Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 519–526
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Anti-tumor activity of calcitriol: pre-clinical and clinical studies☆

Donald L. Trump[a,b,*], Pamela A. Hershberger[c], Ronald J. Bernardi[c], Sharmilla Ahmed[d], Josephia Muindi[a], Marwan Fakih[a], Wei-Dong Yu[b], Candace S. Johnson[b]

[a] *Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA*
[b] *Department of Pharmacology & Therapeutics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA*
[c] *Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15213, USA*
[d] *Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*

Abstract

1,25-Dihydroxycholecalciferol (calcitriol) is recognized widely for its effects on bone and mineral metabolism. Epidemiological data suggest that low Vitamin D levels may play a role in the genesis of prostate cancer and perhaps other tumors. Calcitriol is a potent anti-proliferative agent in a wide variety of malignant cell types. In prostate, breast, colorectal, head/neck and lung cancer as well as lymphoma, leukemia and myeloma model systems calcitriol has significant anti-tumor activity in vitro and in vivo. Calcitriol effects are associated with an increase in G_0/G_1 arrest, induction of apoptosis and differentiation, modulation of expression of growth factor receptors. Glucocorticoids potentiate the anti-tumor effect of calcitriol and decrease calcitriol-induced hypercalcemia. Calcitriol potentiates the antitumor effects of many cytotoxic agents and inhibits motility and invasiveness of tumor cells and formation of new blood vessels. Phase I and II trials of calcitriol either alone or in combination with carboplatin, taxanes or dexamethasone have been initiated in patients with androgen dependent and independent prostate cancer and advanced cancer. Data indicate that high-dose calcitriol is feasible on an intermittent schedule, no dose-limiting toxicity has been encountered and optimal dose and schedule are being delineated. Clinical responses have been seen with the combination of high dose calcitriol + dexamethasone in androgen independent prostate cancer (AIPC) and apparent potentiation of the antitumor effects of docetaxel have been seen in AIPC. These results demonstrate that high intermittent doses of calcitriol can be administered to patients without toxicity, that the MTD is yet to be determined and that calcitriol has potential as an anti-cancer agent.

Keywords: Vitamin D; Anti-tumor; Cancer; Clinical trials; Growth factors; Prostate cancer

1. Introduction

Vitamin D is a steroid hormone, which modulates calcium homeostasis through actions on kidney, bone and the intestinal tract [1]. Vitamin D is synthesized in the skin from 7-dehydro-cholesterol in response to ultraviolet light, is 25-hydroxylated to 25-hydroxycholecalciferol in the liver and 1-hydroxylated to the active form, 1,25-dihydroxycholecalciferol or calcitriol in the kidney [1,2]. In addition to classical effects on bone and mineral metabolism, calcitriol is also involved in the proliferation and differentiation of a variety of different cell types and tissues [1–13].

2. Calcitriol antitumor activities

The VDR is found, not only in classical target organs (intestinal tract, kidney, bone), but also in many other epithelial and mesenchymal cells as well as leukemic cells, and many malignant cell types [1,2]. Calcitriol inhibits growth in vitro and in vivo in murine and human breast and colon cancer models [5,6,14,15]. Calcitriol can induce differentiation, cell cycle arrest and/or apoptosis in leukemic and tumor cells [6,16,17]. Progression through the cell cycle is regulated by cyclins and their associated cyclin dependent kinases (cdk). The cdk inhibitors $p21^{Waf1/Cip1}$ and $p27^{Kip}$ are implicated in G_1 phase arrest [18]. In HL-60 cells, a human myelomonocytic leukemia cell line, calcitriol arrests cells in G_1; this effect is mediated through an increase in p27 [16]. Calcitriol mediated arrest in G_0/G_1 is also observed in human breast cancer lines [19]. In U937, a human myelomonocytic cell line, a functional VDRE has been identified in the p21 promoter region and transcriptional activation of p21 by the

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +1-716-845-3499; fax: +1-716-845-3423.
E-mail address: donald.trump@roswellpark.org (D.L. Trump).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.068

VDR induces differentiation in this cell line [17]. Apoptosis is mediated by biochemically diverse stimuli that activate caspases and bring about cellular destruction through specific cleavage of key cellular proteins [20]. The bcl-2 protein, which is overexpressed in many tumors, suppresses apoptosis and the bax protein promotes apoptotic cell death [21]. Calcitriol induces apoptosis in MCF-7 breast cancer cells as well as in HL-60 leukemic cells and the expression of bcl-2 is down-regulated by calcitriol in HL-60 [22].

3. Growth factor signaling and calcitriol

EGFR is a transmembrane glycoprotein found primarily on cells of epithelial origin [23]. Agents like ZD1839 target the receptor and act by inhibiting tyrosine receptor kinase and preventing the phosphorylation of the receptor [24]. ZD1839 synergizes with platinum agents and taxanes and may act through effects on the Erk pathway [25]. Calcitriol can modulate the expression of the EGFR [26] and calcitriol treatment increases EGF binding [27]. Calcitriol can also inhibit the degradation of EGFR mRNA [28]. Calcitriol treatment can also lead to a reduction in insulin-like growth factor-I (IGF-I) signaling. This effect was determined to be due to an increase in IGF-I binding proteins, which act to titrate the growth factor away from the receptor, as well as direct effects on the IGF-I receptor levels [29]. Similarly, the TGFβ signaling pathway has also been implicated in carrying out the antiproliferative effects of calcitriol via modulation of both the receptor and the ligand [30] which may act independently of EGFR. Inhibition of other growth factor receptor pathways may sensitize cells to EGFR inhibition. In support of this possibility, increased insulin-like growth factor receptor-I signaling has been implicated in mediating resistance to EGFR inhibition [31]. Interestingly, EGF can increase the expression of VDR in the normal rat intestine [32].

4. Glucocorticoids and calcitriol

Treatment of cells with calcitriol and glucocorticoids influences the cellular content of the VDR [33]. While glucocorticoids do not bind the VDR [34], they influence calcitriol-ligand binding to the VDR in normal cells and tissues [33,35]. Glucocorticoids modulate calcitriol effects on Ca^{+2} transport and may alter the metabolism of calcitriol [36]. In addition, glucocorticoids are utilized clinically to ameliorate hypercalcemia in a number of clinical indications, including calcitriol intoxication [37].

5. Antitumor effects in vitro/in vivo

In multiple model systems (murine syngeneic SCC VII/SF, metastatic Dunning rat prostate adenocarcinoma and the human xenograft PC-3 prostate), calcitriol has significant antiproliferative effects in vitro and in vivo [3,4,8,9]. In the metastatic rat Mat-LyLu (MLL) model, calcitriol causes inhibition of tumor growth, but also a significant reduction in the number and size of lung metastases [4]. Calcitriol also causes arrest of tumor cells in G_0/G_1 and is associated with altered expression of cell cycle regulatory proteins [4,10]. In vitro calcitriol treatment results in decreased expression of p21 mRNA and protein, and increased expression of p27 mRNA and protein and Rb dephosphorylation [11]. In vivo, a decrease in tumor volume induced by calcitriol correlates with a significant decrease in the intratumoral p21 expression. Apoptotic cell death occurs through the activation of caspases, which results in specific cleavage of key cellular proteins including poly (ADP-ribose) polymerase or PARP [20]. We demonstrated by Western blot that calcitriol induced 90–100% PARP cleavage in MLL cells treated with 10 μM of calcitriol (half the IC50) [8]. Induction of apoptosis was confirmed using annexin binding as a measure of phosphatidylserine exposure. The caspase inhibitors, ZVAD-fmk and DEVD-fmk had no direct effect on cells but significantly inhibited calcitriol-mediated increase in annexin binding. In addition, bax was unchanged and bcl-2 was decreased at 24 h in MLL treated with calcitriol; this results in an increased bax/bc1-2 ratio, which favors death.

6. Effect of calcitriol and dex on antitumor activity and VDR ligand binding

Dex significantly enhances calcitriol antitumor efficacy, in vitro and in vivo [12,13]. In SCC and PC-3, dex was able to significantly enhance in vitro and in vivo clonogenic cell kill as compared to either agent alone. This combination induces significant tumor regression in this model system. To further examine the effects of calcitriol and dex, we examined antiproliferative, cell cycle and apoptotic effects of this combination in SCC. The glucocorticoid antagonist, RU486 was able to block the dex-induced enhancement of calcitriol antiproliferative activity. Calcitriol induces cell cycle arrest [9,10] and dex plus calcitriol results in a higher percentage of cells in G_0/G_1 phase as compared to either calcitriol or dex alone with significant inhibition with RU486. The combination of calcitriol/dex led to an increase in the cleaved, active form of caspase-3 and a further reduction in full length PARP as compared to calcitriol alone and RU486 blocked this effect. In addition, the levels of P-Erk and P-Akt were reduced in cells treated with calcitriol and a further reduction was observed in combination with dex suggesting that dex enhances calcitriol pro-apoptotic signaling. RU486 inhibited the effects of dex on both Erk and Akt, suggesting that the GR may be required for these activities and that they may be important targets for antitumor activity.

Dex significantly increases VDR receptor content (number) without changing the affinity for ligand (Kd) [12]. In addition, the combination of calcitriol and dex resulted in a

significant increase in VDR protein as compared to calcitriol alone or dex alone. We also examined whether changes could be observed in vivo in animals treated with calcitriol. Tumor-bearing mice were treated for 3 days with calcitriol and tumors harvested 4 h later and whole cell extracts analyzed by Western blot. At 4 h after the last injection of calcitriol, VDR protein was induced in the animals treated with calcitriol. Modulation of VDR demonstrated delivery of drug to the tumor or the presence of calcitriol within the tumor; we also demonstrated that enhanced VDR in the tumor correlated with an increase in antitumor efficacy.

7. Calcitriol enhancement paclitaxel-mediated antitumor efficacy

In vitro, pretreatment with calcitriol or the calcitriol analogue, 1,25-dihydroxy16-ene-23-ynecholecalciferol significantly enhanced cisplatin, carboplatin, docetaxel or paclitaxel mediated clonogenic cell kill as compared to either agent alone [10]. In the in vivo excision clonogenic assay, pretreatment with calcitriol markedly enhanced cisplatin-, carboplatin-, docetaxel- or paclitaxel-mediated clonogenic tumor cell kill, even at low doses of cytotoxic drug as compared to drug alone. As described below, clinical trials were initiated based on these pre-clinical data to examine the combination of calcitriol and either carboplatin or paclitaxel.

The antiproliferative activity of calcitriol is associated with decreased p21 expression in vitro and in vivo [11]. Recent studies indicate p21 suppression increases sensitivity to paclitaxel [38]. We have examined the molecular accompaniments of paclitaxel cytotoxicity with and without calcitriol in the prostate model, PC-3. The in vitro effects of calcitriol and paclitaxel on p21, Bcl-2, caspase-3, and poly (ADP-ribose) polymerase (PARP) in PC-3 were evaluated by Western blot. Treatment in vitro with calcitriol resulted in a decrease in p21 expression in PC-3. Paclitaxel induced apoptosis in PC-3 as evidenced by the time-dependent loss of procaspase-3 and full-length PARP. Paclitaxel caused increase of p21 and loss of Bcl-2. An increase was observed in PARP cleavage in PC-3 treated with calcitriol/paclitaxel as compared to paclitaxel alone. Thus, calcitriol and paclitaxel have enhanced antitumor effects in PC-3 as compared to either agent alone.

8. Calcitriol enhancement of cisplatin-mediated antitumor activity

We previously demonstrated an enhanced in vitro and in vivo antitumor effect when the Vitamin D analog Ro23-7553 was combined with cisplatin [10]. To further explore the nature of the interaction between calcitriol and cisplatin, studies were initiated to define the mechanism by which the combination displays enhanced activity [39]. Median-dose effect analysis demonstrates that calcitriol and cisplatin act synergistically to inhibit SCC growth. When SCC cells were treated with calcitriol and/or cisplatin, greater caspase-3 activation was observed for the combination than for either agent alone. No alterations in cellular platinum concentration or platinum:DNA adducts were observed for calcitriol/cisplatin co-treatment compared to cisplatin treatment alone. Cisplatin induced p53 and its downstream targets, p21 and Bax, in both adherent (non-apoptotic) and floating (apoptotic) cell populations. In contrast, calcitriol reduced p53, p21, and Bax to nearly undetectable levels in adherent cells. In the floating cells, calcitriol reduced levels of p53 and p21, but Bax expression was maintained at control levels. The two agents also had divergent effects on survival and stress signaling pathways. P-Erk and P-Jun levels increased after treatment with cisplatin, but decreased after treatment with calcitriol and calcitriol/cisplatin. Moreover, cisplatin decreased levels of MEKK-1, while calcitriol upregulated and calcitriol/cisplatin further upregulated MEKK-1. We propose that the increased cytotoxicity for calcitriol/cisplatin results from cisplatin enhancement of calcitriol-induced apoptotic signaling through MEKK-1. Preliminary data also demonstrates that in the host cell activation assay, calcitriol may enhance cisplatin-mediated cytotoxicity through inhibitory effects on DNA repair and studies are in progress to explore these mechanisms.

9. Effects of calcitriol on the expression of EGFR family growth factor receptors

Calcitriol regulates the transcription of a large number of target genes via its interactions with the Vitamin D receptor. While the overall effect of calcitriol treatment is antiproliferative in a variety of cancer models, calcitriol may also cause some molecular changes that promote growth and survival. We have observed that treatment of SCC cells with calcitriol leads to an increase in EGFR protein levels [40], particularly in the population of cells that remain adherent to the tissue culture flask. The upregulation of EGFR protein correlates with a recent report indicating that calcitriol inhibits the degradation of EGFR mRNA [28]. In addition, while the levels of P-Erk and P-Akt, two downstream targets of EGFR signaling, are markedly reduced in the subset of cells that detach from the tissue culture plate and are apoptotic after treatment with calcitriol; these effects are much less pronounced in the cells that remain adherent [6]. As reported previously [40], EGFR protein levels were increased in SCC cells after treatment with 10 nM calcitriol. ZD1839 alone did not affect EGFR protein levels; however, it limited the ability of calcitriol to increase EGFR levels. To examine whether the increased EGFR protein in calcitriol-treated cells could lead to increased signaling, we assessed the ability of EGF stimulation to generate P-EGFR in cells treated with calcitriol. Treatment with calcitriol resulted in higher P-EGFR levels

than control; while ZD1839, either alone or in combination with calcitriol, strongly inhibited the formation of P-EGFR. HER2/neu can form heterodimers and cross-phosphorylate EGFR, and recent reports indicate that its expression level can affect the sensitivity to ZD1839 [41]. Therefore, we examined the effects of calcitriol on HER2/neu levels. While the protein levels were not significantly altered by calcitriol or ZD1839, there was a slight shift in mobility in groups treated with calcitriol, perhaps indicating an effect on post-translational modification. However, HER2 containing *p*-tyrosine residues was still detectable in the faster migrating band after calcitriol treatment; therefore, the increased mobility caused by calcitriol cannot be explained simply by the loss of HER2 phosphorylation. Effects of calcitriol on EGFR, EGFR-P and HER2/neu were observed in a number of other cell lines, including PC-3 and LnCAP. When cells were isolated (attached versus floating) and then fractionated, the EGFR was found to be up-regulated by calcitriol in pre-apoptotic cells (attached), but, in contrast to MEKK-1, up-regulated EGFR was localized almost exclusively to the membrane fraction. Furthermore, EGFR levels were nearly undetectable in apoptotic cells (floating cells). Erk, on the other hand, was nearly completely found in the cytosolic fraction, with no change in levels in pre-apoptotic cells and a modest down-modulation in apoptotic cells.

Since calcitriol increased both total EGFR and P-EGFR levels in the viable, adherent cell population, we used an in vitro clonogenic assay to test whether the combination of calcitriol with ZD1839 would lead to enhanced antiproliferative activity. ZD1839 had only limited activity as a single agent in this model. While no enhancement was observed with a single treatment of 10 μM ZD1839, five daily treatments of 2 μM significantly enhanced the effects of calcitriol ($P < 0.001$, Student's *t*-test). The Erk and Akt pathways are both downstream of EGFR and have been observed to be inhibited by calcitriol [6], as a result, we examined the effects of these two agents on the levels of phosphorylated, activated Erk and Akt. Interestingly, while neither agent alone had dramatic effects on either P-Erk or P-Akt, combination of the two agents caused significant reductions in both markers.

In order to determine whether similar antiproliferative effects could be achieved by the combination of calcitriol and ZD1839 against a human SCC cell line, we tested this combination using the SCC-25 cell line. Over a 10 days period, calcitriol alone had little effect on cell proliferation, while ZD1839 caused nearly a six-fold reduction in cell number; however, combination of the two agents held the cell count to near baseline levels. To test the antitumor effects of the combination of calcitriol and ZD1839 in vivo, we treated nude mice bearing SCC-25 xenografts with either agent alone or the combination for 2 consecutive weeks and followed tumor growth. As was seen in vitro, the combination was clearly superior to either agent alone. This enhanced antitumor effect was seen without any apparent increase in toxicity.

10. Clinical trials with calcitriol

Calcitriol has been utilized in a number of clinical studies. The majority of these were in leukemia and myelodisplasia. Although some evidence of response was seen, the results were largely disappointing [42,43]. Calcitriol causes hypercalcemia by increasing intestinal calcium absorption and mobilizing bone stores [44]. The "common wisdom" is that doses of calcitriol sufficient to achieve exposure similar to those seen in in vitro models cannot be safely given because hypercalcemia intervenes.

This is not as clear as commonly believed. Two groups have now demonstrated that high dose intermittent therapy with calcitriol is safe [45–47]. In fact, in neither instance has any limiting hypercalcemia been noted. Beer and colleagues [47] have administered up to 2.6 μg/kg of calcitriol on a 1× per week schedule without toxicity. Apparent limited absorption was encountered and the maximum dose used in continued studies was 0.5 μg/kg, not because of toxicity, but rather because of apparent limited bioavailability. Our group has administered calcitriol doses as high as 22 μg QD × 3 weekly and 38 μg QD × 3 weekly with paclitaxel without any toxicity. Therefore, it appears quite clear that high-dose, intermittent schedule calcitriol is feasible and safe. Our group has completed four clinical trials of high dose oral calcitriol. The first trial was a phase I study to evaluate the pharmacokinetics and MTD of calcitriol following subcutaneous (s.c.) QOD administration [46]. Thirty-six patients were entered at doses ranging from 2 to 10 μg QOD; dose-limiting toxicity (hypercalcemia) occurred in three of three patients entered at the 10 μg QOD dose. Hypercalciuria occurred at all dose levels examined. No other toxicity was seen. Assessment of serum calcitriol concentrations by a radioimmunoassay revealed a decrease in concentration-time curves on the 7th day compared to the first day of therapy. A dose dependent increase in peak serum level and estimated area under the curve (AUC) were seen; the maximum serum levels occurred at the 10 μg QOD dose: 288 ± 74 and 321 ± 36 pg/mL days 1 and 7, respectively. The normal range of calcitriol serum concentrations using this assay is 16–56 pg/mL. Serum calcitriol levels were maintained at near peak concentrations for at least 8 h following s.c. injection. This study indicates that substantial doses of calcitriol can be administered via this route with tolerable toxicity.

11. Calcitriol + glucocorticoids: prostate cancer

We have completed a phase II study of calcitriol and dex in androgen independent prostate cancer (AIPC) [48]. Calcitriol and dex were administered according to the following schedule: calcitriol 8 μg Monday, Tuesday and Wednesday (MTW) weekly X4, then if no toxicity was seen the dose was escalated to 10 μg MTW for 1 month. If no toxicity occurred the dose of calcitriol was increased to 12 μg MTW weekly for the duration of the study. Dex was administered

orally 4 mg sunday MTW each week. Forty-three patients were treated and 35 received 12 μg MTW > 1 month; no patient has required dose reduction because of hypercalcemia. The only calcitriol related toxicity in this trial was the development in two patients of urinary tract stones. All patients undergo pretreatment and Q3 month renal ultrasound to monitor for nephrolithiasis. Thirty-five patients are evaluable and 28% of the 35 evaluable patients experienced a 50% reduction in PSA; patients with bone pain at study entry have experienced pain relief. Eighty percent of patients have experienced a slowing in the rate of PSA rise and 34% have had stable disease or decrease in PSA (>50% reduction). Our studies indicate that modification in the schedule and route of administration of calcitriol and dex permit dose escalation of this agent.

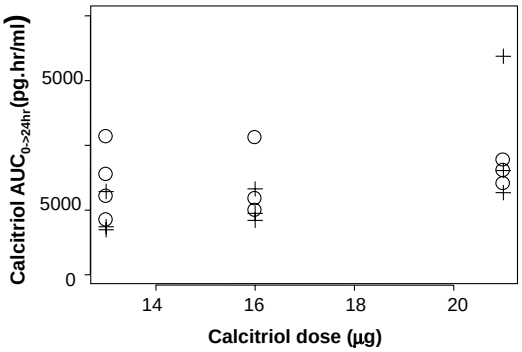

Fig. 2. AUC from patients treated with calcitriol from the phase I trial of calcitriol + paclitaxel where pk_a was determined on day one following calcitriol administration. Patients received either the capsule form (cross symbol) or the liquid form (open symbol).

12. Clinical trials with calcitriol alone and in combination with cytotoxic agents

We conducted two phase I trials of calcitriol + cytotoxic drugs; calcitriol/paclitaxel and calcitriol/carboplatin [45]. Patients with advanced cancer were treated with carboplatin (AUC = 5) Q28 days + escalating doses of calcitriol QDX3 Q28 days. Calcitriol starting dose was 4 μg QDX3. Studies were designed such that in each patient, carboplatin was given on day 1 before calcitriol in one of the first two cycles of treatment and on day 3 after two days of high dose calcitriol on the other. This permits comparison of AUC of carboplatin in the same patient before and after pretreatment with calcitriol. Dose-limiting toxicity was not encountered in this trial. The AUC of carboplatin was higher in each patient following calcitriol than before calcitriol (mean AUC = 7.6 μg/ml h ± 1.8, carboplatin day 3 [DDDC] versus AUC = 6.6 μg/ml h ± 1.4, carboplatin day 1 [CDDD], $P = 0.04$). This increase in AUC was observed with no change with increasing calcitriol dose (4–24 μg, from left to right). While no dose limiting toxicity has been seen, myelosuppression (percentage change in platelet count) following the sequence carboplatin → calcitriol was less than that following calcitriol → carboplatin, consistent with the change in AUC. No clinically detectable renal impairment has been seen with either sequence. These data indicate that potentiation of carboplatin by calcitriol may in part be related to reduced carboplatin clearance.

In another trial, patients with advanced cancer were treated with paclitaxel (80 mg/m^2 weekly × 6) + escalating doses of calcitriol, QDX3 weekly 6×. The starting dose of calcitriol was 4 μg po QDX3, weekly and we entered patients through the 38 μg dose level where it appears that we reached saturable concentrations at 16–20 μg (see pharmacokinetic data below (Fig. 1A and B) [49]. No dose limiting toxicity was encountered. In this study, paclitaxel was administered on day 1, cycle 1 of therapy prior to any calcitriol therapy and on day 3 with the third dose of calcitriol in week 2 and all subsequent weeks. This permitted evaluation of the effect of calcitriol on paclitaxel pharmacokinetics—week 1 versus week 2. No changes in peak concentration, AUC or $T_{1/2}$ were noted. To investigate the issue of bioavailability of calcitriol, patients were given escalating doses of calcitriol starting at 14 μg using a liquid formulation of calcitriol with potentially greater bioavailability (Fig. 2).

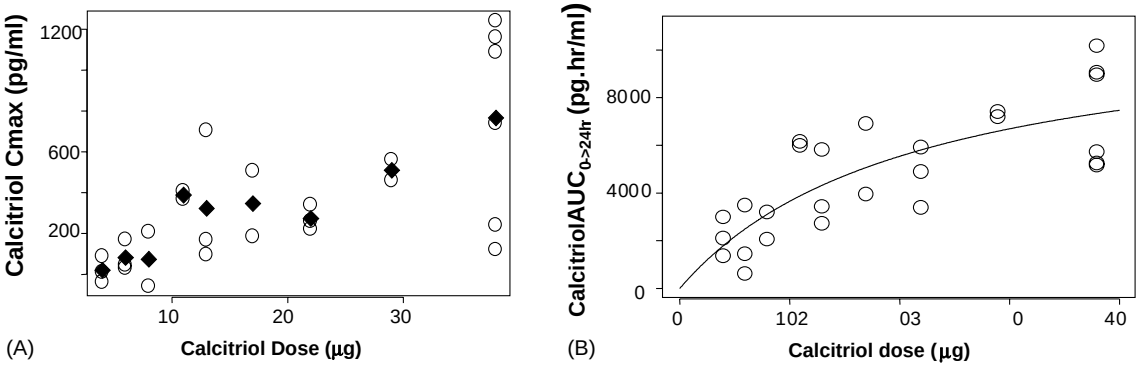

Fig. 1. (A) Scatter plot of the maximum serum calcitriol concentration (C_{max}) vs. calcitriol doses. Closed symbols represent mean values at each dose level. (B) Baseline-subtracted serum calcitriol $AUC_{0\rightarrow 24\,h}$ (area under the concentration-time curve for the 24 h period after calcitriol administration) plotted against dose, and a fit of the Michaelis–Menten function.

13. Calcitriol plasma pharmacokinetics

Pharmacokinetic studies were required in at least two of three patients at each dose level of the calcitriol/paclitaxel clinical trial and were performed in 26 of the 36 patients; six patients at the highest dose level (38 μg) underwent pharmacokinetic studies (from 89 patients). Baseline plasma calcitriol concentrations of the 26 cancer patients resulted in a median concentration of 26 pg/ml (range 13–81). The normal range for this assay is 16–74 pg/ml. Serum calcitriol concentrations higher than baseline occurred within 1 h of oral calcitriol administration. A scatter plot of the maximum concentration of calcitriol (C_{max}) for each patient studied at each dose level is portrayed in Fig. 1A.

As shown in Fig. 1B, baseline-subtracted serum calcitriol $AUC_{0\rightarrow 24\,h}$ (area under the concentration-time curve for the 24 h period after calcitriol administration) is plotted against dose. A fit to the Michaelis–Menten function ($AUC = a \times dose/(1 + b \times dose)$) indicates that $AUC_{0\rightarrow 24\,h}$ is not proportional to dose ($a = 540 \pm 140$ pg h/ml μg; if AUC were proportional to dose, b would equal 0). A statistical test for proportionality gives a P-value of 0.0014. The effect of the nonlinearity over the range of doses studies is large: the fit value of $AUC_{0\rightarrow 24\,h}$ at 38 μg was only four times that at 4 μg, instead of the 9.5 times expected for a proportional relationship. However, no deviation from linearity can be detected up to a dose of 17 μg ($P = 0.4$). In addition, there is no relationship between serum calcium and dose. None of the patients became hypercalcemic.

Clinical studies to date have predominantly utilized oral administration of capsules at 0.5 μg per capsule. At doses of 38 μg, the patients took 72 gel capsules at one time. To investigate decreased bioavailability and lack of absorption due to the mass of gel caps in the stomach, we utilized a liquid formulation of calcitriol in palm oil (1 μg/ml) which was tasteless and easy to swallow. As shown in Fig. 2, no significant difference was observed in C_{max} or AUC in patients given liquid or capsules at various doses.

Beer and colleagues [50] have recently reported that the combination of calcitriol (0.5 μg/kg) day 1 and docetaxel (36 mg/m^2) day 2 yield a PSA response rate (>50% decrease) of 81%. This is twice what one would expect with docetaxel alone. While this is a single institution, phase II trial the results are provocative and consistent with preclinical data indicating potentiation of taxanes by calcitriol. A phase III trial of docetaxel ± calcitriol is underway.

14. Calcitriol pharmacokinetic parameters in mice/man

Key to clinical trial design is relating serum calcitriol levels in animals that resulted in a significant antitumor effect to human serum levels. In both mice and rats, the AUC for calcitriol from both i.v. and i.p. routes of administration was not significantly different with i.v. resulting in a higher C_{max} as compared to i.p. [51]. Peak levels are reached at 1 h and return to normal at 24 h following 0.125 and 0.5 μg of calcitriol. Both 0.125 and 0.5 μg when administered to tumor-bearing mice, result in a significant anti-tumor response. No diurnal variation was observed in serum calcitriol levels during the 24 h sampling period. A similar pattern was obtained for pk_a parameters of calcitriol in tumor-bearing mice. To determine whether the mouse serum calcitriol levels that resulted in an anti-tumor effect could ever be achieved in man, we compared AUC and C_{max} levels obtained in the calcitriol/paclitaxel phase I clinical trial where we have administered 38 μg daily × 3 without toxicity. As shown in Table 1, at 0.125 μg (the lowest dose to consistently result in a significant antitumor effect in mice), the AUC was 37.3 ng/h/ml and this compares in man at 38 μg to 7.5 ng/h/ml. Similarly, in mice the C_{max} was 9.2 ng/ml and this compared to 1.4 ng/ml in man. At the 0.042 μg dose in mice, an antitumor effect could be seen but was not consistently observed. Therefore, effective serum calcitriol levels are five to seven times higher in mice than compared to highest oral dose administered in man (38 μg).

Table 1
Calcitriol pharmacokinetic parameters mice and man

Dose (μg)	$AUC_{0\rightarrow 24\,h}$ (ng/ml)	C_{max} (ng/ml)
Mouse (IP)		
13	3.9 ± 1.4	0.5 ± 0.3
17	5.4 ± 2.1	0.5 ± 2.2
Man (PO)		
0.042	3.6	0.7
0.125	37.3	9.2
38	7.5 ± 2.1	1.4 ± 0.9

These data indicate that serum concentrations in mice that are associated with substantial antitumor effects are only four- to eight-fold higher than those achieved in man without any toxicity. As noted above, further dose escalation of calcitriol will be limited by absorption concerns. Therefore, we have initiated a phase I trial of intravenous calcitriol (Calcijex®) with either docetaxel (in men with AIPC) or gefitinib (in any patient with an advanced, epithelial cancer). These studies will provide more definitive information regarding the MTD of calcitriol on an intermittent schedule as well as pharmacokinetics and dynamics of this dose and schedule.

Acknowledgements

This work is supported by grants from the NCI (CA95045, CA85142 and CA67267) and CaPCURE.

References

[1] D.D. Bikle, S. Pillai, Vitamin D, calcium, and epidermal differentiation, Endocrine Rev. 14 (1993) 3.

[2] H. Reichekl, H.P. Koeffler, A.W. Norman, The role of vitamin D endocrine system in health and disease, New Engl. J. Med. 320 (1989) 980.
[3] M.C. McElwain, M.A. Dettlebach, R.A. Modezelewski, et al., Antiproliferative effects in vitro and in vivo of 1,25-dihydroxyvitamin D_3 and a vitamin D_3 analog in a squamous cell carcinoma model system, Mol. Cell Diff. 3 (1) (1995) 31.
[4] R.H. Getzenberg, B.W. Light, P.E. Lapco, et al., Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system, Urology 50 (1997) 999.
[5] K.W. Colston, S.K. Chander, A.G. Mackay, et al., Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro, Biochem. Pharmacol. 44 (1993) 693.
[6] T.F. McGuire, D.L. Trump, C.S. Johnson, Vitamin D_3-induced apoptosis of murine squamous cell carcinoma cells: selective induction of caspase-dependent MEK cleavage and up-regulation of MEKK-1, J. Biol. Chem. 276 (28) (2001) 26365.
[7] J.Y. Zhou, A.W. Norman, D.L. Chen, 1,25-dihydroxy-16-ene-23-yne-vitamin D3 prolongs survival time of leukemic mice, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 3929.
[8] R.A. Modzelewski, Apoptotic effects of paclitaxel and calcitriol in rat dunning MLL and human PC-3 prostate tumor cells in vitro, Proc. Am. Assoc. Cancer Res. 40 (1999) 580.
[9] P.A. Hershberger, W.D. Yu, R.A. Modzelewski, et al., Enhancement of paclitaxel antitumor activity in squamous cell carcinoma and prostatic adenocarcinoma by 1,25-dihydroxycholecaciferol (1,25-D_3), Cancer Res. 7 (2001) 1043.
[10] B.W. Light, W.-D. Yu, M.C. McElwain, et al., Potentiation of cisplatin anti-tumor activity using a vitamin D analogue in a murine squamous cell carcinoma model system, Cancer Res. 57 (1997) 3759.
[11] P.A. Hershberger, R.A. Modzelewski, Z.R. Shurin, In vitro and in vivo modulation of $p21^{Wafl/Cip1}$ and $p27^{Kip1}$ in squamous cell carcinoma I response to 1,25-dihydroxycholecalciferol (calcitriol), Cancer Res. 59 (1999) 2644.
[12] W.-D. Yu, M.C. McElwain, R.A. Modzelewski, et al., Potentiation of 1,25-dihydroxyvitamin D_3-mediated anti-tumor activity with dexamethasone, J. Natl. Cancer Inst. 90 (1998) 134.
[13] R.J. Bernardi, D.L. Trump, W.-D. Yu, et al., Combination of 1α,25-dihydroxyvitamin D_3 with dexamethasone enhances cell cycle arrest and apoptosis: role of nuclear receptor cross-talk and Erk/Akt signaling, Clin. Cancer Res. 7 (2001) 4165.
[14] L. Frappart, N. Falette, M.F. Lefebvre, et al., In vitro study of effects of 1,25-dihydroxyvitamin D_3 on the morphology of human breast cancer cell line B.T. 20, Differentiation 40 (1989) 63.
[15] M. Shabahang, R.R. Buras, F. Davoodi, et al., 1,25-dihydroxyvitamin D_3 receptors as a marker of human colon carcinoma cell line differentiation and growth inhibition, Cancer Res. 53 (1993) 3712.
[16] Q.M. Wang, J.B. Jones, G.P. Studzinski, Cyclin-dependent kinase Inhibitor p27 as a mediator of the G1-S phase block induced by 1,25-dihydroxyvitamin D_3 in HL60 cells, Cancer Res. 56 (1996) 264.
[17] M. Liu, M.-H. Lee, M. Cohen, et al., Transcriptional activation of the cdk inhibitor p21 by vitamin D_3 leads to the induced differentiation of the myelomonocytic cell line U937, Genes Devel. 10 (1996) 142.
[18] J.R. Biggs, A.S. Kraft, Inhibitors of cyclin-dependent kinase and cancer, J. Mol. Med. 73 (1995) 509.
[19] M.S. Sheikh, H. Rochefort, M. Garcia, Overexpression of $p21^{WAF1/C1P1}$ induces growth arrest, giant cell formation and apoptosis in human breast carcinoma cell lines, Oncogene 11 (9) (1995) 1899.
[20] P.A. Henkart, ICE family proteasees: mediators of all apoptotic cell death? Immunity 4 (1996) 195.
[21] D.M. Hockenbery, The bc1-2 oncogene and apoptosis, Semin. Immunol. 4 (1992) 413.
[22] E. Elstner, M. Linker-Israeli, T. Umiel, et al., Combination of a potent 20-epi-vitamin D_3 analogue (KH 1060) with 9-*cis*-retinoic acid irreversibly inhibits clonal growth, decreases bc1-2 expression, and induces apoptosis in HL-60 leukemic cells, Cancer Res. 56 (1996) 3570.
[23] D.M. Thompson, G.N. Gill, The EGF receptor: structure, regulation, and potential role in malignancy, Cancer Surv. 4 (1985) 767–788.
[24] A.E. Wakeling, Epidermal growth factor receptor tyrosine kinase inhibitors, Science 2 (2002) 382–387.
[25] F. Ciardiello, R. Caputo, R. Bianco, et al., Antitumor effect and potentiation of cytotoxic drugs activity in human cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor, Clin. Cancer Res. 6 (2000) 2053–2063.
[26] W.M. Tong, E. Kallay, H. Hofer, et al., Growth regulation of human colon cancer cells by epidermal growth factor and 1,25-dihydroxyvitamin D_3 is mediated by mutual modulation of receptor expression, Eur. J. Can. 34 (1998) 2119–2125.
[27] P.M. Petkovich, J.L. Wrana, A.E. Grigoriadis, et al., 1,25-Dihydroxyvitamin D_3 increases epidermal growth factor receptors and transforming growth factor beta-like activity in a bone-derived cell line, J. Biol. Chem. 262 (1987) 13424–13428.
[28] E.A. Gonzales, S. Disthabanchong, R. Kowalewski, et al., Mechanisms of the regulation of EGF receptor gene expression by calcitriol and parathyroid hormone in UMR 106-01 cells, Kidney Int. 61 (2002) 1627–1634.
[29] B.J. Boyle, X.Y. Zhao, P. Cohen, Insulin-like growth factor binding protein-3 mediates 1 alpha, 25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1, J. Urol. 165 (2001) 1319–1324.
[30] L. Yang, J. Yang, S. Venkateswarly, et al., Autocrine TGFbeta signaling mediates vitamin D_3 analog-induced growth inhibition in breast cells, J. Cell Physiol. 188 (2001) 383.
[31] A. Chakravarti, J.S. Loeffler, N.J. Dyson, Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling, Cancer Res. 62 (2002) 200.
[32] D.E. Bruns, A.V. Krishnan, D. Feldman, et al., Epidermal growth factor increases intestinal calbindin-D9K and 1,25-dihydroxyvitamin D receptors in neonatal rats, Endocrinology 125 (1989) 478.
[33] T.L. Chen, C.M. Cone, E. More-Holton, et al., Glucocorticoid regulation of 1,25-(OH)2-vitamin D_3 receptors in cultured mouse bone cells, J. Biol. Chem. 257 (1982) 13564.
[34] R. Boullon, W.H. Okamura, A.W. Norman, Structure-function relationships in the vitamin D endocrine system, Endocrine Rev. 16 (1995) 200.
[35] M. Hirst, D. Feldman, Glucocorticoid regulation of 1,25-(OH)2-vitamin D_3 receptors: divergent effects on mouse and rat intestine, Endocrine 111 (1982) 1400.
[36] D.V. Kimberg, R.D. Baerg, E. Gershon, et al., Effect of cortisone treatment on the active transport of calcium by the small intestine, J. Clin. Investig. 50 (1971) 1321.
[37] R.C. Haynes, Agents affecting calcification: calcium, parathyroid hormone, calcitonin, vitamin D and other compounds, in: A.G. Gilman, T.W. Rall, A.S. Nies, P Taylor (Eds.), The Pharmacological Basis of Therapeutics, New York, Pergamon Press, 1990, p. 1496.
[38] M. Barloule, P. Chadebech, V. Baldin, et al., Involvement of p21 in mitotic exit after paclitaxel treatment in MCF-7 breast adenocarcinoma cell line, Oncogene 15 (1997) 2867.
[39] P.A. Hershberger, T.F. McGuire, W.-D. Yu, et al., Cisplatin potentiates 1,2-dihydroxyvitamin D_3-induced apoptosis, Mol. Cancer Therap. 1 (10) (2002) 821–829.
[40] T.F. McGuire, J. Brallier, D.L. Trump, et al., 1,25-Dihydroxycholecalciferol (calcitriol) inhibits mitogen-activated protein kinase activity without significantly affecting MEK activity in murine squamous cell carcinoma cells, Proc. Am. Assoc. Cancer Res. 41 (2000) 428.
[41] J.G. Christensen, R.E. Schreck, E. Chan, et al., High levels of HER-2 expression alter the ability of epidermal growth factor receptor (EGFR) family tyrosine kinase inhibitors to inhibit EGFR phosphorylation in vivo, Clin. Cancer Res. 7 (2001) 4230.

[42] D. Cunningham, N.L. Glichrist, R.A. Cowan, et al., Alfacalcidol as a modulator of growth of low grade non-Hodgkin's lymphomas, Br. Med. J. 291 (1985) 1153.
[43] H.P. Koeffler, K. Hirji, L. Iltri, 1,25-Dihydroxyvitamin D3: in vivo and in vitro effects on human preleukemic and leukemic cells, Cancer Treat Rep. 69 (1985) 1399.
[44] M. Davies, High-dose vitamin D therapy: indications, benefits and hazards, in: P. Walter, G. Brubacher, H. Stahelin (Eds.), Elevated Dosages of Vitamins, Hans Humber, Lewistown, NY, 1989, p. 81.
[45] C.S. Johnson, M.J. Egorin, R. Zuhowski, Effects of high dose calcitriol (1,25-dihydroxyvitamin D_3) on the pharmacokinetics of paclitaxel or carboplatin: results of two phase I studies, Am. Soc. Clin. Oncol. 19 (2000) 210a.
[46] D.C. Smith, C.S. Johnson, C.C. Freeman, et al., A phase I trial of calcitriol (1,25-dihydroxycholecalciferol) in patients with advanced malignancy, Clin. Cancer Res. 5 (1999) 1339.
[47] T.M. Beer, M. Munar, W.B. Henner, A phase I trial of pulse calcitriol in patients with refractory malignancies, Cancer 91 (2001) 2431.
[48] D.L. Trump, S. Serafine, J. Brufsky, et al., High dose calcitriol ($1,25(OH)_2$ vitamin D_3) + dexamethasone in androgen independent prostate cancer (AIPC), Am. Soc. Clin. Oncol. 19 (2000) 337a.
[49] J.R. Muindi, Y. Peng, D.M. Potter, et al., Pharmacokinetics of high dose calcitriol: results obtained during a phase one trial of calcitriol and paclitaxel, Cancer Pharm. Therap. 72 (6) (2002) 648–659.
[50] T.M. Beer, K.M. Eilers, M. Garzotto, et al., Androgen-independent prostate Cancer (AIPC) treatment with weekly high-dose calcitriol and docetaxel, Am. Soc. Clin. Oncol. 21 (2002) 177a.
[51] J.R. Muindi, R.A. Modezlewski, Y. Peng, P.A. Hershberger, D.L. Trump, C.S. Johnson, Pharmacokinetics of 1α,25-dihydroxycholecalciferol pharmacokinetics in normal and tumor bearing mice, Oncol. 66 (1) (2004) 62–66.