SPECIAL GUEST EDITOR SECTION

Role of Phytosterols in Cancer Prevention and Treatment

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Plant sterols or phytosterols have been shown to be effective in improving blood lipid profile and thereby protective against cardiovascular disease. In addition to their cardioprotective effects, phytosterols have gained more insight for their protective effect against various forms of cancer. Phytosterols have been reported to alleviate cancers of breast, prostate, lung, liver, stomach and ovary. Reductions in growth of various cancer cells including liver, prostate and breast by phytosterols treatment have been demonstrated. Although exact mechanisms of phytosterols for their anticancer effects are not very well delineated, there have been several mechanisms proposed such as inhibition of carcinogen production, cancer cell growth and multiplication, invasion and metastasis and induction of cell cycle arrest and apoptosis. Other mechanisms including reduction of angiogenesis, invasion and adhesion of cancer cells and production of reactive oxygen species have also been suggested. However, cancer therapy using phytosterol formulations have yet to be designed, largely due to the gap in the literature with regards to mode of action. Furthermore, most of the studies on anticancer effects of phytosterols were conducted in vitro and animal studies and need to be confirmed in humans.

Phytosterols (plant sterols) have been shown to reduce blood cholesterol levels by reducing intestinal absorption of cholesterol (1–3). In addition to their cholesterol-lowering actions, mounting evidence suggests the anticancer effects of phytosterols (4–7). Phytosterols could be beneficial in reducing cancer of human breast (8–10), prostate (11), lung (12), stomach (13), and ovary (14). Regarding anticancer effect of phytosterols on colon cancer, Marttinen et al. found tumor and colonic cell proliferation enhancing effects of both plant sterols and stanols in Apc^{Min} mice (15, 16). Basker et al., on the other hand, have demonstrated anticarcinogenic effects of β-sitosterol in 1,2-dimethylhydrazine induced colon carcinogenesis in rats (17). Thus, although anticancer effects of phytosterol consumption in some animal (18) and human (19) trials were not observed, the majority of studies indicate that phytosterols possess anticancer effects on various types of cancer in humans and animals. The lack of anticancer effect of phytosterols on colon cancer along with inconsistent results in animals could be due to reductions in intestinal cholesterol absorption, resulting in enhanced colonic cholesterol flow, which might induce and promote development of cancer as suggested by Normen et al. (18). These observations indicate the efficacy of phytosterols in prevention of both cardiovascular disease and cancer. However, by comparison, only limited knowledge exists regarding the anticancer effects of phytosterols and their mode of action. Although phytosterols might be a potent anticancer agent alleviating different types of cancer, in the current paper breast and prostate cancers are focused upon principally, based on availability of current research findings.

Breast Cancer and Phytosterols

Breast cancer is the most common cancer in women and second leading cause of cancer death in women of developed countries. The etiology of breast cancer is multifactorial. Significant breast cancer risk factors include age, early age at menarche, late age of menopause, late age at first pregnancy, obesity, oral contraception, hormone replacement therapy, diet, family history, lactation, and prior history of benign breast disease. Among these various etiological factors, modulation of cholesterol metabolism plays an important role in breast cancer. Lof and Weiderpass have shown that being overweight and gaining weight strongly associate with breast cancer risk (19). Lower high density lipoprotein (HDL)-cholesterol and apolipoprotein A-I and higher very low density lipoprotein (VLDL)-cholesterol levels were observed in serum of women with breast cancer compared with healthy women (20), whereas Qadir and Malik (21) reported that in women with breast cancer, plasma levels of triglycerides, total, and LDL-cholesterol were higher than in healthy women.

Consumption of phytosterols has been shown not only to inhibit breast cancer but also to recuperate the altered lipids levels caused by cancer (9). A case control study carried out in Uruguay examined the role of phytosterols on risk of breast cancer and demonstrated that total phytosterol intake was associated with an anticancer effect [odds ratio = 0.41, 95% confidence interval (CI) = 0.26–0.65; (22)]. Effects of phytosterol consumption on cancer development have also been investigated in several animal models. Reduction in rates of growth and metastasis of human breast cancer cells was found in SCID mice when fed diets rich in phytosterols (23). Effects of phytosterols on breast cancer cell growth and metastasis were studied in female SCID mice fed defined diets supplemented

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with 2% phytosterols, 2% cholesterol, or a 0.2% cholic acid vehicle. Mice were injected with MDA-MB-231 cells into their inguinal mammary fat pads after 2 weeks. Eight weeks after the dietary supplementation, mice receiving phytosterols exhibited a 40% reduction in serum cholesterol levels. Tumor sizes in animals fed the phytosterol diet were 33% smaller than those in animals fed the cholesterol diet. Tumor cell metastasis to lymph nodes and lungs was observed in 57% of the phytosterol-fed animals, whereas it was seen in 71% of cholesterol-fed animals. In another study, the tumor size in ovariectomized athymic mice injected with MCF-7 cells was found to be reduced by 32–42% when supplemented with a β-sitosterol enriched diet. These results suggest that phytosterols inhibit tumor growth by an estrogen independent mechanism of actions (8). Llaverias et al. investigated the effects of dietary phytosterols on tumor onset and progression using a mouse model of inherited breast cancer (24). Results suggested reductions in development of mammary hyperplastic lesions and tumor burden with a phytosterol supplemented diet compared to control animals. Furthermore, phytosterols prevented lipoprotein oxidation in mice fed a high fat diet (24).

Effects of phytosterols have been studied in both estrogen receptor positive and negative human breast cancer cells using MCF-7 and MDAMB-231 cells, respectively. In both cell types, β-sitosterol potentially reduced the growth of both the estrogen receptor positive and negative cells. One of the mechanisms behind this action was found to involve activation of de novo ceramide synthesis by stimulating serine palmitoyl transferase activity (25). Other mechanisms proposed for the anticancer effects of phytosterols include the promotion of cell cycle arrest, induction of apoptosis, and stimulation of sphingomyelin turnover. Awad et al. have studied the effects of β-sitosterol and campesterol on mevalonate and mitogen-activated protein kinase (MAPK) MAP kinase pathways in MDA-MB-231 cells (26). Results demonstrated a reduction in the cell growth upon phytosterol treatment through down-regulation of cholesterol synthesis from mevalonate and stimulation of the MAPK pathway. The authors also demonstrated that phytosterols effectively reduced the cholesterol content of the MDAMB-231 cells (26).

In MDA-MB-231 cells, β-sitosterol induced a dose-dependent cell growth inhibition through cell cycle arrest as well as cell death (27). Treatment of MDA-MB-231 cells with β-sitosterol resulted in G0/G1 cell cycle arrest, which was accompanied by a decrease in CDK4 and cyclin D1 and an increase in p21/Cip1 and p27/Kip1 protein levels. Further, cell death effect of β-sitosterol was associated with induction of apoptosis. β-sitosterol also caused the depolarization of mitochondrial membrane potential and increased the ratio between Bax and Bcl-2 proteins (27).

Treatment with β-sitosterol also increases First Apoptosis Signal (Fas) protein content and caspase activity in breast cancer cells, which suggests that the increased caspase activity after β-sitosterol administration could be due to alteration of structure and function of cancer cell membranes as a result of β-sitosterol incorporation (10). β-sitosterol prevents breast cancer metastasis by inhibiting cell invasion of the basement membrane due to its ability to limit the adhesive interaction of the tumor cell with the basement membrane. Phytosterols are thought to exert these anticancer effects by inducing cell cycle arrest at the G2/M transition in MDAMB-231 cells. After supplementation with β-sitosterol, 43% of the cancer cells were in the G2 phase compared with 12 and 24% of cells supplemented with cholesterol or vehicle, respectively (28). Phytosterols have been shown also to act as a weak estrogen receptor modulators and, thereby, function through hormonal modulation (29). These mechanisms could explain the anticarcinogenic effects of phytosterols on breast cancer.

Prostate Cancer and Phytosterols

Prostate cancer is the second leading cause of cancer-related death in men and is the most frequently diagnosed malignancy in the Western world. Higher prevalence of prostate cancer was shown to be associated with increased intake of saturated fats and cholesterol. Despite extensive research, the etiology of prostate cancer is not clearly defined and is believed to be a multifactorial disease involving genetic, hormonal, dietary, age, race, and environmental causes (30, 31). Prostate cancer, which is an age-dependent malignancy, has high incidence and a long latency period, making it an ideal target for chemoprevention.

Various experiments have demonstrated the anticancer effect of phytosterols against prostate cancer. Von Holtz et al. have shown that β-sitosterol inhibits the growth and stimulates apoptosis of LNCaP cells (32). Phytosterol treatment induced apoptosis in PC-3 hormone insensitive human prostate adenocarcinoma cells (33). The effects of phytosterol and cholesterol treatment for 48 h on cancer cell proliferation and apoptosis were studied in prostate cancer cells (34). Results demonstrated that treatment with phytosterols at physiological doses (16 μM) suppressed mitosis and significantly induced tumor suppression and apoptosis by upregulating the expression of caveolin-1, the mediator of androgen-dependent proto-oncogene, which controls cell growth. β-Sitosterol treatment of prostate cancer cells at 16 μM has been demonstrated to reduce the cell growth and increase apoptosis by at least 9 and 73%, respectively (35).

Mechanistic studies with cancer cells have shown the release of ceramide after β-sitosterol supplementation (36), suggesting the involvement of the sphingomyelin cycle. Previous studies have shown that β-sitosterol stimulates the sphingomyelin cycle and the production of ceramide (32, 36). Ceramide has been shown to increase reactive oxygen species (ROS) in some cells (37). Stimulation of the sphingomyelin cycle elevates ceramide production and inhibits of tumor growth (38, 39). Two enzymes were shown to be involved in the action of ceramide on cell growth, protein phosphatase 2A (PP 2A) and phospholipase D (PLD), upon activation by ceramide and increase cell growth (40, 41). β-Sitosterol (16 μM) treatment to LNCaP prostate cells for 5 days increased the activities of PP 2A and PLD, which was not found with cholesterol treatment (42). Results of this study suggest activation of PP 2A, adding support to the role of the activation of the sphingomyelin cycle by β-sitosterol treatment. However, the authors found an increase in PLD activity that was modest but significant with β-sitosterol supplementation, suggesting that this pathway may be modulated by other mechanisms. Incorporation of β-sitosterol into cell membranes may alter fluidity and, thereby, influence the activation of membrane-bound enzymes including PLD.

Effects of β-sitosterol on ROS and prostaglandin release in PC-3 prostate cancer cells have been studied (35). Results demonstrated an inhibition of cell growth, increase in

prostaglandin release and ROS production, and an induction of apoptosis cell cycle arrest with phytosterol treatment. Phytosterols possess a preventive role against benign prostatic hyperplasia (BPH). BPH is not prostatic cancer but an enlargement of the central area of the prostate that is associated with excessive cellular proliferation in both the glandular and stromal elements. In two multicenter, randomized, double-blind, placebo-controlled clinical trials, administration of as little as either 20 mg β-sitosterol three times a day (43) or 65 mg of β-sitosterol twice a day (44) to patients with BPH was shown to be associated with significant symptomatic improvements in prostate function.

Mechanisms of Anticancer Actions of Phytosterols

Various mechanisms of actions for phytosterols against cancer have been suggested, including inhibition of production of carcinogens, cancer cell growth, invasion, and metastasis and promotion of apoptosis (5). Phytosterols could alleviate cancers of the breast or prostate based on any one or more of the mechanisms explained above. In addition, several other mechanisms of action have been suggested as responsible for the anticancer effects of phytosterols. Park et al. for instance, demonstrated that β-sitosterol enhances apoptosis and inhibits proliferation in human leukemic U937 cells by activating caspase-3 and increasing Bax:Bcl-2 ratio (45).

Another potential mechanism occurs through ROS produced as a result of oxidative stress to cells, which could lead to DNA damage resulting in carcinogenesis. β-Sitosterol elevated activities of antioxidant enzymes in cultured macrophage cells with phorbol 12-myristate 13-acetate induced oxidative stress, suggesting that phytosterols can protect cells from damage by ROS (46). Baskar et al. have suggested that the anticarcinogenic effects of β-sitosterol in experimental colon cancer are through their antioxidant properties and ability to reduce β-catenin and Proliferating Cell Nuclear Antigen **(**PCNA) expression in colonic mucosa (17). Awad et al. investigated the effects of treatment of cultured lipopolysaccharide-activated macrophage cells with phytosterols (33). Results showed decreased production of prostaglandins after treatment with phytosterols. These results suggest that phytosterols can alleviate cancer development by reducing the production of carcinogens.

Phospholipids interact more strongly with cholesterol than with phytosterols, demonstrating that incorporation of phytosterols into membranes could modify the structure of the membranes (47). Lipid rafts of the cell membrane, where sterols are found to be highly concentrated, regulate cascades of cellular phosphorylation that occur from external stimuli (48). Hence, incorporation of phytosterols into lipid rafts may lead to beneficial changes in signal transduction.

One of the other mechanisms by which phytosterols may promote apoptosis is through reducing blood cholesterol concentrations. Reductions in blood cholesterol level could potentially lead to enhanced apoptosis. High intakes and elevated blood levels of cholesterol are thought to possibly be associated with heightened risk of cancer (20, 21). Increased blood cholesterol leads to their accumulation in cell membranes. Mice consuming hypercholesterolemic diets showed increased levels of blood cholesterol, which resulted in elevated cholesterol in lipid rafts of prostate cancer cells (48). Such increased cholesterol levels in cell membranes results in increased survival and reduced apoptosis of cells (49, 50). Increased cholesterol content in lipid rafts of prostate cancer cells lead to increased Akt activity (48). These findings suggest that by reducing blood cholesterol level, phytosterols might suppress the incorporation of cholesterol into the lipid rafts of cancer cells and, thereby, promote apoptosis of cancer cells through reduction of anti-apoptotic signal transduction and diminish cancer risk.

Summary

Administration of phytosterols has been found to be effective against various forms of cancer. Several mechanisms such as inhibition of carcinogen production, stimulation of apoptosis, and induction of the sphingomyelin cell cycle have been suggested as accounting for the anticarcinogenic effects of phytosterols. However, cancer therapy using phytosterol formulations has yet to be designed, largely due to the gap in the literature with regards to mode of action. In addition, most of the anticancer effects of phytosterols have been demonstrated in in vitro and animal studies and need to be confirmed in carefully controlled human trials.

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