analogues of 1$\alpha,25(OH)_2 D_3$ are still needed. Llach et al. (15) reported that 19-nor-$1\alpha,25(OH)_2 D_2$ was as effective as 1$\alpha,25(OH)_2 D_3$ in suppressing parathyroid hormone secretion in hemodialysis patients with secondary hyperparathyroidism without inducing hypercalcemia or hyperphosphatemia. In the current study, we examined the antiproliferative activity of 19-nor-$1\alpha,25(OH)_2 D_3$ in LNCaP cells and in primary cultures of prostate cancer cells. In these two different cultures, 19-nor-$1\alpha,25(OH)_2 D_3$ showed antiproliferative effects similar to those of $1\alpha,25(OH)_2 D_3$ as determined by $[^3]$H thymidine incorporation (Figs. 1 and 2). Both compounds had a greater effect in the primary cultures of prostate cancer cells than in the LNCAp prostate cancer cell line, which suggests that primary cultures may be a more sensitive system to differentiate the effectiveness of different vitamin D compounds in vitro. $1\alpha,25(OH)_2 D_3$ was previously shown (21) to decrease cyclin-dependent kinase 2 activity, resulting in decreased retinoblastoma protein phosphorylation and accumulation of LNCAp cells in G1 phase of the cell cycle. Because a functional retinoblastoma pathway seems to be required for the maximal antiproliferative effects of $1\alpha,25(OH)_2 D_3$, primary prostate cultures may exhibit increased growth inhibition by $1\alpha,25(OH)_2 D_3$, 25(OH)D$_3$, and 19-nor-$1\alpha,25(OH)_2 D_3$ because, compared with the cell lines, these cultures are less likely to have mutations in this pathway.

Because the clinical use of $1\alpha,25(OH)_2 D_3$ in cancer therapy is limited by the risk of hypercalcemia, many investigators have attempted to duplicate the antiproliferative effects of 1$\alpha,25(OH)_2 D_3$ in vitro using analogues of 1$\alpha,25(OH)_2 D_3$ that are less calcemic, such as 19-nor-1$\alpha,25(OH)_2 D_2$, 16-ene-23-yner-1$\alpha,25(OH)_2 D_3$ (29), and EB1089 (30), or they have used a combination of 1$\alpha,25(OH)_2 D_3$ or 1$\alpha,25(OH)_2 D_3$ analogues with other drugs (31, 32). However, our discovery that prostate cells in primary culture express high 1$\alpha$-OHase activity and can synthesize 1$\alpha,25(OH)_2 D_3$ from 25(OH)D$_3$ suggests that 25(OH)D$_3$ may offer another potential solution to the problem of hypercalcemia caused by the systemic administration of 1$\alpha,25(OH)_2 D_3$. This is because 1$\alpha,25(OH)_2 D_3$ would be synthesized intracellularly, act in an autocrine fashion, and be degraded, and would not be expected to leak into the systemic circulation and cause hypercalcemia. Our results confirm that 25(OH)D$_3$ is highly active in inhibiting prostate cells proliferation in vitro (Figs. 3 and 4). Similar antiproliferative effects are observed when 25(OH)D$_3$ is administered to primary cultures of prostate cells in clonogenic assays. Because 25(OH)D$_3$ binds to

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the VDRs with only 0.001 to 0.002 of the binding affinity of 1α,25(OH)2D3 (33), we consider it unlikely that these results are due to the direct actions of 25(OH)D3 on the VDRs. Rather, we suggest that these results reflect the conversion of 25(OH)D3 to 1α,25(OH)2D3 by 1α-OHase present in prostate cells. This conclusion is further supported by experiments in which LNCaP cells were transfected with 1α-OHase cDNA. These cells, and not untransfected cells, responded to 25(OH)D3 by an inhibition of [3H]thymidine incorporation. 5 Experiments to determine whether similar effects can be produced in vivo using human tumor cells xenografted into athymic mice are presently underway.

Although kidney cells are the “classic” cells that possess 1-OHase, 1α,25(OH)2D levels produced by the kidney are very tightly regulated by serum levels of parathyroid hormone (34). Thus, in normal individuals, even large increases in serum 25(OH)D will not result in increased systemic levels of 1α,25(OH)2D (35, 36). However, the extrarenal synthesis of 1α,25(OH)2D is generally unregulated (13, 37). This suggests that increases in systemic levels of 25(OH)D could result in increased local production of 1α,25(OH)2D in some extrarenal sites (i.e., prostate) without producing hypercalcemia.

Two commonly used assay methods were used to study the antiproliferative activity of 25(OH)D3 in the primary cultures of prostate cells: [3H]thymidine incorporation and cell count. The former involved a short-term, 18-h incubation with the hormone and studied the effect of the drugs on DNA synthesis. [3H]thymidine incorporation is an index of cell division. In certain cell types, decreases in [3H]thymidine incorporation may also reflect increases in cell differentiation (38). Conversely, cell count, which requires longer-term incubation (7 days) with the hormone, reflects cell growth only. The difference in dose-response curves that we observed in the two assays may reflect differences in the incubation time of these assays because: (a) with longer incubation times, more 25(OH)D3 would be converted to 1α,25(OH)2D3 intracellularly, and, thus, the effects of 25(OH)D3 would be similar to the effects of the direct addition of 1α,25(OH)2D3; and (b) more exogenously added 1α,25(OH)2D3 would likely increase its own degradation by inducing the expression of 24-hydroxylase (34).

Most of the antiproliferative effects of 1α,25(OH)2D3 and its analogues are believed to be mediated through the functional expression of VDR. We, therefore, compared the transactivation activity of 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 in PC-3 cells cultured with 1α,25(OH)2D3 or 19-nor-1α,25(OH)2D2 at the indicated concentrations. CAT activity was assessed by TLC using cellu...
cells that were stably transfected with VDR (PC-3/VDR), using the reporter plasmid MOPV-DREtkCAT. This plasmid, which contains two copies of the VDRE found in the MOP gene, was chosen because previous studies in other well-characterized prostate cancer cell lines indicated that VDR transcriptional activity could be detected using this VDRE-containing reporter, even in cell lines that expressed extremely low levels of VDR (9, 21). Using this system, we demonstrated that 1α,25(OH)2D3 and 19-nor-1α,25(OH)2D2 had almost identical transactivation activity (Fig. 5) in agreement with our [3H]thymidine incorporation data (Figs. 1 and 2). 25(OH)D3 at 5 × 10^{-8} M showed a comparable transactivation activity as that caused by 10^{-8} M 1α,25(OH)2D3, consistent with our finding that PC-3 cells have the capacity to convert 25(OH)D3 to 1α,25(OH)2D3 (12).

In summary, this report demonstrates that, like 1α,25(OH)2D3, 19-nor-1α,25(OH)2D2, and 25(OH)D3 possess potent antiproliferative effects on human prostate cancer cell lines and on primary cultures of human prostate cancer cells. Both 19-nor-1α,25(OH)2D2 and 25(OH)D3 are equipotent to the parent hormone in their ability to transactivate the VDR. Although both of these vitamin D compounds act ultimately on the VDR, their proximal biological targets are different: 25(OH)D3 requires the presence of 1α-OHase, whereas 19-nor-1α,25(OH)2D2 does not. Our studies of prostate cancer cell lines have shown a large variation in the expression of 1α-OHase. For example, although LNCaP cells showed profound growth inhibition by 1α,25(OH)2D3, these cells do not express measurable levels of 1α-OHase message and activity (12, 13) and, accordingly, are not growth-inhibited by 25(OH)D3 (39). This suggests that prostatic tumors that do not express 1α-OHase should be treated with 1α,25(OH)2D3 ana-
logues, such as 19-nor-1α,25(OH)$_2$D$_3$ or EB1089. Because both 25(OH)D$_3$ and 19-nor-1α,25(OH)$_2$D$_3$ are known to be noncalcemic within a wide dosing range (14) and both are approved for human use (for other indications), these vitamin D 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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REFERENCES


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