Minireview

Calcitriol in cancer treatment: From the lab to the clinic

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Abstract

1,25-Dihydroxyvitamin D (calcitriol), the most active metabolite of vitamin D, has significant antineoplastic activity in preclinical models. Several mechanisms of activity have been proposed. These include inhibition of proliferation associated with cell cycle arrest and, in some models, differentiation, reduction in invasiveness and angiogenesis, and induction of apoptosis. Proposed mechanisms differ between tumor models and experimental conditions, and no unifying hypothesis about the mechanism of antineoplastic activity has emerged. Synergistic and/or additive effects with cytotoxic chemotherapy, radiation, and other cancer drugs have been reported. Significantly supraphysiological concentrations of calcitriol are required for antineoplastic effects. Such concentrations are not achievable in patients when calcitriol is dosed daily due to predictable hypercalcemia and hypercalcuria; however, phase I trials have demonstrated that intermittent dosing allows substantial dose escalation and has produced potentially therapeutic peak calcitriol concentrations. Recently, a phase II study reported encouraging levels of activity for the combination of high-dose calcitriol and docetaxel administered on a weekly schedule in patients with androgen-independent prostate cancer. This regimen is now under study in a placebo-controlled randomized trial in androgen-independent prostate cancer and in phase II studies in several other tumor types. Further work is needed to elucidate the molecular mechanisms of antineoplastic activity and optimal clinical applications of calcitriol in cancer. [Mol Cancer Ther. 2004;3(3):373–381]

Introduction

Vitamin D is a steroid hormone synthesized in human skin from 7-dehydrocholesterol in the presence of UV light. It is also commonly ingested from dietary sources such as fortified milk products. Vitamin D is primarily metabolized in the liver and subsequently in the kidney into 1,25-dihydroxyvitamin D (calcitriol), the most biologically active metabolite of vitamin D (1). Calcitriol in turn controls calcium and phosphate homeostasis and is essential for the development and maintenance of healthy bones.

Numerous *in vitro* and *in vivo* studies have shown that vitamin D potently inhibits cell proliferation in a wide range of cell types, including carcinomas of the breast, prostate, colon, skin, and brain, myeloid leukemia cells, and others (2, 3). Furthermore, vitamin D receptors (VDRs) as well as enzymes involved in the synthesis and degradation of vitamin D have now been identified in many non-neoplastic peripheral tissues, including colon, pancreas, brain, lymph nodes, and keratinocytes, suggesting a role for vitamin D in the regulation of normal cellular growth at a local level (4, 5). Recently, vitamin D has also been demonstrated to induce apoptosis and to inhibit angiogenesis, tumor invasion, and metastases. These preclinical data suggest that vitamin D (alone or in combination with other agents) has potential applications in cancer prevention and treatment.

Vitamin D-Mediated Signaling

Vitamin D and its analogues exert their activity through both genomic and non-genomic pathways. The classic genomic response is mediated through the VDR, a member of the steroid hormone superfamily (6). VDRs are present in more than 30 tissues, including intestine, kidney, bone, brain, stomach, heart, pancreas, skin, activated T and B lymphocytes, colon, ovary, breast, and prostate (7, 8). VDR is a ligand-activated transcription factor that, in combination with the retinoid-X receptor (RXR) and in some cases the retinoid A receptor (RAR), binds to the vitamin D response element (VDRE) in the promoters of target genes (9). The high-affinity VDR/RXR receptor heterodimer interacts with coactivator complexes that link VDR to the RNA polymerase complex and initiate transcription. A number of genes are recognized to contain functional vitamin D response elements. These include several bone-related genes [osteocalcin, osteopontin, bone sialoprotein, the calcium binding proteins calbindin-D28k and D9K, fructose 1,6-bisphosphatase, parathyroid hormone, parathyroid hormone-related protein (10, 11), human growth hormone (12), and receptor activator of NF-κB ligand (RANKL) (13)], as well as the cell cycle regulator p21 (14), the insulin receptor (15), 25(OH)D_3 24-hydroxylase (16), GADD45 (17), tumor necrosis factor α (18), CYP3A4 (19), urokinase plasminogen activator, protein lipase C γ (PLCγ), transforming growth factor β2, fibronectin, β3 integrin (11), and involucrin (20).

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In addition to the classic genomic effects of VDR, vitamin D regulates a number of cytoplasmic signaling pathways through protein kinase C (21–23), ras and mitogen-activated protein kinase (MAPK) (23–25), protein lipase A and prostaglandins (26, 27), cyclic AMP and protein kinase A (28), phosphatidyl inositol 3, the ceramide pathway (29), and Ca^{2+}-regulated voltage-sensitive (VSCC) or insensitive (VICC) channels (30). Activation of the cytoplasmic signaling pathways often results in rapid changes in intracellular calcium and the activation or deactivation of proteins such as bcl-2 and c-jun. A number of these pathways ultimately affect cellular growth, differentiation, and apoptosis and may cooperate with the classical genomic pathway to transactivate the VDR.

Antineoplastic Activity in Preclinical Models

In 1981, Abe *et al*. (31) were first to demonstrate the potential of VDR ligands to treat cancer. They reported that mouse myeloid leukemia cells possessed VDR and their exposure to vitamin D led to terminal differentiation. Since then, antineoplastic activity of VDR ligands has been demonstrated in both *in vitro* and/or *in vivo* models of carcinoma of the bladder (32), breast (33), colon (34), endometrium (35), kidney (36, 37), lung (38), pancreas (39), prostate (40–46), sarcomas of the soft tissues (47) and bone (48, 49), neuroblastoma (50, 51), glioma (52), melanoma (53), squamous cell carcinoma (SCC) (54, 55), and others.

Mechanisms of Antineoplastic Activity

Based largely on investigation carried out in *in vitro* models of cancer, several mechanisms have been suggested for $1,25(OH)_2D_3$'s antineoplastic activity. Proposed mechanisms differ between tumor models and experimental conditions, and no unifying hypothesis about the mechanism of activity has emerged.

Inhibition of Proliferation and Differentiation

Inhibition of proliferation, in some tumor models associated with induction of differentiation, is the most extensively studied mechanism of $1,25(OH)_2D_3$'s antineoplastic activity. $1,25(OH)_2D_3$ induces arrest in the G_1 phase of the cell cycle in numerous cell lines (56–60). In several tumor models, including HL-60 leukemia cells and U937 myelomonocytic cells, transcriptional activation of cyclin-dependent kinase (CDK) inhibitors $p27^{Kip1}$ and $p21^{Waf1}$, respectively, has been implicated as the mechanism responsible for cell cycle arrest in response to vitamin D (56, 57). The effects of vitamin D on CDK inhibitors are not universal, however. In PC-3 prostate cancer cells, the effects appear to be quite time dependent with $p21^{Waf1}$ expression increased after 24 h and reduced at 72 h (61), while in SCC, p21 expression appears to be reduced in response to calcitriol treatment at 24 h (54).

Other mitogenic signals may also be inhibited by vitamin D, including signaling through the mitogenic ERK/mitogen-activated protein kinase pathway (25). Reduced expression of c-*myc* has been observed (62, 63); however, the effects of $1,25(OH)_2D_3$ on c-*myc* vary in different preclinical model systems.

$1,25(OH)_2D_3$ also leads to the dephosphorylation of the retinoblastoma protein in normal human keratinocytes (64), in the murine syngeneic SCC model (54), and in several other tumor model systems including breast cancer (65, 66), squamous carcinoma of the head and neck (67), leukemia (68), and others.

$1,25(OH)_2D_3$ may also inhibit proliferation by interfering with growth factor signaling. In some models, $1,25(OH)_2D_3$ decreases the expression of epidermal growth factor receptors (EGFRs) (69), induces transforming growth factor β (70, 71), and alters components of the insulin-like growth factor (IGF) system (72–74). Differentiation can accompany growth inhibition; however, there are examples of $1,25(OH)_2D_3$-induced differentiation in cells that are resistant to $1,25(OH)_2D_3$-induced cell cycle arrest or growth inhibition (75, 76). Proposed mechanisms of the effects of vitamin D on proliferation are illustrated in Fig. 1.

Apoptosis

Vitamin D induces apoptosis in several tumor models, including carcinomas of the breast, colon, and prostate as well as myeloma, and (B-cell chronic) lymphocytic leukemia (25, 77–83); however, the underlying processes are only beginning to be elucidated.

The anti-apoptotic protein Bcl-2, which is overexpressed in many tumors, is down-regulated by $1,25(OH)_2D_3$ or its analogues in several prostate cancer cell lines (80), as well as in B-cell chronic lymphocytic leukemia cells (83), MCF-7 breast cancer cells, and retinoblastoma cells undergoing apoptosis in response to vitamin D treatment (84, 85). In invasive breast cancer cells (SUM-159PT cells), the reduction in Bcl-2 protein is accompanied by an increase in the pro-apoptotic protein Bax and a release of cytochrome *c* from the mitochondria followed by PARP cleavage (86). *In vitro* studies of MCF-7 breast cancer cells, LNCaP prostate cells, and B-cell chronic lymphocytic leukemia cells indicate that vitamin D-mediated apoptosis in these cell lines is independent of p53 status (83, 87, 88). In the prostate cancer cell lines LNCaP and ALVA-31, as well as

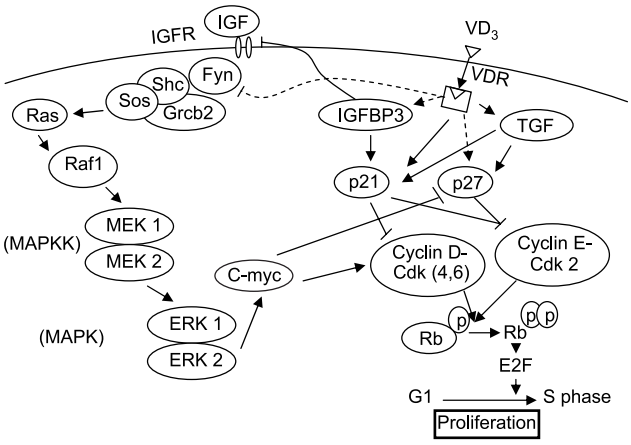

Figure 1. Proposed mechanisms of vitamin D-mediated antiproliferative effects. *Solid lines* illustrate known direct effects. *Dashed lines* illustrate effects that are not fully characterized and may be indirect.

in the MCF-7 breast cancer cells, vitamin D stimulates cytochrome *c* release from mitochondria by a caspase-independent mechanism. Other suggested mechanisms for the apoptotic effects of vitamin D include down-regulation of the anti-apoptotic insulin-like growth factor receptor (89), up-regulation of the pro-apoptotic signaling molecule MEK kinase-1 (55), activation of the sphingomyelin-ceramide-ganglioside GD3 signaling pathway (29), reduced expression of Akt, a kinase which regulates an important survival pathway (90, 91), increased activity of the pro-apoptotic agent tumor necrosis factor α (92), and increased mobilization of cytosolic calcium (93). Surprisingly, there are also examples of anti-apoptotic effects by vitamin D following cellular stress (94) and UV damage (95). Mechanisms behind these observations are not fully understood. Proposed mechanisms of the effects of vitamin D on apoptosis are illustrated in Fig. 2.

Invasiveness and Angiogenesis

$1,25(OH)_2D_3$ may also affect tumor invasion and metastasis. *In vitro* assays show that $1,25(OH)_2D_3$ is able to inhibit the invasiveness of breast (96), lung (97), and prostate carcinoma cells (98, 99). *In vivo* inhibition of tumor metastasis has also been demonstrated in several rodent tumor models including prostate cancer (46), melanoma (100), and bladder cancer (32).

Proposed mechanisms for the anti-invasive effects of $1,25(OH)_2D_3$ include inhibition of serine proteinases (such as components of the plasminogen activator system) and decreases in the activity of metalloproteinases (98, 101), as well as decreased expression of α6 and β4 integrins (99), increased expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells (102), and inhibition of tenascin-C, an extracellular matrix protein which promotes growth, invasion, and angiogenesis and is up-regulated in many cell types during tumorigenesis (103).

Inhibition of angiogenesis may also contribute to the observed anti-metastatic activity of $1,25(OH)_2D_3$. *In vitro*, $1,25(OH)_2D_3$ inhibits the proliferation of some tumor-derived endothelial cells (91) and inhibits sprouting and elongation of endothelial cells induced by vascular endothelial growth factor (104). $1,25(OH)_2D_3$ also has been shown to inhibit tumor-induced angiogenesis in mice (104, 105).

Vitamin D with Other Agents

Recognition of the antineoplastic effects of calcitriol has stimulated an interest in combinations with other agents. Synergistic or additive effects have been observed with a number of such combinations and may be explained by increases in signaling through VDR, by targeting multiple components of the same pathway, or by targeting redundant survival or death pathways simultaneously.

Steroids

Dexamethasone potentiates the antitumor effects of $1,25(OH)_2D_3$ *in vitro* and *in vivo* (90, 106). In SCC cells, the effect appears to be associated with increases in VDR protein and in ligand binding, without changes in VDR mRNA levels or ligand affinity (106). In PC-3 cells, dexamethasone enhances vitamin D-mediated antineoplastic activity. Vitamin D-induced cell cycle arrest, reduction of phospho-Erk1/2 and phospho-Akt levels, and apoptosis are increased with the addition of dexamethasone (54, 90, 106). Furthermore, dexamethasone increases calcitriol-mediated inhibition of tumor-derived endothelial cell growth (91).

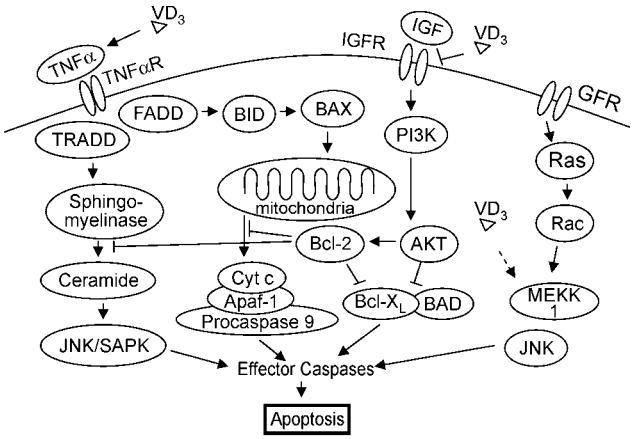

Figure 2. Proposed mechanisms of vitamin D-mediated pro-apoptotic effects. *Solid lines* illustrate known direct effects. *Dashed lines* illustrate effects that are not fully characterized and may be indirect.

Cytotoxic Chemotherapy

Calcitriol and other VDR ligands have also been examined in combination with several cytotoxic agents. In prostate cancer cell lines, calcitriol enhances antitumor activity of docetaxel (107), paclitaxel (61), platinum compounds (108), and mitoxantrone (109). Similar results are seen in prostate cancer animal models with taxanes (61) and mitoxantrone (109). Such interactions are not unique to prostate cancer and have been described in preclinical models of SCC (110), breast cancer (111, 112), and myeloid leukemia (113). The mechanisms by which VDR ligands potentiate the activity of cytotoxic agents are incompletely understood and are likely to be complex.

Retinoid Receptor Ligands

Because VDR heterodimerizes with RXR, it is not surprising that $1,25(OH)_2D_3$ and RXR ligands interact to induce synergistic growth inhibition in several tumor models (114, 115). In addition, tumor-induced angiogenesis is synergistically inhibited when $1,25(OH)_2D_3$ is combined with all-*trans* retinoic acid, 9-*cis* retinoic acid, and 13-*cis* retinoic acid in a human transformed keratinocyte model (116) and additive and/or synergistic induction of apoptosis have been reported in several *in vitro* tumor models (115).

Tamoxifen

The addition of a calcitriol analogue significantly enhanced tamoxifen inhibition of mammary carcinogenesis induced by *N*-nitroso-*N*-methylurea (NMU) in Sprague-Dawley rats (117). Combined treatment with $1,25(OH)_2D_3$ and tamoxifen enhances apoptosis in MCF-7 breast cancer cells (118) and similar results were seen *in vivo* with 22-oxa-calcitriol and tamoxifen in nude mouse xenografts of MCF-7 cells (119).

Radiation

Several effects of vitamin D on cancer cells may be radiosensitizing. For example, induction of p21 expression has been shown to be associated with increased sensitivity to radiation (120). In the LNCaP model, $1,25(OH)_2D_3$ inhibited growth synergistically with ionizing radiation (IR) and potentiated IR-induced apoptosis (121). ILX 23-7553, a calcitriol analogue, produced additive growth inhibition and apoptosis when combined with IR in MCF-7 breast cancer cells (122).

Ketoconazole

The P450 inhibitor ketoconazole also inhibits the 25-hydroxyvitamin D3 24-hydroxylase enzyme which catalyzes the initial step in the conversion of active vitamin D into less active metabolites. A recent study shows that ketoconazole enhances the growth inhibitory effects of vitamin D in prostate cancer cells (123, 124).

Clinical Development of Calcitriol for Cancer Treatment

Daily Administration of Calcitriol Alone and in Combination

Initial efforts to explore the activity of calcitriol in patients with cancer focused on daily dosing. In prostate cancer, this approach was tested in both hormone-naïve and androgen-independent disease (125, 126). Only minimal dose escalation above physiologic replacement doses was possible before hypercalcemia and/or hypercalcuria developed. No confirmed responses were seen, although in the hormone-naïve study, slowing of the rate of rise of serum prostate specific antigen (PSA) was suggested. The significance of such changes in tumor marker kinetics is not known. Daily calcitriol has also been examined in pilot trials in myelodysplastic syndrome (127) and ovarian cancer (128) with little activity. Pilot studies have also examined daily calcitriol in combination with cytarabine (129) and with cytarabine and hydroxyurea (130) in the treatment of both myeloid and lymphoid leukemia. While activity was seen with the combinations, the studies were not designed to isolate the effect of calcitriol. An underpowered randomized study that compared cytarabine to cytarabine with 13-*cis* retinoic acid and calcitriol in myelodysplastic syndrome did not show an improvement with the combination (131). Daily calcitriol together with isotretinoin was also inactive in patients with ovarian cancer (128).

On the basis of the calcitriol pharmacokinetics studied in normal volunteers, the calcitriol concentrations achieved in these studies would be expected to be only modestly above the physiologic range (132).

Intermittent Dosing and Parenteral Administration

Preclinical systems suggest that the antineoplastic activity of calcitriol is dose dependent and, in most systems, concentrations of 1 nM or higher are associated with significant antineoplastic activity *in vitro*. Because such concentrations are not achievable with daily dosing, both intermittent dosing and parenteral administration have been examined in dose escalation studies. Subcutaneous administration allowed dose escalation to 8 μg every other day and produced peak blood calcitriol concentrations of approximately 0.7 nM (133).

Weekly administration of oral calcitriol allowed substantial dose escalation. In the initial phase I trial, doses ranging from 0.06 to 2.8 μg/kg were examined (134). A plateau in peak calcitriol concentrations (C_{max}) and area under the concentration curve (AUC) was seen at doses above 0.48 μg/kg. Peak blood calcitriol concentrations at the higher doses ranged from 3.7 to 6.0 nM. This trial demonstrated that potentially therapeutic calcitriol concentrations are achievable with weekly dosing. The maximum tolerated dose was not defined, however, because no dose-limiting toxicity was encountered. This initial trial examined treatment for only 4 weeks, thus additional safety data were provided by a phase II trial of weekly calcitriol dosed at 0.5 μg/kg in hormone-naïve prostate cancer patients with a rising serum PSA (135). In this trial, patients were treated for a median of 10 months and no toxicity exceeded grade 2. Average peak calcitriol concentrations were approximately 2 nM. In this non-randomized study, no responses were seen; however, several patients had modest reductions in serum PSA. In the entire population, the rate of rise of the serum PSA was slower on treatment than before therapy. Because these end points have not been validated, it is not clear if such observations would translate into meaningful clinical benefit in a randomized trial. The pharmacokinetic profile of calcitriol administered on this schedule is illustrated in Fig. 3.

Another weekly schedule, with calcitriol given on three consecutive days every 7 days, has also been tested in a phase I trial of calcitriol combined with paclitaxel (136). Doses up to 38 μg × 3 were given without dose-limiting toxicity. On this schedule, the calcitriol AUC also did not increase linearly with increasing dose. Calcitriol C_{max} ranged from 1.4 to 3.5 nM. Only the pharmacokinetic results of this trial have been reported to date.

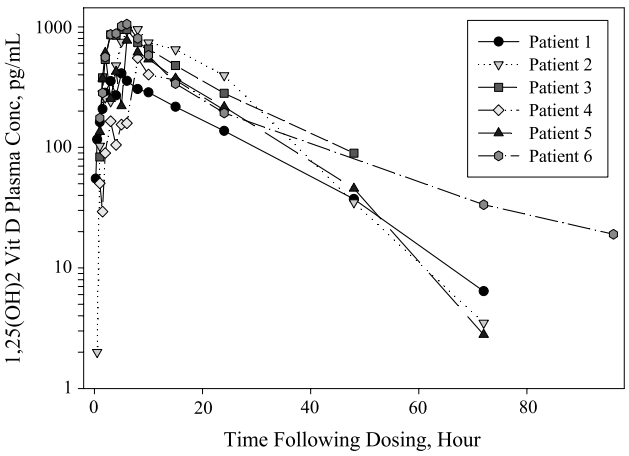

Figure 3. Plasma calcitriol concentrations (pg/ml) over time in six patients treated with 0.5 μg of calcitriol administered over 4 h starting at time 0. Reprinted with permission from Ref. 135.

These studies established that weekly oral administration of calcitriol permits substantial dose escalation and produces potentially therapeutic peak concentrations of calcitriol. The apparent limitation in bioavailability at higher doses precluded the determination of the MTD of weekly calcitriol. The reason for this limitation was not determined in these studies and could be related to the formulation or to the parent compound.

The commercially available calcitriol formulation used in these trials (Rocaltrol 0.5 μg capsules, Roche Pharmaceuticals, Nutley, NJ) was developed for use at much lower doses and has potential limitations. A large number of capsules (typically 70–100) are required for each dose. This is inconvenient and may impair adherence with therapy. Although potentially therapeutic concentrations are achievable with this formulation, considerable inter-patient variability in calcitriol pharmacokinetics is seen and, at higher doses, neither the peak concentrations nor the AUC increases in a dose-proportional fashion. To date, this apparent absorption limitation has prevented investigators from escalating weekly calcitriol to dose-limiting toxicity, thus the MTD had not been defined.

A high concentration formulation of calcitriol (DN-101, Novacea, Inc., South San Francisco, CA) has been developed recently and is under study both as a single agent and in combination with chemotherapeutic agents in several malignancies.

Intermittent Dosing in Combination with Other Antineoplastic Agents

Encouraged by preclinical evidence, our group tested the combination of weekly calcitriol and docetaxel in patients with metastatic androgen-independent prostate cancer (AIPC). Docetaxel has significant single-agent activity in AIPC. In four phase II studies that enrolled 138 patients, an overall response rate of 42% by PSA and 28% by measurable disease assessment was identified (137–140).

Thirty-seven patients with chemotherapy-naïve metastatic AIPC were enrolled in a phase II trial of oral calcitriol (0.5 μg/kg) on day 1 followed by docetaxel (36 mg/m^2) on day 2 delivered weekly on a 6 of 8 weeks schedule (141). The activity of the regimen as measured by PSA response rate (81%, 95% CI 68–94%) and measurable disease response rate (53%, 95% CI 27–79%) was encouraging relative to historical controls, while treatment-related toxicity was generally similar to that expected with single-agent docetaxel. In an exploratory analysis, neither agent appeared to affect the pharmacokinetics of its companion. These results led to the development of a multi-institutional placebo-controlled, double-blinded randomized trial designed to test the findings of this initial phase II study.

Because preclinical data suggest that calcitriol's antineoplastic activity is not restricted to prostate cancer, phase II trials of calcitriol and docetaxel in breast and pancreatic cancer have recently been initiated at the OHSU Cancer Institute.

Studies of 3× weekly calcitriol with dexamethasone, paclitaxel, and carboplatin are under way at Roswell Park Cancer Institute (3).

Alternative Routes of Administration

Finlay *et al.* (142) examined hepatic regional administration of calcitriol in patients with hepatic metastases from colorectal cancer and recently reported initial safety data in patients with hepatocellular carcinoma (143). It is too early to determine if regional administration of calcitriol will prove useful.

Calcitriol Analogues

The development of analogues of calcitriol that might have antineoplastic activity but cause less hypercalcemia has been proposed as another strategy to target VDR for cancer treatment. Several hundred such analogues that most commonly differ in their side chain have been synthesized. Differences in their calcemic activity may be attributable to lesser affinity for the VDR, differences in drug metabolism, and differences in binding to the vitamin D binding protein (144). A detailed discussion of calcitriol analogues is beyond the scope of this review, but several excellent reviews can be recommended (145, 146). Several analogues, dosed daily, have entered clinical trials.

Bower *et al.* (147) treated 19 patients with locally advanced or cutaneous metastatic breast cancer with topical calcipotriol, a vitamin D analogue. Three of the 14 patients who completed 6 weeks of treatment showed a 50% reduction in the bidirectional diameter of the treated lesions and 1 other patient showed minimal response.

In a phase I trial, the dose-limiting toxicity of 1-α-hydroxyvitamin D2 was hypercalcemia and renal insufficiency. The proposed phase II dose was 12.5 μg/day on a daily schedule. Two of 25 AIPC patients had objective partial responses (148).

After phase I evaluation (149), Seocalcitol (EB1089, Leo Pharmaceuticals, Ballerup, Denmark) was evaluated in a phase II study in patients with inoperable pancreatic cancer. Dose-dependent hypercalcemia was reported. No objective responses were seen (150).

Conclusions

Calcitriol exerts potent antineoplastic activity in a broad range of tumor models. Several mechanisms of activity have been proposed. Growth inhibition and accumulation in G_0-G_1, associated with transcriptional activation of CDK inhibitors $p27^{Kip1}$ and/or $p21^{Waf1}$ has been the most extensively studied mechanism; however, effects on other mitogenic signals, induction of apoptosis, and inhibition of invasiveness and angiogenesis have also been reported. Additive and/or synergistic activity has been reported with cytotoxic chemotherapy, radiation, and other cancer drugs. At present, no unifying hypothesis for calcitriol's anticancer effects has been formulated. Indeed, it is likely that different mechanisms predominate in different neoplasms. The translation of these preclinical finding into patient care had been hampered by predictable hypercalcemia and hypercalcuria when calcitriol is dosed daily. Calcitriol concentrations that, based on preclinical data, are

thought to be necessary for antineoplastic activity, are not achievable with conventional daily dosing. In contrast, intermittent administration of calcitriol has allowed substantial dose escalation. This approach has moved forward in clinical trials. Recently, a phase II study reported encouraging levels of activity for the combination of high-dose calcitriol and docetaxel administered on a weekly schedule in patients with AIPC. This regimen is now under study in a placebo-controlled randomized trial. Further work is needed to fully understand the molecular mechanisms of activity and optimal clinical applications of calcitriol in cancer.

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