Calcitriol and Genistein Actions to Inhibit the Prostaglandin Pathway: Potential Combination Therapy to Treat Prostate Cancer

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Abstract

We present an overview of the prostaglandin (PG) pathway as a novel target for the treatment of prostate cancer (PCa) using a combination of calcitriol and genistein, both of which have known antiproliferative properties. Calcitriol inhibits the PG pathway in PCa cells in 3 separate ways: by decreasing cyclooxygenase-2 (COX-2) expression, stimulating 15-hydroxyprostaglandin dehydrogenase (15-PGDH) expression, and decreasing EP (PGE₂) and FP (PGF₂α) receptors. These actions of calcitriol result in reduced levels of biologically active PGE₂, leading ultimately to growth inhibition of the PCa cells. We also demonstrate the advantages of using calcitriol in combination with genistein for the treatment of PCa.

Genistein, a major component of soy, is a potent inhibitor of the activity of CYP24, the enzyme that initiates the degradation of calcitriol. This leads to increased half-life of bioactive calcitriol, thereby enhancing all of calcitriol’s actions including those on the PG pathway. In addition to inhibiting CYP24 enzyme activity, genistein has its own independent actions on the PG pathway in PCa cells. Like calcitriol it inhibits COX-2 expression and activity, leading to decreased synthesis of PGE₂. It also inhibits the EP and FP receptors, thereby reducing the biological function of PGE₂. Thus, the combination of calcitriol and genistein acts additively to inhibit the PG pathway. Both calcitriol and genistein are relatively safe and have little toxicity associated with their intake. We postulate that the combination of calcitriol and genistein is an attractive therapeutic option for the treatment of PCa.


Prostate cancer (PCa) is the second most common cancer, after skin cancer, in men in the United States, causing ~30,000 deaths in 2005 (1). Initially, PCa is characterized by androgen-dependent growth, and androgen-deprivation therapy remains the most important therapy for patients with advanced PCa (2). However, as the disease progresses, the cancer eventually evolves into androgen-independent PCa that is no longer responsive to androgen-deprivation therapy (2). Effective treatment options for patients with androgen-independent PCa are limited, and the cancer eventually leads to metastasis and death (3,4). Despite the clinical importance of PCa and the large number of men harboring the disease, the mechanisms underlying the development and progression of the PCa are poorly understood.

Here we present an overview of the prostaglandin (PG) pathway as a contributing factor to PCa carcinogenesis and progression (5,6). Because of their role in driving the growth and spread of the cancer, PGs have become an attractive target for chemoprevention and treatment (7,8). Calcitriol, the hormonally active form of vitamin D (9), and soy and genistein, 1 of its active ingredients (10), have been investigated for their potential to exert beneficial effects on the progression of PCa (11–21). We now present further supporting evidence for the hypothesis that 1 of the activities of calcitriol and genistein contributing to their anticancer efficacy is that they individually and in combination target the PG pathway. Although both calcitriol and soy have many actions that add to their anticancer activity, we believe that targeting the PG pathway is a novel action of calcitriol and genistein in PCa, and we focus on this newly recognized activity in this article.
**PG signaling in prostate cancer**

Prostate tumorigenesis is a multistep process in which environmental and dietary factors play a significant role (22). Chemoprevention and treatment strategies for many cancers involve interruption of multistage carcinogenesis by targeting some growth-promoting signaling pathways such as those modulated by transcription factors (e.g., NF-κB, AP-1, STAT-3), antiapoptotic proteins (e.g., Bcl-2, Bcl-xl, Akt), cell cycle proteins (such as cyclins and cyclin-dependent kinases), and numerous growth factor signaling pathways. One such pathway is the PG pathway, which has become a novel target for the treatment and prevention of cancer (23,24). PGs are members of a large group of hormonally active fatty acids synthesized from arachidonic acid (AA) by the action of cyclooxygenases (COX). They play a significant role in the development and progression of PCa, and ample evidence implicates PGs as potent mediators of PCa proliferation (5,25,26). The steady-state levels of cellular PGs depend on the relative rates of COX-dependent biosynthesis of active PGs and 15-hydroxyprostaglandin dehydrogenase (15-PGDH)–dependent degradation to inactive metabolites.

**COX-2**

COXs are prostaglandin G/H synthases that catalyze the synthesis of PGs from AA released by membrane phospholipases. They exist in the following 2 forms: as COX-1, which is constitutively expressed in most tissues, and COX-2, which is inducible and regulated by mitogens, growth factors, and cytokines (27–29). COX-2 is overexpressed in cancers of the colon, liver, pancreas, stomach, breast, head and neck, and esophagus (30). The expression and role of COX-2 in PCa, however, has been somewhat controversial. Although early reports indicated overexpression of COX-2 in prostate cancer cells (31–33), some recent studies could detect low or no expression of COX-2 in prostatic tissue (34,35). Zha et al. (34) found low expression of COX-2 in established PCa but appreciable expression of COX-2 in proliferative inflammatory atrophy lesions that have been implicated in prostate carcinogenesis. Similar observations about these lesions were made in other studies (36,37). In a recent study of 90 PCa patients, Rubio et al. (38) reported that COX-2 protein expression could be detected in both biopsy cores and surgically removed prostate specimens by immunohistochemistry. Many investigators believe that regardless of the source (prostate cancer cells or infiltrating inflammatory cells), PGs contribute to prostate carcinogenesis. Several investigators postulated a chemopreventive role for nonsteroidal antiinflammatory drugs (NSAIDs), especially the COX-2-selective inhibitors, further implicating COX-2 in prostate carcinogenesis (39–42). Recent evidence also indicates that the expression of COX-2 is an independent predictor of prostate cancer recurrence (43), suggesting an important role for COX-2 in PCa progression. PGE$_2$, 1 of the metabolic products of COX-2, exhibits several biological actions that promote both tumorigenesis and tumor progression. These actions include increased cell proliferation (25,44), promotion of angiogenesis (39), elevated expression of Bcl-2, resistance to apoptosis (45), and altered immune surveillance (46,47).

**PG receptors**

PGE$_2$ is the most significant PG produced in human prostate tissue (48). Its actions are mediated through the EP receptors, which are G-protein–coupled receptors that activate second messenger systems within the cell (49,50). There are 4 EP receptors (receptors for PGE$_2$): EP1 and EP4 are expressed in prostate cells (26,51). We have also shown the presence of FP receptors (the receptor for PGF$_{2α}$) in PCa cells (26). A number of in vivo studies demonstrated the vital role played by EP receptors in tumorigenesis. Disruption of the EP2 receptor gene decreases the number and size of intestinal polyps in APC$^{−/−}$ knockout mice (52). Partial resistance to carcinogen-mediated induction of aberrant crypt foci was demonstrated in mice with homozygous deletions of EP1 and EP4 receptor genes (53,54). EP1, EP2, and EP4 receptors were elevated in mammary tumors in COX-2-MMTV mice. All of these studies suggest an important role for the EP receptors in mediating PG functions and contributing to carcinogenesis.

### 15-PGDH

15-PGDH is the key enzyme involved in the biological inactivation of PGs (55). Hormones and drugs known to regulate the expression of 15-PGDH include progesterone, androgens, interleukin-6, and thiazolidine drugs (56–59). Yan et al. (60) demonstrated that 15-PGDH, which is expressed uniformly in most normal colon tissue, was either reduced or totally absent in colon cancer specimens and that restoring 15-PGDH expression strongly inhibited the ability of colon cancer cells to form tumors in immune-deficient mice. The study concluded that 15-PGDH was a tumor suppressor that antagonized the effects of COX-2 and also inhibited angiogenesis in vivo. In LNCaP human PCa cells, androgens increase 15-PGDH expression in a dose- and time-dependent manner (56,59). Androgens are well known to exhibit a biphasic growth effect in LNCaP cells. The induction of 15-PGDH was demonstrated at concentrations of androgen that are known to inhibit the growth of LNCaP cells (56,59), suggesting a link between growth inhibition and increased expression of 15-PGDH. Preliminary reports in certain other cancers suggest that loss of 15-PGDH expression contributes to malignancy (61,62). We (26) showed that calcitriol, which is known to inhibit growth of androgen-sensitive LNCaP cells, induces the expression of 15-PGDH. Taken together, these data suggest an important role for 15-PGDH to inhibit tumor growth and progression.

### Calcitriol and genistein actions and advantages of combination therapy

Natural and synthetic dietary agents have drawn immense attention from both the scientific community and the public because of their role in cancer prevention and suppression. Epidemiologic studies suggested that the progression of latent PCa to clinically relevant disease is delayed among Asians who consumed diets rich in soy and vitamin D (63–65). 1,25-Dihydroxyvitamin D$_3$ (calcitriol), the hormonally active form of vitamin D$_3$, is a potent inhibitor of a number of cancers (66). Extensive laboratory and clinical studies demonstrated the antiproliferative effects of calcitriol in PCa (11–13,16–18,67). Likewise, genistein, a major component of soy, inhibits the growth of several types of cancers including PCa (68–71). An international study involving 50 countries identified soy products as functional foods with substantial protective effects against PCa (72).

Although the antiproliferative effects of calcitriol have been impressive on PCa cell lines, its effects on PCa patients has been modest. Patients administered calcitriol as a single agent have responded with a stabilization of their rise in serum prostate-specific antigen with minimal tumor regression (67,73). However, significantly improved responses were achieved when calcitriol was combined with drugs (74–77). The growth-inhibitory effects of calcitriol appear to correlate inversely with the inducible expression of CYP24, the P-450 enzyme that initiates the degradation of calcitriol (78,79). We (80,81) showed that in cells...
that are resistant to the effects of calcitriol, a combination of calcitriol and potent P-450 enzyme inhibitors such as liarozole and ketoconazole enhances calcitriol's growth-inhibitory effects. These compounds inhibit the CYP24 enzyme activity, thereby increasing the bioavailability of calcitriol, leading to enhancement of its actions.

Genistein's inhibition of CYP24 expression has been well documented. Several studies showed that high concentrations of genistein inhibit the expression of the enzymes CYP24 and CYP27B1, both of which are involved in calcitriol metabolism (82–84). Wiertzke and Welsh (85) reported increases in vitamin D receptor (VDR) expression in T47D human breast cancer cells treated with genistein. Extensive studies from our laboratory showed that genistein potentiates the action of calcitriol by directly inhibiting CYP24 enzyme activity, leading to an increase in the half-life of calcitriol and amplifying the homologous up-regulation of VDR, the receptor that mediates the actions of calcitriol (86). Thus, the increases in the bioavailability of the active hormone coupled with increased VDR levels sensitize the cells to the growth-inhibitory actions of calcitriol in the presence of genistein (86). Rao et al. (87) showed that calcitriol and genistein together cause synergistic growth inhibition of PCa cells through cell cycle arrest. They attributed this to genistein's modulation of vitamin D signaling (88). In this article we present further evidence for our hypothesis that the PG pathway is a novel target for the actions of both calcitriol and genistein in PCa (26).

**PG pathway as a target for the actions of calcitriol and genistein**

We recently showed that the regulation of PG metabolism and biological actions constitutes a novel pathway contributing to the antiproliferative effects of calcitriol in PCa (26). We demonstrated that calcitriol had 3 effects on the PG pathway in PCa cells: it reduced the expression of COX-2 in both prostate cancer cell lines (LNCaP and PC-3) and primary cultures of normal and cancerous prostatic epithelial cells; stimulated the expression of 15-PGDH, the enzyme initiating PG catabolism; and decreased the expression of EP and FP receptors in PCa cells. These 3 effects led to decreased PGE2 levels and attenuation of PG-mediated functional responses in calcitriol-treated PCa cells. We also showed that combined treatments with calcitriol and NSAIDs (both selective and nonselective inhibitors of COX-2) exhibited synergistic inhibitory action of PCa cell growth (26). Effects of genistein to decrease COX-2 transcriptional activity were mediated by calcitriol and genistein through cell cycle arrest. They attributed this to genistein's modulation of vitamin D signaling (88). In this article we present further evidence for our hypothesis that the PG pathway is a novel target for the actions of both calcitriol and genistein in PCa (26).

Precursor AA stimulated cell growth. Both calcitriol and genistein blocked this growth stimulation, and the combination significantly better growth inhibition then either agent alone.

Chen and Hughes-Fulfurd (51) showed that AA increases the expression of the immediate-early gene c-fos by undergoing a COX-2-mediated conversion to PGE2, binding of PGE2 to its receptors (EP2/EP4 receptors), and subsequent activation of the protein kinase A pathway, which eventually leads to the stimulation of cell growth. Therefore, we evaluated the effect of calcitriol, genistein, and the combination on AA-stimulated c-fos expression in PCa cells. As shown in Figure 2, the expression of c-fos mRNA as measured by qRT-PCR increased >5-fold when PC-3 cells were treated with AA, and this increase was attenuated by calcitriol and genistein, with the combination being more effective than the individual agents.

Although we have focused this article on inhibition of the PG pathway by calcitriol and genistein, both are known individually to exert pleiotropic effects in PCa cells to regulate cell growth, differentiation, and apoptosis. Figure 3 is a schematic diagram...
Figure 3  Schematic representation of the actions of calcitriol and genistein on the prostaglandin pathway. Actions of calcitriol are represented as 1) inhibition of COX-2 expression, 2) inhibition of PG receptors (EP and FP), and 3) stimulation of 15-PGDH. Actions of genistein are represented as a) inhibition of COX-2 expression and activity, b) inhibition of EGF-stimulated COX-2 expression, and c) inhibition of CYP24 activity.

showing our view of the interaction of calcitriol and genistein on the PG pathway. It does not depict the many other actions of both calcitriol and genistein that contribute to the antiproliferative activity of these drugs but focuses on their interactions regarding inhibition of PG synthesis and action. Our data suggest that the effects of calcitriol and genistein reflect their multiple actions to reduce biologically active PGE2 levels (through COX-2 suppression and 15-PGDH induction for calcitriol and COX-2 suppression for genistein) and inhibit PGE2 signaling (through reduction in EP receptor levels). In addition, genistein, by inhibiting CYP24 activity, will prolong the half-life of calcitriol, thereby enhancing all of calcitriol's actions, including inhibition of the PG pathway. Genistein also inhibits COX-2 expression stimulated by epithelial growth factor, providing yet another useful target in this complex pathway.

Conclusions

The ability of calcitriol and genistein to inhibit the synthesis and biological effects of proinflammatory PGs suggests that their combination may be useful as a chemopreventive or therapeutic strategy in PCa. Until recently, NSAIDs were actively investigated as cancer-preventive agents because of their ability to inhibit COX-2 activity and PG synthesis. However, NSAIDs, especially the COX-2–selective inhibitors, are associated with adverse cardiovascular events, and their use in cancer chemoprevention has come under a cloud (92,93). Despite the promising results, many trials using COX-2–selective NSAIDs have been discontinued. Our research suggests that calcitriol and genistein may be a relatively safer option for inhibiting the PG pathway. Because calcitriol regulates multiple genes involved in the PG pathway, it is very effective in inhibiting PG generation and actions, and the combination with genistein enhances calcitriol’s ability to inhibit the PG pathway. Calcitriol is a safe agent with hypercalcemia as the only side effect, and even this untoward effect is greatly reduced by intermittent calcitriol therapy (18,67,75–77,94). Genistein is a major component of soy and, as a dietary agent, soy exhibits little or no toxicity. Therefore, the combination of these relatively nontoxic agents that are available for use, orally administered, and economical offers a potentially effective therapeutic option for PCa therapy that can be moved easily and swiftly to clinical trials.

Literature Cited


