Review

Inhibition of prostaglandin synthesis and actions contributes to the beneficial effects of calcitriol in prostate cancer

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Key words: calcitriol, prostaglandins, COX-2, 15-PGDH, NSAIDs, combination therapy, prostate cancer

Our research is aimed at obtaining a better understanding of the molecular mechanisms of the anti-proliferative and cancer preventive effects of calcitriol with the goal of developing strategies to improve the treatment of prostate cancer (PCa). In PCa cells calcitriol inhibits the synthesis and biological actions of prostaglandins (PGs) by three actions: (i) the inhibition of the expression of cyclooxygenase-2 (COX-2), the enzyme that synthesizes PGs, (ii) the upregulation of the expression of 15-prostaglandin dehydrogenase (15-PGDH), the enzyme that inactivates PGs and (iii) decreasing the expression of EP and FP PG receptors that are essential for PG signaling. Since PGs have been shown to promote carcinogenesis and progression of multiple cancers, we hypothesize that the inhibition of the PG pathway contributes to the ability of calcitriol to prevent or inhibit PCa development and growth. We have shown that the combination of calcitriol and non-steroidal anti-inflammatory drugs (NSAIDs) result in a synergistic inhibition of the growth of PCa cell cultures and this combination therapy offers a potential therapeutic strategy. These findings led us to embark on a clinical trial combining the non-selective NSAID naproxen with calcitriol in men with early recurrent PCa. The results indicate that the combination of high dose weekly calcitriol with naproxen slows the rate of rise (doubling time) of PSA in most patients indicating the slowing of disease progression. Further studies are warranted to determine the role of this combination therapy in the management of recurrent PCa.

Introduction

Calcitriol (1,25-dihydroxyvitamin D³), the active metabolite of vitamin D, is an important regulator of calcium homeostasis and bone metabolism through its actions in intestine, bone, kidney and the parathyroid glands.¹ However, calcitriol also exerts antiproliferative and pro-differentiating effects in a number of tumors and malignant cells raising the possibility of its use as an anti-cancer agent. Extensive research supports an inhibitory role for calcitriol in

the development and progression of prostate cancer (PCa).²⁻⁵ UV light is essential for the synthesis of vitamin D in the skin and epidemiological studies suggest that mortality rates due to PCa in the U.S are inversely related to sunlight exposure.^{6,7} Vitamin D deficiency has also been shown to increase PCa risk.⁸ Several studies on PCa cell cultures have demonstrated the anti-proliferative and prodifferentiating effects of calcitriol.²⁻⁵ However, relatively higher concentrations of calcitriol are required for these anti-cancer effects that may cause hypercalcemia in vivo.

Therefore investigators have focused on the development of calcitriol analogs that exhibit increased anti-proliferative activity and reduced tendency to cause the hypercalcemic side-effects.^{9,10} In vivo studies have demonstrated inhibitory effects of calcitriol and its analogs on the growth of human PCa xenografts in immunocompromised mice causing significant reduction in tumor size and volume.^{5,11-13} Transgenic models of PCa have also been developed in animals to study the chemopreventive effects of calcitriol and its analogs.¹⁴ These in vivo models provide a valuable tool to study the tumor inhibitory effects of calcitriol and analogs while monitoring their tendency to cause hypercalcemia and to validate their potential use in clinical trials.

Molecular Mechanisms of the Anti-Cancer Actions of Calcitriol

Many important mechanisms have been implicated in calcitriol-mediated growth inhibition. Calcitriol inhibits the proliferation of PCa cells through cell cycle arrest in the G₁/G₀ phase,^{5,13,15} by increasing the expression of the cyclin-dependent kinase inhibitors p21^{Waf/Cip1} and p27^{Kip1},¹⁵⁻¹⁸ decreasing cyclin-dependent kinase 2 (Cdk2) activity¹⁵ and causing the hyperphosphorylation of the retinoblastoma protein (pRb).¹⁹ Calcitriol induced G₀ arrest appears to be p53 dependent.^{5,13,20} Calcitriol induces apoptosis in some PCa cells and suppresses the expression of anti-apoptotic genes such as *bcl-2*.²¹ Other mechanisms include the stimulation of differentiation, modulation of growth factor actions and the inhibition of invasion and metastasis.^{5,11-13,20,22} The inhibition of angiogenesis also contributes to the anti-tumor effects of calcitriol in vivo.^{5,11-13,20} Using cDNA microarrays we have identified novel molecular targets of calcitriol involved in the regulation of prostate epithelial cell growth.^{23,24} Our studies have identified known, important target genes as well as several novel genes regulated by calcitriol in primary prostate epithelial cells and LNCaP cells. Interestingly calcitriol

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Submitted: 10/01/08; Accepted: 10/02/08

Previously published online as a *Dermato-Endocrinology* E-publication: <http://www.landesbioscience.com/journals/dermatoendocrinology/article/7106>

regulates multiple genes involved in prostaglandin (PG) metabolism and signaling.^{23,24}

Prostaglandins and PCa

PGs play a role in the development and progression of several cancers including PCa^{25,26} by stimulating cellular proliferation, inhibiting apoptosis, promoting angiogenesis and by activating carcinogens.^{27,28} Cyclooxygenase (COX)/prostaglandin endoperoxidase synthase (prostaglandin G/H synthase), the rate-limiting enzyme that catalyzes the conversion of arachidonic acid to PGs, exists as two isoforms, COX-1 which is constitutively expressed in many tissues and cell types and COX-2, which is rapidly induced by a variety of mitogens, cytokines, tumor promoters and growth factors.²⁹ Compelling evidence from genetic and clinical studies indicates that increased expression of COX-2 is one of the key steps in carcinogenesis.^{26,29} Several studies have demonstrated COX-2 overexpression in prostate adenocarcinoma^{26,30-36} suggesting a positive role for COX-2 in prostate tumorigenesis. However, not all PCa are associated with elevated COX-2 expression.^{37,38} Although Zha et al.³⁸ did not find consistent overexpression of COX-2 in established PCa, they detected appreciable COX-2 expression in areas of proliferative inflammatory atrophy (PIA), lesions that have been implicated in prostate carcinogenesis. All investigators agree that local production of PGs by the infiltrating inflammatory cells increases the risk of carcinogenesis and/or progression.^{26,38-40} Silencing of COX-2 in metastatic PCa cells induces cell growth arrest and causes morphological changes associated with enhanced differentiation highlighting the role of COX-2 in prostate carcinogenesis.⁴¹ COX-2 protein expression in prostate biopsy cores and PCa surgical specimens is inversely correlated with disease-free survival.⁴² Elevated expression of COX-2 in radical prostatectomy specimens has also been shown to be associated with an increased risk of disease recurrence and worse outcome.⁴³

The key enzyme responsible for the metabolic inactivation of PGs is 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catalyzes the conversion of PGs to their corresponding 15-keto derivatives with greatly reduced biological activities.⁴⁴ 15-PGDH is described as an oncogene antagonist that functions as a tumor suppressor in colon cancer⁴⁵ as well as in breast cancer.⁴⁶

PGs bind to G-protein coupled membrane receptors (prostanoid receptors), which activate different signal transduction pathways⁴⁷ to mediate the diverse effects of PGs.⁴⁷ PGE₂ and PGF₂ are the major PGs stimulating the proliferation of PCa cells and PCa cells express EP and FP PG receptors.^{48,49}

Inhibition of the PG Pathway by Calcitriol in Prostate Cells

Following up on the observations from our microarray studies, we went on to demonstrate calcitriol regulation of the PG pathway genes in multiple PCa cell lines as well as primary prostatic epithelial cells established from surgically removed prostate tissue from PCa patients.⁴⁸ We found measurable amounts of COX-2 mRNA and protein in various PCa cell lines as well as primary prostatic epithelial cells derived from normal and cancerous prostate tissue, which were significantly decreased by calcitriol treatment.⁴⁸ We also found that calcitriol significantly increased the expression of 15-PGDH mRNA and protein in various PCa cells.⁴⁸ We further showed that by decreasing the expression of COX-2 and stimulating that of

15-PGDH, calcitriol decreased the levels of biologically active PGs in PCa cells thereby reducing the growth stimulation by PGs.⁴⁸

Calcitriol also decreased the expression of EP and FP PG receptors, providing another mechanism for decreasing the biological activity of PGs.⁴⁸ The calcitriol-induced decrease in PG receptor levels resulted in the attenuation of PG mediated functional responses even when exogenous PGs were added to the cultures.⁴⁸ Calcitriol suppressed the induction of the immediate-early gene *c-fos* and the growth stimulation seen following the addition of exogenous PGs or the PG precursor arachidonic acid to PCa cell cultures.⁴⁸ Thus calcitriol inhibits the PG pathway in PCa cells by three separate mechanisms, decreasing COX-2 expression, increasing 15-PGDH expression and reducing PG receptor levels. We believe that these actions contribute to the suppression of the proliferative stimulus provided by PGs in PCa cells. The regulation of PG metabolism and biological actions constitute an additional novel pathway of calcitriol action contributing to its antiproliferative effects in prostate cells.

Combination of Calcitriol and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) as a Therapeutic Approach in PCa

NSAIDs are a class of drugs that decrease PG synthesis by inhibiting COX-1 and COX-2 enzymatic activities. Several NSAIDs inhibit both the constitutively expressed COX-1 and the inducible COX-2 while others have been shown to be more selective in inhibiting the COX-2 enzyme activity. Our hypothesis is that the action of calcitriol at the genomic level to reduce COX-2 expression leading to decreased COX-2 protein levels will allow the use of lower concentrations of NSAIDs to inhibit COX-2 enzyme activity. In addition, an increase in the expression of 15-PGDH due to calcitriol action will lower the levels of biologically active PGs and enhance the NSAID effect. Therefore, we hypothesized that the combination of calcitriol and NSAIDs would exhibit additive/synergistic effects to inhibit PCa cell growth. In cell culture studies we examined the growth inhibitory effects of the combinations of calcitriol with the COX-2-selective NSAIDs NS398 and SC-58125 or the non-selective NSAIDs, naproxen and ibuprofen.

The combinations caused a synergistic enhancement of the inhibition of PCa cell proliferation, compared to the individual agents.⁴⁸ These results led us to further hypothesize that the combination of calcitriol and NSAIDs would have clinical utility in PCa therapy and is worthy of evaluation in a clinical trial.⁴⁸

Clinical Trials with Calcitriol

Several clinical trials have been carried out in PCa patients to evaluate the safety and efficacy of treatment with calcitriol or its analogs.^{2,4,5,11,13} Calcitriol is an FDA approved drug (for indications other than PCa) and has been administered at the highest dose tolerated, limited by persistent hypercalcemia or hypercalciuria. A decrease in the rate of rise of serum prostate specific antigen (PSA) levels has been observed in PCa patients following daily modest doses of calcitriol⁵⁰ indicating a beneficial effect of calcitriol in slowing the progression of the disease. Recent investigations have followed the approach of administering calcitriol intermittently in very high doses where it still elicits its antiproliferative effects but causes only transient hypercalcemia.⁵¹⁻⁵³

Preclinical⁵⁴ and clinical studies⁵⁵ on colon and other cancers have successfully used the strategy of combining low doses of two active drugs to achieve a more effective chemoprevention and therapeutic outcome than those using the individual agents.⁵⁶ The combination approach would also minimize the toxicities of the individual drugs by allowing them to be used at lower doses while achieving a significant therapeutic effect.

Based on our preclinical observations, we proposed that a combination of calcitriol with a NSAID would be a beneficial approach in PCa therapy. The combination strategy allows the use of a lower concentration of NSAIDs and thereby minimizing their undesirable side-effects. It has very recently become clear that long-term use of COX-2-selective inhibitors such as rofecoxib (Vioxx) causes an increase in cardiovascular complications in patients.⁵⁷⁻⁶⁰ In comparison, non-selective NSAIDs such as naproxen may be associated with fewer cardiovascular adverse effects.⁶¹ Our preclinical data show that the combination of calcitriol with non-selective or selective NSAIDs is equally effective in inducing synergistic growth inhibition. We therefore proposed that the combination of calcitriol with a non-selective NSAID would be a useful therapeutic approach in PCa that would allow both drugs to be used at reduced dosages leading to increased safety.⁴⁸

Calcitriol, in fact is already being used in combination therapy with other agents that may enhance its antiproliferative activity while reducing its tendency to cause hypercalcemia.¹² The results of the ASCENT I clinical trial in advanced PCa patients who failed other therapies demonstrated that the administration of a very high dose (45 micrograms) of calcitriol (DN101, Novacea, South San Francisco, CA) once weekly along with the regimen of the chemotherapy drug taxotere in use at the time of that trial (once weekly) caused a very significant improvement in overall survival and time to progression, providing evidence indicating that calcitriol can enhance the efficacy of active drugs in cancer treatment.⁶² The ASCENT I trial did not meet its primary endpoint, i.e., a lowering of serum PSA. However, based on the promising survival results, (16.4 months in the taxotere arm versus 24.5 months in the taxotere plus calcitriol arm) a larger, phase III trial (ASCENT II) with survival as an endpoint was designed.

At the time of the ASCENT I trial there was no standard established in docetaxel dosing. Since then two large randomized trials have established every three week dosing of docetaxel rather than weekly dosing to be the standard of care.^{63,64} This new, improved docetaxel regimen was used in the ASCENT II trial in the control arm, which was compared to DN101 plus the older taxotere weekly dosing regimen in the DN101 arm, resulting in an asymmetric study design. To reiterate, ASCENT II compared the taxotere alone arm dosed with the new regimen of taxotere (every three weeks plus prednisone) to the combination arm of calcitriol plus the older ASCENT I taxotere weekly dosing regimen.^{65,66} Unfortunately the improved survival due to the combination demonstrated in the ASCENT I trial could not be confirmed in the ASCENT II trial.⁶⁵ In fact, the trial was prematurely stopped by the data safety monitoring committee after 900 patients were enrolled, when an excess number of deaths were noted in the study (DN101) arm. Since the trial was stopped, further analysis (unpublished) suggests that the increased deaths in the treatment arm compared to the control arm were not due to calcitriol toxicity but due to better survival in the control arm that

received the new and improved docetaxel regimen. From the information available, we conclude that in the ASCENT II trial, DN101 did not cause the increased death rate but, on the other hand, did not improve the efficacy of taxotere, thus failing to confirm the improved survival seen in the ASCENT I trial. The FDA has recently released the hold it had placed on DN101.

In a different, more recent study, in PCa patients who developed resistance to taxotere, addition of weekly high dose calcitriol (32 micrograms per week) restored responsiveness to taxotere.⁶⁷

We recently carried out a single arm, open label phase II study evaluating the combination of the non-selective NSAID naproxen and calcitriol in patients with early recurrent PCa.⁶⁸ The trial was prematurely stopped when the FDA put a temporary hold on DN101 based on the data from ASCENT II. Patients had no evidence of metastases.

All the patients received 45 micrograms of calcitriol (DN101) orally once a week and 375 mg naproxen twice a day for one year. We monitored serum PSA levels every eight weeks. Bone scans were done every three months along with ultrasound of the kidney to assess asymptomatic renal stones. Serum testosterone levels were not affected by the therapy so there were no sexual side-effects. There was mild gastro-intestinal toxicity in three patients presumably from the naproxen and one patient had to be removed from the study. One patient developed a small asymptomatic renal stone and was removed from the study. He required no intervention for his renal stone. Changes in PSA doubling time (PSADT) post intervention were compared to baseline PSADT values. The therapy was well tolerated by most patients and a prolongation of the PSADT was achieved in 75% of the patients suggesting a beneficial effect of the combination therapy.⁶⁸

Conclusions

Our recent research has led us to the identify several new calcitriol target genes in prostate cells revealing novel molecular mechanisms of calcitriol action including multiple effects to inhibit the PG pathway. Calcitriol reduces both PG production (by suppressing COX-2 and increasing 15-PGDH expression) and PG biological actions (by PG receptor downregulation). We propose that calcitriol inhibition of the PG pathway contributes significantly to its anti-proliferative effects in PCa cells. Further the inhibition of the expression of the pro-inflammatory enzyme COX-2 suggests that calcitriol has anti-inflammatory actions that may play an important role in the prevention of PCa development and progression. Combinations of calcitriol with non-selective as well as COX-2 selective NSAIDs exhibit synergistic enhancement of growth inhibition in PCa cell cultures suggesting the combination of calcitriol and a NSAID may have therapeutic utility in PCa. The results of our recent clinical trial in patients with early recurrent PCa indicate that the combination of high dose weekly calcitriol with naproxen has activity to slow the rate of rise of PSA in most patients. Using PSA kinetics, the best available tool for monitoring patients with biochemical relapse, the majority of patients in the trial appeared to exhibit a slowing of disease progression. The development of a therapy that delays the progression of PCa in this group of patients would be an advantage in postponing the need to use androgen deprivation therapy or other therapeutic modalities with side-effects and would therefore represent an advance over current therapy.

However, the role of this combination therapy will require further study to determine its rightful place in the management and or prevention of PCa.

Acknowledgements

This work was supported by grants from the American Institute for Cancer Research (06A114) and from the NIH (DK42482) to D.F.

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