1α,25-Dihydroxyvitamin D₃ exerts tissue-specific effects on estrogen and androgen metabolism

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Abbreviations:
17β-HSD, 17β-hydroxysteroid dehydrogenase
ELISA, enzyme-linked immunosorbent assay
SAM, selective aromatase modulator
TBP, TATA box binding protein
VDR, vitamin D receptor

ABSTRACT

It is well-known that 1α,25-dihydroxyvitamin D₃ and analogs exert anti-proliferative and pro-differentiating effects and these compounds have therefore been proposed to be of potential use as anti-cancer agents. Due to its effects on aromatase gene expression and enzyme activity, 1α,25-dihydroxyvitamin D₃ has been proposed as an interesting substance in breast cancer treatment and prevention. In the present study, we have examined the effects of 1α,25-dihydroxyvitamin D₃ on estrogen and androgen metabolism in adrenocortical NCI-H295R cells, breast cancer MCF-7 cells and prostate cancer LNCaP cells. The NCI-H295R cell line has been proposed as a screening tool to study endocrine disruptors. We therefore studied whether this cell line reacted to 1α,25-dihydroxyvitamin D₃ treatment in the same way as cells from important endocrine target tissues. 1α,25-Dihydroxyvitamin D₃ exerted cell line-specific effects on estrogen and androgen metabolism. In breast cancer MCF-7 cells, aromatase gene expression and estradiol production were decreased, while production of androgens was markedly increased. In NCI-H295R cells, 1α,25-dihydroxyvitamin D₃ stimulated aromatase expression and decreased dihydrotestosterone production. In prostate cancer LNCaP cells, aromatase expression increased after the same treatment, as did production of testosterone and dihydrotestosterone. In summary, our data show that 1α,25-dihydroxyvitamin D₃ exerts tissue-specific effects on estrogen and androgen production and metabolism. This is important knowledge about 1α,25-dihydroxyvitamin D₃ as an interesting substance for further research in the field of breast cancer prevention and treatment. Furthermore, the observed cell line-specific effects are of importance in the discussion about NCI-H295R cells as a model for effects on estrogen and androgen metabolism.

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1. Introduction

Vitamin D₃ is a multifunctional hormone, known to affect a broad range of physiological functions, including calcium homeostasis, insulin secretion, immune system and fetal development [1]. Vitamin D₃ is bioactivated in two subsequent steps to produce the active hormone 1α,25-dihydroxyvitamin D₃ (calcitriol) [2]. We have recently reported that 1α,25-dihydroxyvitamin D₃ affects the hormone production and the activity and expression of key steroidalogenic enzymes in human adrenocortical NCI-H295R cells [3].

NCI-H295R cells are widely used as a model for human adrenal cortex. It has been proposed that this adrenocortical carcinoma cell line could be suitable in a screening assay to study the effects of different chemicals on estradiol and testosterone production [4-7]. In this study, we compare NCI-H295R cells with cell lines derived from important target tissues in estradiol and testosterone production.

It is well-known that 1α,25-dihydroxyvitamin D₃ and analogs exert anti-proliferative and pro-differentiating effects and they have therefore been proposed to be of potential use as anti-cancer agents [8-11]. Epidemiological studies have revealed relationships between solar UV-B exposure, which is needed for vitamin D₃ production, and decreased incidence and mortality in breast cancer. Low 25-hydroxyvitamin D₃ serum level has also been found to be correlated to breast cancer risk [12]. Furthermore, 1α,25-dihydroxyvitamin D₃ has been reported to affect apoptosis and expression of tumor suppressor genes [9,12].

Estrogens are produced from androgenic precursors in a reaction catalyzed by aromatase (CYP19A1). Important enzyme-catalyzed reactions in the metabolism of androgens and estrogens are shown in Fig. 1. 5α-Reductase and aromatase are two key enzymes in determining the balance between androgen production and estrogen production. The principal site of estrogen production in premenopausal women is the ovaries. Aromatase is also expressed in other tissues including breast and osteoblasts [9]. A large group of all breast cancers involves estrogen-dependent mechanisms, i.e., they rely on estrogens to proliferate. For estrogen-dependent carcinomas arising

Abbreviations: 17β-HSD, 17β-hydroxysteroid dehydrogenase; ELISA, enzyme-linked immunosorbent assay; SAM, selective aromatase modulator; TBP, TATA box binding protein; VDR, vitamin D receptor

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Fig. 1. Important enzyme-catalyzed reactions in the production of estrogens and androgens.

in postmenopausal women, when the ovarian estrogen production is decreased, the local estrogen production in breast is crucial for the tumor development [13]. The aromatase expression is higher in breast cancer tissue than in normal breast tissue and the local estrogen levels in breast cancer tissue are higher than the circulating levels [14,15]. Aromatase inhibitors and antiestrogens have therefore become important drugs in breast cancer treatment.

1,25-Dihydroxyvitamin D₃ has been shown to increase aromatase activity in placental cells [16,17], prostate cells [18] and osteoblasts [19]. Kinuta et al. [20] have reported that vitamin D receptor null mutant mice have a decreased aromatase activity in the ovary, testis and epididymis. Interestingly, it was recently reported that 1α,25-dihydroxyvitamin D₃ exerts the opposite effect on aromatase in breast cancer cells. Both aromatase gene expression and aromatase enzyme activity were down regulated following treatment with 1α,25-dihydroxyvitamin D₃ [21].

The tissue-selective regulation of aromatase activity is of particular interest for 1α,25-dihydroxyvitamin D₃ as a potential agent to prevent or treat breast cancer. The aim of the current study was to further clarify the effects of 1α,25-dihydroxyvitamin D₃ on estrogen and androgen hormone production and the activity of key steroidogenic enzymes in different cell lines.

2. Materials and methods

2.1. Chemicals

1α,25-Dihydroxyvitamin D₃ was obtained from Solvay (Duphar, The Netherlands). The vitamin D receptor antagonist TEI-9647 was a kind gift from Teijin Pharma, Tokyo, Japan. Other chemicals were of analytical grade and purchased from various commercially available sources.

2.2. Cell culture and treatment

Human adrenocortical carcinoma NCI-H295R cells (ATCC CRL-2128) were cultured in Dulbecco’s modified Eagle’s medium/Nutrient Mixture F-12 Ham (Sigma) supplemented with 1% ITS Plus premix (BD Biosciences), 2.5% NuSerum (VWR), 1% l-glutamine (Gibco) and 1% antibiotic/antimycotic (Gibco). Human breast adenocarcinoma MCF-7 cells (ATCC HTB-22) were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% antibiotic/antimycotic (Gibco). Human prostate adenocarcinoma LNCaP cells (ATCC CRL-1740) were cultivated in RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco) and 1% antibiotic/antimycotic (Gibco).

All cells were cultured as monolayers in a humidified environment at 37 °C with 5% CO₂. Cells were treated with 1α,25-dihydroxyvitamin D₃ dissolved in ethanol for 6–48 hours in concentrations from 1 nM to 100 nM. The control group was treated with the same amount of ethanol.

2.3. RT-PCR

Expression of mRNA for vitamin D receptor (VDR) in NCI-H295R cells was examined using RT-PCR. RNA was isolated using RNeasy Mini Kit (Qiagen) and reverse transcribed to cDNA by Reverse Transcription System (Promega). Amplification was performed using the AmpliTaq Gold system (Applied Biosystems) in accordance with the manufacturer’s recommendations. Primers and program were as described by Pascussi et al. [22]. Human liver RNA was used as positive control.

2.4. Real-time RT-PCR

Expression of mRNA for aromatase, 5α-reductase type II and promoter-specific aromatase transcripts was measured using real-time RT-PCR. RNA was isolated using RNeasy Mini kit (Qiagen) and reverse transcribed to cDNA by Reverse Transcription System (Promega). The real-time PCR analysis was performed with iQ SYBR Green Supermix (Bio-Rad) using an iQ5 Real-Time PCR Detection System (Bio-Rad). All experiments were conducted in accordance to the manufacturer’s recommendations. Human TATA box binding protein (TBP) was used as endogenous control. All real-time RT-PCR data were normalized to the endogenous control. The relative mRNA expression was calculated with the standard curve method and expressed as fold change compared to the untreated group.

Primers and programs for aromatase and promoter-specific aromatase transcripts were as previously described by Díaz-Cruz et al. [23] and for 5α-reductase type II and for previously described negative VDREs [26].

2.5. In silico analysis of aromatase promoters

Aromatase promoters I.3, I.4 and II were analyzed for putative vitamin D response elements (VDRE) using Consite. The promoters were also manually searched for the consensus sequences for VDREs described by Matilainen et al. [25] and for previously described negative VDREs [26].

2.6. Analysis of hormone production

Cells were cultured as described above and treated with 10 nM 1α,25-dihydroxyvitamin D₃ for 24 hours. The cell culture medium was then collected and the hormone levels analyzed. Production of androstenedione, estradiol, testosterone and dihydrotestosterone was measured by enzyme-linked immunosorbent assay (ELISA). The ELISA kits (DE3265, DE2693, DE1559 and DE2330) were purchased from Demeditec Diagnostics GmbH, Germany. The absorbance was measured at 450 nm, using a Polarstar Optima (BMG Labtech) plate reader. The analyses were performed in accordance with the manufacturer’s recommendations.

2.7. Aromatase enzyme activity

To determine the aromatase enzyme activity, the aromatase-catalyzed conversion of testosterone to estradiol was measured. Cells were cultured as described above and treated with 10 nM 1α,25-dihydroxyvitamin D₃ for 24 hours. MCF-7 cells were also treated with a combination of 10 nM 1α,25-dihydroxyvitamin D₃ and 1 μM TEI-9647 (a VDR antagonist) to examine if the effects of 1α,25-dihydroxyvitamin D₃ on aromatase enzyme activity were mediated by VDR.
Following the treatment, cell culture medium was changed and the aromatase substrate testosterone (10 μM) was added. To prevent the 5α-reductase-catalyzed conversion of testosterone to dihydrotestosterone, the cells were treated with 10 μM finasteride (an inhibitor of 5α-reductase). After 16 hours incubation, the cell culture medium was collected and the estradiol concentration was measured using an ELISA kit purchased form Demeditec Diagnostics GmbH, Germany, adopting the methodology described in Section 2.6.

To examine time course effects of 1α,25-dihydroxyvitamin D₃, NCI-H295R cells were treated with 10 nM 1α,25-dihydroxyvitamin D₃ for 6 hours, 24 hours or 48 hours. In a separate experiment, the dose response effects of 1α,25-dihydroxyvitamin D₃ were studied in NCI-H295R cells. The cells were treated with 1, 10 or 100 nM 1α,25-dihydroxyvitamin D₃ for 24 hours. Following these treatments, the conversion of testosterone to estradiol was measured as described above.

2.8. Statistical analysis

Analysis of statistical significance was performed using Student’s t-test in Microsoft Excel. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Effects of 1α,25-dihydroxyvitamin D₃ on gene expression of important enzymes in the production of estrogens and androgens

Aromatase and 5α-reductase are two important enzymes in estrogen and androgen production (Fig. 1). 1α,25-Dihydroxyvitamin D₃ has been reported to have tissue-selective effects on aromatase activity, with increased activity in placental cells, prostate cells and osteoblasts, while the activity was decreased in breast cancer cells [16–19,21]. In order to study the effect of 1α,25-dihydroxyvitamin D₃ on gene expression level, adrenocortical NCI-H295R cells, breast cancer MCF-7 cells and prostate cancer LNCaP cells were treated with 1α,25-dihydroxyvitamin D₃ and the mRNA level of aromatase was measured using real-time RT-PCR.

The aromatase gene expression was markedly increased by 1α,25-dihydroxyvitamin D₃ in H295R cells and in LNCaP cells while it was decreased in MCF-7 cells (Fig. 2). The suppressive effect in MCF-7 cells was expected since it has been reported recently [21]. The stimulating effects in NCI-H295R cells and LNCaP cells on the other hand are novel findings. Lou et al. [18] have reported that 1α,25-dihydroxyvitamin D₃ increases aromatase activity in LNCaP cells but that the gene expression was unaffected. Our findings do not support their results on gene expression level.

The gene expression of 5α-reductase was measured using real-time RT-PCR. No statistically significant effects of 1α,25-dihydroxyvitamin D₃ on 5α-reductase expression were observed in NCI-H295R cells or MCF-7 cells (data not shown). In LNCaP cells, the 5α-reductase expression was increased by 17% (±0.004; p < 0.001).

3.2. Expression of vitamin D receptor (VDR) and in silico analysis of the aromatase promoters

The effects of 1α,25-dihydroxyvitamin D₃ on gene expression are mediated by the vitamin D receptor (VDR). RT-PCR with mRNA from...
NCI-H295R cells revealed expression of VDR (Fig. 3). Expression of VDR in LNCaP cells has been previously shown by Ellfolk et al. [27] and in MCF-7 by Ooi et al. [28].

The *in silico* analysis, where the sequences for the aromatase promoters I,3, I,4 and II were analyzed for putative VDREs, showed no sequence identical to the consensus VDRE sequence described by Matilainen et al. [25] nor to the previously described negative VDREs [26]. This might reflect the fact that the mechanisms for vitamin D-induced repression of gene expression, as well as the sequences for negative VDREs, are not fully understood.

3.3. Effects of 1α,25-dihydroxyvitamin D₃ on androstenedione production

We have previously reported that 1α,25-dihydroxyvitamin D₃ treatment decreases the androstenedione production in NCI-H295R cells due to decreased 17,20-lyase activity [3]. In the present study, the effect of 1α,25-dihydroxyvitamin D₃ on androstenedione production was measured in LNCaP cells, MCF-7 cells and NCI-H295R cells.

In contrast to the situation in NCI-H295R cells, 1α,25-dihydroxyvitamin D₃ did not affect the androstenedione production in LNCaP cells and MCF-7 cells (Fig. 4). This indicates that the 1α,25-dihydroxyvitamin D₃-mediated down regulation of 17,20-lyase activity is a cell line-specific effect. It is well-known that 17,20-lyase activity can be regulated by posttranscriptional mechanisms in a tissue-selective manner [29–31].

3.4. Effects of 1α,25-dihydroxyvitamin D₃ on estrogen production

To study whether 1α,25-dihydroxyvitamin D₃ affects the estrogen production, NCI-H295R, MCF-7 and LNCaP cells were cultured as described above and treated with 10 nM 1α,25-dihydroxyvitamin D₃ and the estradiol production was then measured using ELISA. Interestingly, the estradiol production was affected in a cell line-specific manner. In breast cancer MCF-7 cells, the estradiol production was significantly decreased (Fig. 5A). No statistically significant changes were observed in prostate LNCaP cells following vitamin D treatment (Fig. 5C). The results in MCF-7 cells were in accordance with the effects of 1α,25-dihydroxyvitamin D₃ on aromatase gene expression, since aromatase catalyzes estradiol synthesis. The estradiol production in adrenocortical NCI-H295R cells was not affected by treatment with 1α,25-dihydroxyvitamin D₃, even though aromatase gene expression was increased by the same

![Graphs](https://via.placeholder.com/150)
A possible explanation for this could be cell line-specific effects of 1α,25-dihydroxyvitamin D3 on the production of the estradiol precursor androstenedione. We have previously reported that treatment with 1α,25-dihydroxyvitamin D3 resulted in markedly decreased production of androstenedione in NCI-H295R cells due to decreased 17,20-lyase activity [3], while the androstenedione production was unaltered in MCF-7 cells and LNCaP cells following the same treatment (see above). To study the effect of 1α,25-dihydroxyvitamin D3 on estradiol production alone, isolated from the effects on androstenedione production, NCI-H295R cells, LNCaP cells and MCF-7 cells were cultured and treated as described above with the addition of 10 μM androstenedione. If the discrepancy between the results on gene expression level and estradiol production level in NCI-H295R cells is caused by the decreased androstenedione production, 1α,25-dihydroxyvitamin D3 treatment should lead to increased estradiol production in this assay. Indeed, treatment with 1α,25-dihydroxyvitamin D3 increased the estradiol production significantly in NCI-H295R cells (Fig. 5F), which was in agreement with the increase in aromatase gene expression. In LNCaP cells and MCF-7 cells, no statistically significant differences between the control group and the treated group were observed after the addition of androstenedione (Fig. 5B and D).

3.5. Effects of 1α,25-dihydroxyvitamin D3 on androgen production

The production of the two androgens testosterone and dihydrotestosterone was measured in cell culture medium from NCI-H295R, MCF-7 and LNCaP cells treated with 1α,25-dihydroxyvitamin D3 as described above.

Treatment with 1α,25-dihydroxyvitamin D3 resulted in an increased testosterone production in MCF-7 and LNCaP cells while the production was unaltered in NCI-H295R cells (Fig. 6A–C).

The production of dihydrotestosterone in MCF-7 cells was induced 4-fold following treatment with 1α,25-dihydroxyvitamin D3. In LNCaP cells, the production was increased while it was decreased in NCI-H295R cells (Fig. 7).

In conclusion, 1α,25-dihydroxyvitamin D3 alters the androgen production in a cell line-specific manner where the production is increased in breast cancer MCF-7 cells and prostate cancer LNCaP cells while it is decreased in adrenocortical NCI-H295R cells.
The aromatase enzyme activity was not altered by treatment with 1α,25-dihydroxyvitamin D₃ in LNCaP cells (Fig. 8B), which was in accordance with the effect on estradiol secretion (see section 3.4). In NCI-H295R cells, on the other hand, 1α,25-dihydroxyvitamin D₃ treatment resulted in increased aromatase activity after 24 hours treatment (Fig. 8C). Prolonged treatment resulted in a further increase in aromatase activity (Fig. 9A). The effects of 1α,25-dihydroxyvitamin D₃ on aromatase enzyme activity were most pronounced in the group treated with the highest concentration (Fig. 9B).

3.7. Effects of 1α,25-dihydroxyvitamin D₃ on aromatase promoters

It is well-known that the gene expression of aromatase is regulated by different promoters in a tissue-specific way [32,33]. We therefore wanted to study if the observed cell line-specific effects of 1α,25-dihydroxyvitamin D₃ on aromatase gene expression could be the result of such a cell line-specificity in promoter activity.

The effects of 1α,25-dihydroxyvitamin D₃ on different promoter regions of aromatase exon I were examined using real-time RT-PCR (Fig. 10). 1α,25-Dihydroxyvitamin D₃ increased the level of promoter-specific aromatase transcripts for promoter I.4 and promoter I.3 while it decreased the level of promoter II transcripts in NCI-H295R cells. In MCF-7 cells, expression of all three transcripts was decreased following the same treatment, which is in accordance with a previous report [21]. The alterations in promoter-specific expression in LNCaP cells resembled those in NCI-H295R cells, but did not reach statistical significance.

This supports our findings that 1α,25-dihydroxyvitamin D₃ exerts tissue-specific effects on aromatase gene expression. The discrepancy

![Fig. 8. Effects of treatment with 10 nM of 1α,25-dihydroxyvitamin D₃ on aromatase enzyme activity in MCF-7 cells (A), LNCaP cells (B) and NCI-H295R cells (C). Cells were treated with 1α,25-dihydroxyvitamin D₃ dissolved in 99% ethanol or with vehicle only (Control) for 24 hours. After the treatment, 10 μM testosterone was added and the aromatase-catalyzed conversion of testosterone to estradiol was measured, as described in Materials and methods. A: α,25-Dihydroxyvitamin D₃ increased the level of aromatase enzyme activity in MCF-7 cells. B: α,25-Dihydroxyvitamin D₃ increased the level of aromatase enzyme activity in LNCaP cells (Fig. 8B), which was in accordance with the effect on estradiol secretion. C: In NCI-H295R cells, on the other hand, 1α,25-dihydroxyvitamin D₃ treatment resulted in increased aromatase activity after 24 hours treatment (Fig. 8C). Prolonged treatment resulted in a further increase in aromatase activity (Fig. 9A). The effects of 1α,25-dihydroxyvitamin D₃ on aromatase enzyme activity were most pronounced in the group treated with the highest concentration (Fig. 9B).](image-url)

![Fig. 9. Effects of different treatment times (A) and concentrations (B) of 1α,25-dihydroxyvitamin D₃ on aromatase enzyme activity in NCI-H295R cells. Cells were treated with 10 nM 1α,25-dihydroxyvitamin D₃ dissolved in 99% ethanol or with vehicle only (Control) for 6 hours, 24 hours or 48 hours. In a separate experiment, cells were treated with 1 nM, 10 nM or 100 nM 1α,25-dihydroxyvitamin D₃ dissolved in 99% ethanol or with vehicle only (Control) for 24 hours. After the treatment, 10 μM testosterone was added and the aromatase-catalyzed conversion of testosterone to estradiol was measured, as described in Materials and methods. α,25-Dihydroxyvitamin D₃ increased the level of promoter I.4 and promoter I.3 while it decreased the level of promoter II transcripts in NCI-H295R cells. In MCF-7 cells, expression of all three transcripts was decreased following the same treatment, which is in accordance with a previous report [21]. The alterations in promoter-specific expression in LNCaP cells resembled those in NCI-H295R cells, but did not reach statistical significance. This supports our findings that 1α,25-dihydroxyvitamin D₃ exerts tissue-specific effects on aromatase gene expression. The discrepancy](image-url)
between decreased levels of promoter II transcripts and increased total aromatase gene expression in NCI-H295R cells (see section 3.1.) could be a result of tissue differences in promoter activity, i.e., promoter I.4 and promoter I.3 might be more active than promoter II in NCI-H295R cells.

4. Discussion

The human adrenocortical NCI-H295R cell line has been proposed as a model to study estrogenic and androgenic pathways. In the present study, we have examined whether NCI-H295R cells react in the same way as cell models derived from prostate and breast when they are treated with 1α,25-dihydroxyvitamin D$_3$. Interestingly, we found that both estrogen and androgen metabolism were affected in a cell line-specific way. The largest differences were observed between NCI-H295R cells and MCF-7 cells, where aromatase gene expression, estradiol production, aromatase enzyme activity, aromatase promoter activity, testosterone production and dihydrotestosterone production were affected in opposite ways in the two cell lines. The discrepancies between NCI-H295R cells and LNCaP cells were smaller, but still noteworthy. Production of both testosterone and dihydrotestosterone was affected differentially in the two cell lines, as was the gene expression of 5α-reductase.

Our analysis of effects of 1α,25-dihydroxyvitamin D$_3$ on aromatase promoter activities revealed differences between NCI-H295R cells and MCF-7 cells, where promoter I.3 and promoter I.4 were stimulated and promoter II was down regulated in NCI-H295R cells while all three promoters were down regulated in MCF-7 cells. Our results on MCF-7 cells are in agreement with the data obtained by Krishnan et al. [21]. The results from the current study show that NCI-H295R cells respond in a different way than cells derived from important target tissues in estrogen and androgen production and metabolism. These differences between NCI-H295R cells and cells derived from key endocrine target tissues need to be addressed and clarified if NCI-H295R cells should be used as a model for effects on estrogen and androgen metabolism.

This study reports the expression of VDR in NCI-H295R cells. Expression of VDR in MCF-7 cells and LNCaP cells has been reported previously [27,28]. The lack of putative VDREs in the in silico analysis of the aromatase promoters demonstrates the need for further research in the field of mechanisms for vitamin D-induced repression of gene expression. The mechanisms, as well as the consensus sequence for negative VDREs, have not yet been fully clarified.

Regulation of estradiol production and estrogen signaling is key strategy in breast cancer treatment. It has been reported that 1α,25-dihydroxyvitamin D$_3$ stimulates aromatase enzyme activity in placental cells, prostate cells and osteoblasts. Novel data in this study show that 1α,25-dihydroxyvitamin D$_3$ exerts the same effects in adrenocortical NCI-H295R cells with increased gene expression and increased estradiol production.

1α,25-Dihydroxyvitamin D$_3$ exerts tissue-specific effects on aromatase gene expression and activity. To the recent results reported by Krishnan et al. [21] showing that aromatase gene expression and activity are down regulated in different breast cancer cell lines, our results add the knowledge that 1α,25-dihydroxyvitamin D$_3$ decreases estradiol hormone production in MCF-7 cells, but not in NCI-H295R cells or LNCaP cells. The fact that 1α,25-dihydroxyvitamin D$_3$ decreases production of estradiol, a key component in breast carcinogenesis, in breast cancer MCF-7 cells is interesting in the context of 1α,25-dihydroxyvitamin D$_3$ as an anti-cancer agent.

The aromatase enzyme activity was found to be affected in the same manner as the estradiol production. The aromatase enzyme activity was decreased following 1α,25-dihydroxyvitamin D$_3$ treatment in breast cancer MCF-7 cells while it was increased by the same treatment in adrenocortical NCI-H295R cells. When MCF-7 cells were cotreated with 1α,25-dihydroxyvitamin D$_3$ and a VDR antagonist, the suppressing effect of 1α,25-dihydroxyvitamin D$_3$ was abolished. This lends further support to the hypothesis that 1α,25-dihydroxyvitamin D$_3$ affects aromatase in a tissue-selective manner and that the effect, at least in MCF-7 cells, is mediated by VDR.

Interestingly, we also found that 1α,25-dihydroxyvitamin D$_3$ increased androgen production in MCF-7 cells. The production of testosterone was increased by 60% and the production of dihydrotestosterone was increased 4-fold. The markedly increased production of dihydrotestosterone in MCF-7 cells after 1α,25-dihydroxyvitamin D$_3$ treatment was apparently not the result of increased 5α-reductase
expression. An explanation for this effect could be that the decreased aromatase activity increases the concentration of testosterone, which is the precursor for dihydrotestosterone. The increased androgen production in breast cancer cells following vitamin D treatment needs to be studied further to elucidate its potential physiological roles.

In summary, our results reveal previously unknown cell-specific differences in effects of 1α,25-dihydroxyvitamin D3. Furthermore, the current study adds data supporting that 1α,25-dihydroxyvitamin D3 acts as a selective aromatase modulator (SAM) [21] and therefore is an interesting substance for further research in the field of breast cancer prevention and treatment.

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References


