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Review

Vitamin D Compounds: Clinical Development as Cancer Therapy and Prevention Agents

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Abstract. *While 1,25 dihydroxycholecalciferol (calcitriol) is best recognized for its effects on bone and mineral metabolism, epidemiological data indicate that low vitamin D levels may play a role in the genesis and progression of breast, lung, colorectal and prostate cancer, as well as malignant lymphoma and melanoma. Calcitriol has strong antiproliferative effects in prostate, breast, colorectal, head/neck and lung cancer, as well as lymphoma, leukemia and myeloma model systems. Antiproliferative effects are seen in vitro and in vivo. The mechanisms of these effects are associated with G_0/G_1 arrest, induction of apoptosis, differentiation and modulation of growth factor-mediated signaling in tumor cells. In addition to the direct effects on tumor cells, recent data strongly support the hypothesis that the stromal effects of vitamin D analogs (e.g., direct effects on tumor vasculature) are also important in the antiproliferative effects. Antitumor effects are seen in a wide variety of tumor types and there are few data to suggest that vitamin D-based approaches are more effective in any one tumor type. Glucocorticoids potentiate the antitumor effect of calcitriol and decrease calcitriol-induced hypercalcemia. In addition, calcitriol potentiates the antitumor effects of many cytotoxic agents. Preclinical data indicate that maximal antitumor effects are seen with pharmacological doses of calcitriol and that such exposure can be safely achieved in animals using a high dose, intermittent schedule of administration. AUC and C_{max} calcitriol concentrations of 32 ng.h/ml and 9.2 ng/ml are associated with striking antitumor effects in a murine squamous cell carcinoma model and there is increasing evidence from clinical trials that such exposures can be safely attained in patients. Another approach to maximizing intra-tumoral exposure to vitamin D analogs is to inhibit their catabolism. The data clearly indicate that agents which inhibit the major vitamin D catabolizing enzyme, CYP24 (24 hydroxylase), potentiate calcitriol killing of prostate tumor cells in vitro and in vivo. Phase I and II trials of calcitriol, either alone or in combination with carboplatin, taxanes or dexamethasone, as well as the non-specific CYP24 inhibitor, ketoconazole, have been initiated in patients with androgen-dependent and -independent prostate cancer and other advanced cancers. The data indicate that high-dose calcitriol is feasible on an intermittent schedule, no dose-limiting toxicity has been encountered, but the optimal dose and schedule remain to be delineated. Clinical responses have been seen with the combination of high-dose calcitriol + dexamethasone in androgen-independent prostate cancer (AIPC) and, in a large randomized trial in men with AIPC, potentiation of the antitumor effects of docetaxel were seen.*

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1,25 Dihydroxycholecalciferol (calcitriol) is a potent antiproliferative agent in a wide variety of malignant cell types (1-13). Calcitriol and its analogs have significant antitumor activity *in vitro* and *in vivo* in murine squamous cell carcinoma (SCC), human xenograft prostatic adenocarcinoma (PC-3), rat metastatic prostatic adenocarcinoma Dunning (MLL), human pancreatic, lung, breast and multiple myeloma model systems (3, 4, 11-14 unpublished observations). Calcitriol induced G_0/G_1 arrest, modulating $p27^{Kipl}$ and $p21^{Waf1/Cipl}$, the cyclin-dependent kinase (cdk) inhibitors implicated in G_1 arrest (4, 13, 14). Calcitriol also induced cleavage of caspase 3, polyadenylated ribose-1 phosphate (PARP) and the growth-promoting/pro-survival signaling molecule mitogen-activated protein kinase (MEK) in a caspase-dependent manner (9, 11). The phosphorylation and expression of Akt, a kinase regulating a second cell survival pathway, was also inhibited after treatment with calcitriol. In contrast to changes that occur during cytotoxic drug-induced apoptosis, the pro-apoptotic signaling molecule MEKK-1 was significantly up-regulated by calcitriol (9).

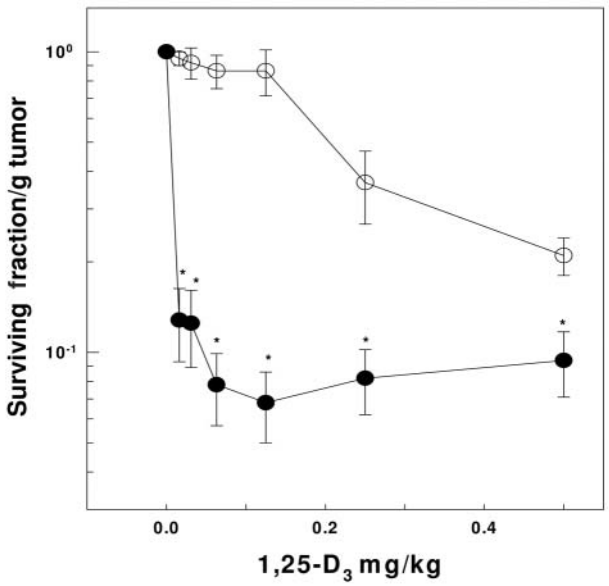

Figure 1. *In vivo clonogenic cell survival in murine SCC tumor following calcitriol alone (○) or calcitriol + dexamethasone (●).*

Dexamethasone (dex) potentiated the antitumor effects of calcitriol and decreased calcitriol-induced hypercalcemia (17) (Figure 1). Both *in vitro* and *in vivo*, dex increased vitamin D receptor (VDR) ligand binding in the tumor while decreasing binding in intestinal mucosa, the site of calcium absorption (17). Dex also potentiated calcitriol-induced suppression of phospho-Erk (P-Erk) and phospho-Akt (P-Akt) (18).

Calcitriol significantly enhanced the *in vitro* and *in vivo* antitumor efficacy of the platinum analogs, cisplatin and carboplatin, as well as the taxanes, paclitaxel and docetaxel (11, 14). In addition, we have shown potentiation of antimetabolites (5FU, gemcitabine), alkylating agents (4HC), and topoisomerase inhibitors (irinotecan, etoposide). In a detailed study of synergy using an excision clonogenic assay and isobologram analysis, synergy with all these agents was achieved by calcitriol pretreatment in intact, tumor-bearing animals. The only agent we have found with which synergy was not detected was the nitrosourea, carmustine (Table I). Enhancement of drug-mediated apoptosis by calcitriol was associated with an increase in PARP-, MEK- and caspase-cleavage and MEKK-1 and a decrease in P-Erk and P-Akt. In addition, the expression of the p53 homolog, p73, was strongly induced by calcitriol and p73, sensitizing tumor cells to the cytotoxic effects of platinum and taxanes. This review summarizes the considerations important in translating these preclinical findings regarding the antitumor effects of vitamin D into clinical practice.

Table I. *Synergistic interactions in vivo: calcitriol + cytotoxic agents.*

Combination Index <1		
Carboplatin	Gemcitabine	Irinotecan
Cisplatin	5FU	Etoposide
Paclitaxel	Cytosine arabinoside	Doxorubicin
Docetaxel		Mitoxantrone
Combination Index >1		
Carmustine (BCNU)		

1 Combination index <1 indicates synergy (Adv Enzyme Regul *22*: 27-55, 1984).
2 Studies in SCC (murine syngeneic tumor, subcutaneous): calcitriol administered QD x 3 (0.125 μg QD x 3) and cytotoxic given as LD_{10} on day 3.

Choice of Vitamin D Analog

Thousands of vitamin D analogs have been synthesized; however, four have been the focus of most studies of the antitumor effects of vitamin D analogs, namely: calcitriol (1,25 dihydroxycholecalciferol), paricalcitol (19-nor, $1,25(OH)_2D_2$), seocalcitol (19-nor, $1,25(OH)_2D_2$, EB1089) and 1alpha hydroxyl ergocalciferol. There are very limited data indicating that one analog has clear superiority over another. Seocalcitol, paricalcitol and 1alpha D2 are less prone to induce hypercalcemia in animal models than calcitriol and this observation has been argued to present a strong advantage for these analogs, which have a substantially lower affinity for the VDR. The mechanism(s) of the antitumor effects of vitamin D analogs are uncertain, and the importance of VDR affinity or any other of the ways in which these analogs differ is unclear. Our own studies have focused predominantly on calcitriol, since it is the most potent and readily available analog for clinical studies.

Calcitriol Dose and Schedule Considerations

Calcitriol, 1alpha D2 and seocalcitol have been utilized in a number of clinical studies. The earliest were studies of calcitriol in leukemia and myelodysplasia (19-21). More recent studies of seocalcitol, calcitriol and 1alpha D2 have been carried out in prostate, breast, colorectal and hepatocellular carcinoma. Although some evidence of response was seen, the results of these studies were largely disappointing (22-27). Most studies have administered vitamin D analogs orally on a continuous daily dosing schedule. On this schedule, either mild hypercalcemia or hypercaliuria was often encountered and limited dose escalation. Calcitriol causes hypercalcemia by increasing intestinal calcium absorption and mobilizing bone stores. It is important to remember that many preclinical studies which demonstrated substantial antitumor effects of

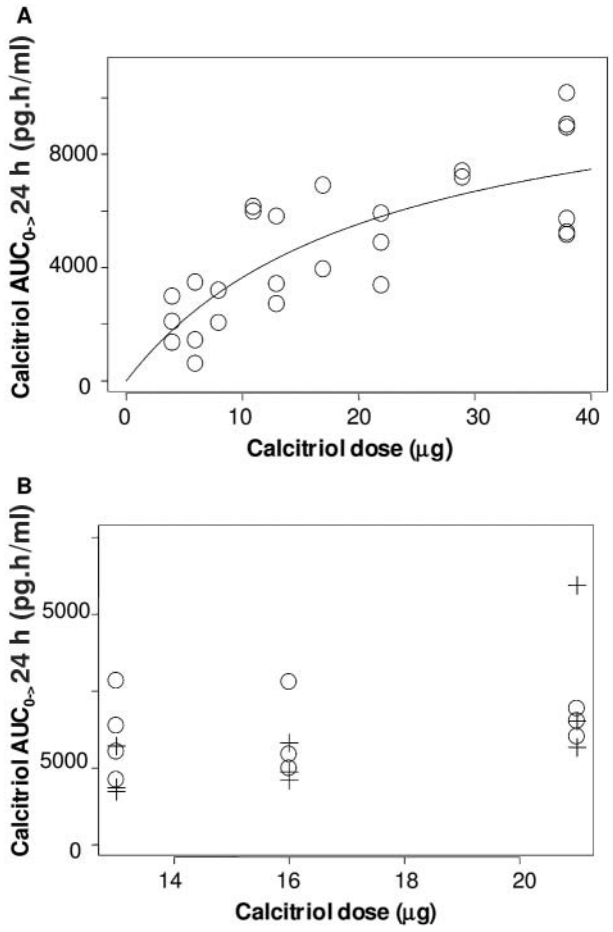

Figure 2. *Clinical pharmacokinetics: oral calcitriol (Caplet-panel A) vs. (liquid – panel B). Panel A demonstrates loss of dose proportional increase in exposure (AUC) with increasing dose; panel B illustrates no difference between the caplet (○) and liquid (+) formulations.*

Table II. *Clinical trials of calcitriol completed by Roswell Park investigators.*

Study	Route/Schedule	Agent[2]	MDA[3]	DLT[4]
1	QD, oral daily	0	2 mcg	yes
2	QOD, SQ[1] QOD	0	10 mcg	yes
3	QDx3 weekly	dex	12 mcg	no
4	QDx3 monthly	carboplatin	24 mcg	no
5	QDx3 weekly	paclitaxel	38 mcg	no
6	QDx3 weekly	0	24 mcg	no

[1]Subcutaneous.
[2]Agent administered with calcitriol:
carboplatin – AUC 5, day 3 monthly;
paclitaxel – 80 mg/m^2 day 3 weekly x 4;
dex – 4 mg QD x 4, weekly.
[3]MDA=maximum dose administered.
Trials 3-6 suspended when bioavailability issues were recognized.
[4]DLT=dose-limiting toxicity; only occurred in trials 1 and 2.

vitamin D analogs used an intermittent dosing schedule and administered very high doses of calcitriol. Two groups have taken these preclinical data and reasoned that high-dose, intermittent dosing schedules should be evaluated clinically. Beer and colleagues demonstrated that up to 2.6 μg/kg calcitriol could be administered once weekly to patients with advanced cancer without any limiting toxicity (28). Further dose escalation was limited not by toxicity, but rather by the observation that commercially available calcitriol preparations were pharmaceutically unfavorable: administration of 2.6 μg/kg to a 70 kg patient requires ingestion of more than 300 caplets since the largest caplet size is 0.5 μg. At doses above 0.5 μg/kg, the desired linear relationship between dose and exposure was lost. Our group has explored QD X3 weekly oral administration. We studied calcitriol as a single agent, with dexamethasone as well as with paclitaxel or carboplatin. We administered 24 μg QD X3 weekly to patients with AIPC with no limiting toxicity and up to 38 μg QD X3 weekly with paclitaxel with no dose-limiting toxicity (29). We too demonstrated the loss of the linear relationship between dose and exposure. We studied a liquid (palm oil) formulation and found that this preparation was not different from caplet formulations with regard to apparent reduced bioavailability (30) (Figure 2). Table II summarizes the studies we have performed using intermittent administration. Intermittent schedules of calcitriol permit the administration of very high doses of drug without toxicity. We have neither achieved a maximum tolerated dose of calcitriol or any dose-limiting toxicity. It is important to emphasize that the patients in all our studies had serum electrolytes, calcium, phosphorus and creatinine measured weekly, usually on the day following the last calcitriol ingestion. In all studies except our initial trials (Studies 1 and 2), computer-assisted tomographic radiographs of the abdomen or ultrasound examinations of the kidneys, ureters and bladder were completed to examine for the development of urinary tract stones. In more than 200 patients studied, one symptomatic and two asymptomatic urinary tract stones were diagnosed.

The dose limiting toxicity and maximum tolerated dose of calcitriol when administered on an intermittent schedule are unknown. By inference, it seems likely that other vitamin D analogs would behave in a similar manner, but no dose escalation of seocalcitol, paricalcitol and 1alpha D2 using an intermittent schedule has been reported.

In view of the pharmaceutical and pharmacokinetic shortcomings of current calcitriol formulations for cancer therapy, Novocea Pharmaceuticals has developed a new calcitriol formulation in 15 and 45 μg caplets. Phase I studies of this formulation have been completed (31). Doses as high as 180 μg were administered orally without toxicity and the pharmacokinetic properties of this formulation were favorable. There is a linear relationship between dose and

C_{max} and AUC. A phase III trial of this agent, called DN101, has recently been completed (see below).

We have studied the exposure to calcitriol in animal models at an effective antitumor dose and find that the AUC and C_{max} are 1/7 -1/9 that of the AUC and C_{max} seen in our human trials at 38 μg orally QD X3. The exposure seen with the higher doses of DN101 approximate the exposure required for antitumor activity in animal studies. We are also studying intravenous (*i.v.*) calcitriol. In a phase I study of *i.v.* calcitriol + gefitinib (Iressa), a weekly dose of 96 μg of calcitriol was administered without clear cut dose-limiting toxicity (32). Exposure (AUC and C_{max}) was similar to that seen following DN101 administration at comparable doses. Formal bioavailability studies have not been performed.

These data suggest that the optimal antitumor effects of calcitriol and, by inference, other vitamin D analogs may be optimized by high-dose, intermittent administration schedules. Such schedules and doses are safe and it seems likely that clinical exposure comparable to that which is associated with single-agent activity in animal models is achievable. Exploration of this concept will require the use of new formulations such as that developed by Novocea or the use of currently available intravenous formulations.

Vitamin D Catabolizing Enzymes as Therapeutic Targets

If exposure to high concentrations of calcitriol is necessary to optimize antitumor effects, another approach with merit is the use of agents which slow the catabolism of calcitriol. CYP24 (24 vitamin D hydroxylase) is the rate-limiting enzyme in the degradation of calcitriol and the calcitriol precursor, 25 hydroxycholecalciferol. A number of recent reports have implicated dysregulation of CYP24 in the pathogenesis of esophageal and other cancers (33-37). Epidemiological evidence suggests that vitamin D insufficiency may be a risk factor for tumor occurrence and progression, and such insufficiency could arise through the inappropriate catabolism of vitamin D through CYP24 pathways, as well as through "environmentally-mediated" insufficient vitamin D synthesis. Cross and colleagues demonstrated loss of vitamin D synthesizing activity 1alpha hydroxylase (CYP27B) as neoplastic lesions of the colon develop (38-40). CYP24 has not been studied sufficiently in this regard. Peehl and colleagues demonstrated that inhibition of CYP24 with the non-specific inhibitor, ketoconazole, potentiated the antitumor activity of calcitriol against the human prostate line, DU-145 (41). Zhao *et al.* and Ly *et al.* demonstrated similar effects in other systems (42, 43). We have extended these studies in a human prostate cancer model (PC-3) and have shown that the combination of calcitriol + dexamethasone + ketoconazole was synergistic by isobologram analysis and that *in vivo* potentiation of the antitumor activity of calcitriol by ketoconazole was possible without limiting toxicity (unpublished results).

We have initiated a phase I clinical trial of high-dose ketoconazole (400 mg TID) + replacement doses of dexamethasone (0.5 mg BID) + escalating doses of calcitriol administered by mouth QD X3 weekly. Ketoconazole is a non-specific P450/CYP enzyme inhibitor and interferes with the administration of a number of other commonly used drugs (*e.g.,* statins, antibiotics, psychotropics and anticoagulants). Other azole compounds and vitamin D analogues which bind irreversibly and inactivate CYP24, but do not activate the VDR, are under development (44-46). It is possible that combinations of vitamin D analogs with CYP24 inhibitors, administered on an optimal schedule, may be another approach to the use of vitamin D as an antitumor agent. Alternatively, CYP24 antagonists may be effective as chemopreventive agents.

Antitumor Responses to Vitamin D Analogs in Clinical Trials

While many clinical trials have been conducted with vitamin D analogs in cancer patients, the therapeutic results have generally been disappointing. Many of these studies might now be criticized for having used inappropriately small doses of vitamin D analogs and employing continuous rather than intermittent schedules. Gross *et al.* and Vieth *et al.* each studied men with rising PSA despite definitive surgery or irradiation (47, 48). Gross *et al.* administered calcitriol 1.5-2.0 μg daily, while Vieth *et al.* administered 25hydroxy vitamin D_3. Both investigations reported a decrease in the rate of PSA rise with vitamin D treatment. The former study was terminated because many patients developed moderate hypercalciuria which was totally asymptomatic.

Beer *et al.* studied patients with rising PSA following local therapy, as well as patients prior to prostatectomy, seeking in the latter trial to delineate antitumor effects by PSA change as well as histological change in the resected prostate (26, 27). A weekly 0.5 μg/kg schedule was employed, but in neither trial were significant antitumor effects noted. Morris *et al.* administered escalating intermittent doses of calcitriol + the bisphosphonate, zoledronic acid, to men with progressive prostate cancer despite androgen deprivation. Likewise, no antitumor effects were seen (49). In a phase II trial in AIPC using high-dose (12 μg/day QD X3, weekly) oral calcitriol and dex (4 mg QD X4, weekly), we saw a 50% reduction in PSA in 28% of the patients and no hypercalcemia (50). This study suggested that glucocorticoids may differentially modulate calcitriol-mediated effects while the PSA response rate in our study may represent a combined antitumor effect of dex + calcitriol; dex as a single agent could explain these results. We believe this has important therapeutic implications and should be examined

further. In each of these trials of calcitriol, the commercially available formulation (Rocaltrol®, Roche Pharmaceuticals) was used. The maximum doses administered provided substantially less blood calcitriol levels than those predicted to be necessary for the induction of antitumor effects, based on animal studies.

The most striking therapeutic effects of vitamin D-based anticancer therapy were seen in a recent trial by Beer *et al*: in 250 men with prostate cancer progressing despite androgen deprivation (51). The men were randomized to receive weekly docetaxel + placebo or weekly docetaxel + 0.5 μg/kg oral calcitriol as DN101 the day prior to docetaxel. Among the 250 patients, there were no clinically significant toxicities attributable to DN101. The primary end-point of this trial was a frequency of >50% change in PSA DND101 *vs.* placebo patients. While DN101 patients achieved this response more often (63% *vs.* 52%, $p=0.073$), the difference was not statistically significant. A planned secondary end-point was the survival difference between the two treatments; the DN101 group had a median survival of 23.5 months, and the docetaxel group had a median survival of 16.4 months ($p=0.035$). These data are encouraging and will be further explored. This study suggests that vitamin D analogs may play important roles as potentiators of cytotoxic therapies.

Substantial preclinical, *in vitro* and *in vivo*, data support the hypothesis that vitamin D compounds may play an important role in cancer therapy and perhaps prevention. Given these preliminary data, it is our firm conviction that vitamin D must be developed in the same way as any other anticancer therapy:

i) An optional formulation must be studied across several diseases.

ii) The optimal biologically effective dose should be explored and maximum tolerated doses must be determined.

iii) Preclinical studies should delineate the most effective and reasonable dosing schedules.

iv) Molecular end-points and vitamin D targets should be further delineated and tested in the clinic.

v) Vitamin D drug-drug interactions, which may be therapeutically advantageous, must be explored: a. vitamin D + cytotoxics; b. vitamin D + other signal modifying agents; c. vitamin D + modulators of vitamin D metabolism.

Despite decades of research on the effects of vitamin D in cancer, aims i-v still require considerable delineation.

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