Phytosterols as Anticancer Dietary Components: Evidence and Mechanism of Action[1,2]

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ABSTRACT Phytosterols (PS) or plant sterols are structurally similar to cholesterol. The most common PS are β-sitosterol, campesterol and stigmasterol. Epidemiologic and experimental studies suggest that dietary PS may offer protection from the most common cancers in Western societies, such as colon, breast and prostate cancer. This review summarizes the findings of these studies and the possible mechanisms by which PS offer this protection. These include the effect of PS on membrane structure and function of tumor and host tissue, signal transduction pathways that regulate tumor growth and apoptosis, immune function of the host and cholesterol metabolism by the host. In addition, suggestions for future studies to fill the gaps in our knowledge have been given. J. Nutr. 130: 2127–2130, 2000.

KEY WORDS: • phytosterols • β-sitosterol • membrane sphingomyelin • apoptosis • tumor growth • ceramide • cancer

Phytosterols (plant sterols, PS)[4] are the counterparts of cholesterol in animal products. They have a structure similar to that of cholesterol but with some modifications. These modifications involve the side chain and include the addition of a double bond and/or methyl or ethyl group. The most common dietary PS are β-sitosterol (SIT), campesterol and stigmasterol (**Fig. 1**). The Western diet contains 80 mg PS/d, whereas vegetarian and Japanese diets contain 345 and 400 mg/d, respectively (1). The best dietary sources of PS are unrefined plant oils, seeds, nuts and legumes (2,3). Processing of plant oils (such as refining and deodorization) reduces PS content, but the loss varies with the type of oil (3). Hydrogenation of refined oils, however, has little effect on PS content (3).

The interest in studying PS was due initially to their effectiveness in reducing the absorption of dietary cholesterol and thus offering protection from cardiovascular diseases (4,5). Several review articles on this subject have appeared in the literature (6,7) and thus will not be addressed here.

Anticancer Properties of Phytosterols. In recent years, a great deal of interest has been given to the role of PS in the protection from cancer. Raicht et al. (8) suggested that dietary SIT may offer protection from chemically induced colon cancer. These authors examined the growth of methylnitrosourea-induced tumor in rats fed 0.2% SIT in the diet for 28 wk. There was a 39% reduction in the number of rats that developed the tumor and a 60% reduction in the number of tumors per rat with SIT feeding.

Usually, the development of colon cancer is preceded by an increase in cell proliferation in the colonic mucosa, i.e., hyperplasia. Accordingly, this condition is considered to be a risk factor for the development of colon cancer (9). Several investigators examined the effect of dietary PS on colonocyte proliferation in mice (10) and rats (11). In these studies, cell proliferation was stimulated by including cholic acid in the diet and monitored by using ^{3}H-thymidine or bromodeoxyuridine. Feeding a 1–2% PS mixture, which was made up of 56% SIT, 28% campesterol, 10% stigmasterol and 6% dihydrobrassicasterol by weight, for 22 d resulted in normalizing the cholic acid–induced hyperproliferation of colonocytes (11). In rats fed colon cancer–inducing chemical carcinogens, such as methylnitrosourea, SIT resulted in reduction of the proliferative compartment of the crypt and cell proliferation (12).

In vitro studies using established human tumor cell lines have revealed an inhibitory effect of SIT on tumor growth of HT-29 cells, a human colon cancer cell line with 16 μmol/L SIT supplementation for 5 d (13). SIT concentration used was the maximum dose within its solubility range and within the physiologic range (4–70 μmol/L) in the blood (7). However, this concentration is lower than that available to colonocytes in vivo because we absorb only 5% of dietary intake (7). Similar results to those obtained in HT-29 cells, but at a lower magnitude, were also observed using 16 μmol/L SIT in LNCaP, a human prostate cancer cell line (14). LNCaP preserves most of the in vivo characteristics of prostate cancer (15). The tumor cells produce prostate-specific antigen (PSA) and acid phosphatase in culture (15) and grow and metastasize in nude16 and SCID mice (17). Thus, it offers a unique model to study human prostate cancer in vitro. SIT (16 μmol/L) has been shown to be effective in reducing tumor growth as judged by reduction in cell number and PSA production in the media (14). The effect of SIT and campesterol compared with cholesterol on the growth and apoptosis of MDA-MB-231, a human breast cancer cell line, has been investigated (18). SIT and campesterol were the only two PS detected in the blood (19,20). In these studies, 16 μmol/L of both cholesterol and campesterol was found to be without effect on tumor growth, whereas 16 μmol/L SIT inhibited the growth of the tumor by 66–80% after 3–5 d of supplementation. The results obtained support the epidemiologic studies that suggest a protective role of PS in the development of cancer (1).

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[4] Abbreviations used: PKC, protein kinase C; PP2A, protein phosphatate 2A; PS, phytosterols; PSA prostate-specific antigen; SIT, β-sitosterol.

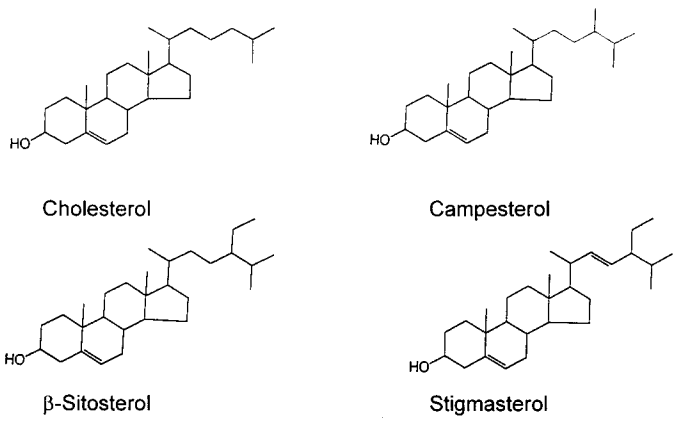

FIGURE 1 Chemical structures of sterols.

Very little in vivo work has been done on the effect of PS on tumor growth and metastasis. To examine the effect of PS on the growth of human tumors in SCID mice, mice were fed a diet supplemented with 2% of either a PS mixture or cholesterol and 0.2% cholic acid to stimulate sterol absorption. Mice were xenographed with MDA-MB-231 cells after 15 d of consuming the diets and the growth of the tumor was monitored weekly (21). After 8 wk, mice fed the PS had 33% smaller tumors ($P < 0.03$) and 20% lower metastasis than those fed the cholesterol diet.

Dietary supplementation of SIT at 60 mg/d for 6 mo has been shown to improve the clinical symptoms of prostatic hyperplasia in humans (22). This disorder, which is benign and does not lead to prostate cancer, is common among older men and results in restricted urinary flow and polyuria due to the enlargement of the gland. In Europe, prostatic hyperplasia is treated clinically with SIT-containing products (22), but these products have not been approved by the Food and Drug Administration in the U.S.

Mechanism of Action of SIT on Tumor Development.

The exact mechanism by which SIT offers protection from cancer is not known. However, several theories have been proposed and the findings in these areas are summarized in the following.

Effect of SIT on membrane structure. Because SIT has a structure similar to cholesterol, an integral lipid component of biological membranes, its incorporation into membranes has been investigated. SIT incorporation into HT-29 cell membranes did not affect total phospholipid concentration or the cholesterol/phospholipid ratio and had very little effect on the fatty acid composition (13). However, the incorporation of SIT resulted in a significant effect on the concentration of two phospholipids, i.e., a 50% decrease in sphingomyelin and an 8% increase in phosphatidylcholine. This suggests alteration in some signal transduction pathways, which will be discussed later.

Recently, the incorporation of SIT and campesterol into several tissues of rats was investigated (19). Rats were fed a diet containing 2% PS mixture, containing by weight, 56% SIT, 28% campesterol, 10% stigmasterol and 6% dihydrobrassicasterol, plus 0.2% cholic acid to enhance the absorption of PS, for 22 d. There was a fivefold increase in plasma PS compared with controls. PS was found to accumulate in adipose tissue and liver microsomes. There was no effect of PS incorporation on microsomal cholesterol concentration of the liver. However, PS reduced the cholesterol concentration by 25% in the testis. There was an increase in some polyunsaturated fatty acids and a decrease in 16:1 fatty acid with PS accumulation in membranes of the liver, testis and prostate. There was no effect of PS incorporation on phospholipid composition of membranes studied.

Effect of PS on membrane fluidity. Membrane fluidity has been shown to be influenced by the lipid composition of membranes (23). For proper function of membranes, fluidity should be maintained at a very narrow range. Incorporation of SIT into liver membranes by feeding rats 5% PS for 21 d decreased the fluidity (24). There was an increase in the activities of several hepatic fatty acid desaturases (Δ9, Δ6 and Δ5) with SIT incorporation, probably as a compensatory mechanism for the decreased fluidity. The significance of these functional alterations in terms of tumor development has not been investigated.

Effect of PS on membrane-bound enzymes. As mentioned above, the incorporation of PS into membranes results in increases in the activities of some fatty acid desaturases in the liver (24). Recent work indicates that there was a 33–44% decrease in the activities of hepatic and prostatic 5 α-reductase and 55% in prostatic aromatase in rats fed a 2% PS mixture in the diet (25). These two membrane-bound enzymes are involved in the metabolism of testosterone. Higher levels of both androgens and estrogens, the end products of the action of these two enzymes, respectively, on testosterone, have been implicated in the development of prostate hyperplasia and prostate cancer (26,27). These results may support the epidemiologic studies that suggest an association between lower levels of prostatic cancer in Asians and vegetarians with diets high in PS compared with the Western diet (28).

Hirano et al. (29)demonstrated that in vitro incubation of some PS with prostatic membranes of benign prostatic hyperplasia patients at concentrations from 10^{-3} to 10^{-6} mol/L inhibited Na^+,K^+-ATPase activity by 23–67%. These authors suggested that PS may suppress prostate metabolism and growth through this mechanism.

Effect of PS on signal transduction pathways. Two pathways have been investigated to explain the inhibition of tumor growth by SIT, protein kinase C (PKC) (30) and the sphingomyelin cycle (31). In vivo work demonstrated the lack of effect of SIT incorporation on the activity of PKC in rat mucosa (11). Moreover, in vitro work on HT-29 cells demonstrated the lack of effect of SIT on phospholipase C, a key enzyme in the PKC pathway, which catalyzes the generation of the two second messengers, inositol trisphosphate and diacylglycerol (32). These in vitro results confirm the in vivo observations on the lack of effect of SIT on the PKC pathway.

The observed changes in membrane phospholipid pattern in HT-29 cells supplemented with SIT, mainly in sphingomyelin, suggest an effect of SIT on the sphingomyelin cycle (**Fig. 2**). Investigating this pathway in HT-29 cells and LNCaP cells revealed the activation of the cycle and increased production of ceramide, the second messenger (14). Several studies suggest activation of protein phosphatase 2A (PP2A) by ceramide as an intermediate step for the action of ceramide on cell growth and apoptosis (33). SIT supplementation increased the activity but not the amount of PP2A in LNCaP cells (3). The effect of SIT on other signal transduction pathways has not been investigated.

Effect of PS on apoptosis. The rate of tumor growth is dependent upon a balance between the rates of cell proliferation and apoptosis. Apoptosis or programmed cell death, as influenced by PS, has been investigated in two tumor cell lines, MDA-MB-231 and LNCaP cells (34,35). In both cell lines, 16 μmol/L SIT was found to stimulate apoptosis by four- to sixfold above control levels after 3–5 d of treatment (34,35).

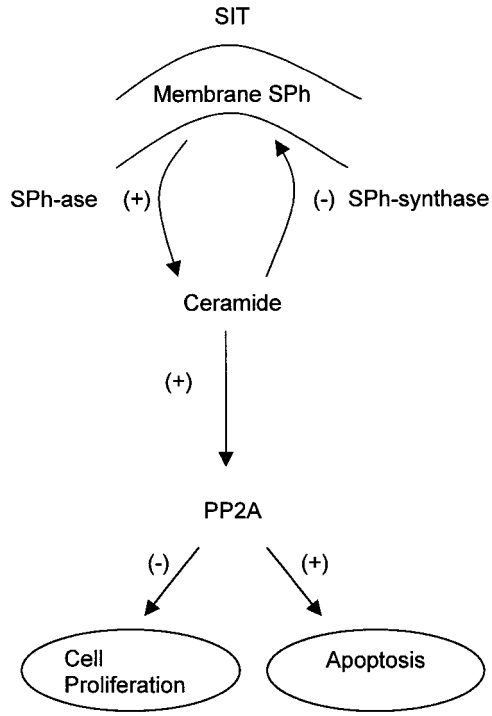

FIGURE 2 Proposed mechanism by which β-sitosterol inhibits tumor growth and stimulates apoptosis. Abbreviations: SIT, β-sitosterol; SPh, sphingomyelin; PP2A, protein phosphatase 2A; SPh-ase, sphingomyelinase; SPh-synthase, sphingomyelin synthase.

Apoptosis was assessed by measuring the release of nucleosomes into the cytoplasm. SIT treatment elevated PP2A activity only in the LNCaP line (35). This suggests that activation of PP2A may not be a unified mechanism for the action of SIT on apoptosis. The mechanism by which SIT stimulates apoptosis requires further investigation.

Effect of PS on membrane integrity. Recent studies demonstrated the lack of cytotoxicity of PS at physiologic levels on cells (34). Treatment of a breast cancer cell line with SIT and campesterol at the highest concentration within the solubility range (16 μmol/L) had no effect on the release of lactic dehydrogenase from cells in culture. Lactic dehydrogenase release is used as an index for membrane integrity.

Effect of PS on immune function. In a recent publication (36), a mixture of SIT and its glucoside at a mass ratio of 100:1 was shown to stimulate human peripheral blood lymphocyte proliferation in vitro. In addition, the ingestion of 60 mg/d of this mixture by volunteers for 4 wk resulted in enhancement of T-cell proliferation upon stimulation in vitro. The specific contribution of the SIT glucoside component in this supplement was not clear. Additional studies are required to examine the mechanism by which PS may stimulate immune system function.

Effect of PS on tissue esterogenic properties. Because estrogen receptors play a role in the development of sex organ tumors, estrogenic properties of PS were assessed. It has been shown that fish develop infertility when exposed to high levels of wood pulp, which is rich in SIT, in the water (37). However, studies in mammals such as rats using plant sterols or stanols did not demonstrate any estrogenic effect in vivo or in vitro (38). These studies showed that PS do not bind to estrogen receptors and do not affect uterine wet weight of immature rats. Furthermore, PS did not stimulate transcriptional activity of human estrogen receptors in yeast (39). Free or esterified stanols at high levels (100 μmmol/L) did not stimulate the growth of estrogen-responsive MCF-7 cells in culture nor increase uterine wet weight of rats when fed at 8.3% in the diet for 4 d (39). Accordingly, the reported results on fish could be due to other components in wood pulp.

Effect of PS on neutral and acidic sterols in the colon. Cholesterol and primary bile acids are converted in the large intestine by bacterial action to coprostanol and secondary bile acids, respectively. The presence of high levels of these modified sterols in the colonic content has been suggested to play a role in the development of colon cancer (40). Dietary PS have been shown to alter the level of fecal sterols (41,42). The mechanisms by which PS influence colonic fecal sterols may include the action of PS on colonic bacteria and alteration of cholesterol absorption (43).

Future Research. The mechanisms by which PS influence cell growth and apoptosis of tumor cells require additional study. This is especially urgent because the public has been advised concerning the effectiveness of dietary PS in reducing blood cholesterol and encouraged to consume PS-enriched products such as margarine and salad dressing. It is intriguing to mention that the added protection from several types of cancer afforded by PS treatment occurs at the same levels of PS used to lower blood cholesterol. The interaction between PS and other phytochemicals such as phytoestrogen, vitamin E and antioxidants, which have also been shown to protect from cancer, should be explored.

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