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Vitamin D Inhibition of the Prostaglandin Pathway as Therapy for Prostate Cancer

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Prostaglandins have been shown to play a role in carcinogenesis and the progression of many cancers, including prostate cancer.[1,2] We have recently discovered that the biologically active form of vitamin D, $1,25(OH)_2D$ or calcitriol, regulates multiple genes involved in the prostaglandin pathway.[3] These effects include decreasing prostaglandin synthesis, increasing prostaglandin catabolism, and inhibiting the expression of prostaglandin receptors. In combination, these three mechanisms reduce prostaglandin levels and signaling, thereby attenuating the growth-stimulatory effects of prostaglandins in prostate cancer.

OVERVIEW OF PROSTAGLANDIN SIGNALING

Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme that catalyzes the conversion of arachidonic acid to prostaglandins and related eicosanoids. The expression of COX-2 is rapidly induced by a variety of mitogens, cytokines, tumor promoters, and growth factors.[2] Compelling evidence from genetic and clinical studies indicates that increased expression of COX-2 is one of the key steps in carcinogenesis.[2] Several studies have demonstrated COX-2 overexpression in prostate adenocarcinoma[4,5] and suggest a positive role for COX-2 in prostate tumorigenesis. However, not all prostate cancers are associated with elevated COX-2 expression.[6,7] Although Zha et al.[6] did not find consistent overexpression of COX-2 in established prostate cancer, they detected appreciable COX-2 expression in areas of proliferative inflammatory atrophy, lesions that have been implicated in prostate carcinogenesis. In a retrospective analysis of prostate cancer outcome, Cohen et al.[8] found that elevated levels of COX-2 in resected prostate strongly predicted recurrence, while low levels were associated with a low incidence of recurrence.

The key enzyme responsible for the metabolic inactivation of prostaglandins, 15-hydroxyprostaglandin dehydrogenase (15-PGDH), catalyzes the conversion of prostaglandins to their corresponding 15-keto derivatives that exhibit greatly reduced biological activity. A recent study describes 15-PGDH as an oncogene antagonist that functions as a tumor suppressor in colon cancer.[9] The study showed that 15-PGDH, which physiologically antagonizes COX-2, is universally expressed in normal colon specimens, but is routinely absent or severely reduced in cancer specimens. Stable transfection of a 15-PGDH expression vector into cancer cells greatly reduced the ability of the cells to form tumors and/or slowed tumor growth in nude mice.[9]

Prostaglandins bind to G-protein-coupled membrane receptors (prostanoid receptors), which activate signal transduction pathways.[10] There are eight members in the prostanoid receptor subfamily that are distinguished by their ligand-binding profile and the signal transduction pathways that they activate upon ligand binding. This diversity accounts for some of the variable, tissue-specific, and often opposing actions of prostaglandins.[10] PGE and PGF are the major prostaglandins stimulating the proliferation of prostate cancer cells.[3] PGE acts through four different PGE receptor (EP) subtypes (EP1–EP4), while PGF activates the FP receptor. Prostate cancer cells express EP and FP prostaglandin receptors.[3,11]

CALCITRIOL EFFECTS ON THE PROSTAGLANDIN PATHWAY IN PROSTATE CELLS

Initial analyses from our laboratory using cDNA microarrays to study changes in the gene expression

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profile following calcitriol treatment of prostate cells indicated that calcitriol up-regulated the expression of 15-PGDH and down-regulated COX-2 expression.[12,13] Further study revealed that calcitriol regulated the prostaglandin pathway genes in multiple prostate cancer cell lines as well as primary prostatic epithelial cells established from surgically removed prostate tissue from prostate cancer patients.[3] We found measurable amounts of COX-2 mRNA and protein in various prostate cancer cell lines and in primary prostatic epithelial cells derived from normal and cancerous prostate tissue, which were significantly decreased by calcitriol treatment.[3] We also found that calcitriol significantly increased the expression of 15-PGDH mRNA and protein in various prostate cancer cells.[3] We further showed that by inhibiting COX-2 and stimulating 15-PGDH expression, calcitriol decreased the levels of biologically active prostaglandins in prostate cancer cells, thereby reducing the growth stimulation by prostaglandins.[3] Interestingly, our data also revealed that calcitriol decreased the expression of the EP and FP prostaglandin receptors. The calcitriol-induced decrease in prostaglandin receptor levels resulted in the attenuation of prostaglandin-mediated functional responses even when exogenous prostaglandins were added to the cultures.[3] Calcitriol suppressed the induction of the immediate-early gene *c-fos* and the growth stimulation seen following the addition of exogenous prostaglandins or the prostaglandin precursor arachidonic acid to prostate cancer cell cultures.[3] Thus, calcitriol inhibits the prostaglandin pathway in prostate cancer cells by three separate mechanisms: 1) by decreasing COX-2 expression, 2) by increasing 15-PGDH expression, and 3) by reducing prostaglandin receptors. We believe that these actions contribute to the suppression of the proliferative stimulus provided by prostaglandins in prostate cancer cells. The regulation of prostaglandin metabolism and biological actions constitute an additional novel pathway of calcitriol action in addition to its many other actions to mediate anti-proliferative effects in prostate cells.

COMBINATION OF CALCITRIOL AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AS THERAPY FOR PROSTATE CANCER

Non-steroidal anti-inflammatory drugs (NSAIDs) have the capacity to decrease prostaglandin 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 designed to be more selective for COX-2. Since calcitriol inhibits COX-2 expression and NSAIDs act on the COX-2 protein to inhibit enzyme activity, we hypothesized that calcitriol and an NSAID would exhibit increased potency when given in combination. We predicted that the combination would allow the use of lower and safer concentrations of NSAIDs to inhibit COX-2 enzyme activity.[3] In addition, an increase in the expression of 15-PGDH due to calcitriol action will lower the levels of biologically active prostaglandins and enhance the NSAID effect. Therefore, we hypothesized that the combination of calcitriol and NSAIDs would exhibit synergistic effects to inhibit prostate cancer cell growth. When calcitriol was combined with the COX-2-selective NSAIDs NS398 and SC-58125 or the non-selective NSAIDs naproxen and ibuprofen, we found a synergistic enhancement of growth inhibition. These results led us to further hypothesize that the combination of calcitriol and NSAIDs may have clinical utility in prostate cancer therapy and the combination was worthy of evaluation in a clinical trial.[3]

The combination therapy approach will allow the use of lower concentrations of NSAIDs and thereby minimize their undesirable side effects. It has recently become clear that continued use of COX-2-selective inhibitors such as rofecoxib (Vioxx) causes an increase in cardiovascular complications in some patients.[14] In comparison, non-selective NSAIDs such as naproxen may be associated with fewer cardiovascular adverse effects.[15] Our data show that the combination of calcitriol with a non-selective NSAID 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 prostate cancer that would allow both drugs to be used at reduced dosages, leading to increased safety.[3]

CLINICAL TRIAL OF CALCITRIOL AND NAPROXEN IN EARLY RECURRENT PROSTATE CANCER

Based on the data described above in prostate cancer cells, we decided to advance the concept of combination therapy of calcitriol with a NSAID to a clinical trial and treat men with early recurrent prostate cancer with calcitriol and naproxen. The calcitriol regimen was chosen to be the very high-dose intermittent calcitriol (DN-101) obtained from Novacea and described by Beer et al.[16,17] The dose was 45 μg administered once per week. Naproxen was given as 400 mg twice per day, a standard dose of this drug. The prostate-specific antigen (PSA) doubling time was the biomarker that we followed. The study is still ongoing, but at this time there are 13 evaluable patients. Two patients have had a drop in serum PSA, but it is too early to tell if the PSA will fall 50% and represent a true PSA response. The PSA doubling time has increased 2- to 10-fold in 9 of the 11 remaining patients. Overall, the treatment is well tolerated. One patient did develop a small asymptomatic kidney stone at 10 months and was removed from the

study. The trial will complete its planned accrual of 33 patients.

CONCLUSIONS

Calcitriol has multiple actions to inhibit and/or prevent cancer cell growth. As described in this review, a newly recognized action to inhibit the prostaglandin pathway can be added to the more commonly described calcitriol anti-cancer actions.[18-21] We believe that calcitriol actions to inhibit the prostaglandin pathway reflect more generally on calcitriol's ability to mediate a wide range of anti-inflammatory effects.[22,23] Paired with an NSAID, the combination has synergistic activity in vitro and can inhibit prostate cancer cell growth in patients based on a slowing of the PSA doubling time. We believe calcitriol will be a useful addition to the drugs available to treat and/or prevent prostate cancer.

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