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Minireview

Pharmacological properties of plant sterols
In vivo and in vitro observations

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Abstract

Plant sterols have been investigated as one of the safe potential alternative methods in lowering plasma cholesterol levels. Several human studies have shown that plant sterols/stanols significantly reduce plasma total and LDL cholesterol. In this article, pharmacological characteristics of plant sterols/stanols have been summarized and discussed. In particular, experimental data that demonstrate the effects of dietary phytosterols on lipid metabolism and development of atherosclerotic lesions have been critically reviewed. Despite their similar chemical structures, phytosterols and cholesterol differ markedly from each other in regard to their pharmacological characteristics including intestinal absorption and metabolic fate. Compared to cholesterol, plant sterols have poor intestinal absorption. The most and best studied effects of plant sterols are their inhibition of intestinal cholesterol absorption. Other biological activities of phytosterols such as effects on lecithin:cholesterol acyltransferase activity, bile acid synthesis, oxidation and uptake of lipoproteins, hepatic and lipoprotein lipase activities and coagulation system have been linked to their anti-atherogenic properties. Moreover, evidence for beneficial effects of plant sterols on disorders such as cutaneous xanthomatosis, colon cancer and prostate hyperplasia has been discussed. Finally, the potential adverse effects of plant sterols as well as pathophysiology of hereditary sitosterolemia are also reviewed. In conclusion, more pharmacokinetic data are needed to better understand metabolic fate of plant sterols/stanols and their fatty acid esters as well as their interactions with other nutraceutical/pharmaceutical agents.

Keywords: Pharmacology; Phytosterols; Atherosclerosis; Cholesterol; Sitosterolemia

Introduction

Plant sterols are found in significant amounts in various parts of plants including seeds, nuts, fruits and vegetable oils (1). Thus, eating habits and availability of the source of plant

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sterols govern human intake of plant sterols/stanols; in the United States the daily intake of phytosterols has been estimated to be approximately 180 mg, while it may be 400 mg in Japan (2–4). Sitosterol and campesterol account for up to 95% of dietary phytosterols (approximately 65% and 30%, respectively); the remainder 5% consists of the other plant sterols/stanols, mainly stigmasterol (5). In addition to their dietary consumption, the use of plant sterols as cholesterol-lowering agents has been recently re-considered. This resulted in marketing phytosterol-enriched food products ("functional foods") in North America and Europe. These products are intended to be used by a wide range of subjects including those with dyslipidemia.

Although their pharmacological properties have not been fully explored, phytosterols have been effective in reducing plasma cholesterol levels without causing any serious side-effects (6–10). Since 1951, when Peterson (11) demonstrated the cholesterol-lowering effect of β-sitosterol in cholesterol-fed chickens, many investigators (6–10,12–15) have studied the effects of these natural substances on disorders of lipid metabolism and atherogenesis in both humans and laboratory animals. A recent clinical review showed that the average reduction in plasma total and low density lipoprotein (LDL)-cholesterol was 10% and 13%, respectively, in a total of 590 subjects treated with phytosterols (16). Gylling et al. (17) observed similar cholesterol-lowering effects of sitostanol ester margarine in children with familial hypercholesterolemia. Gylling and Miettinen (18) studied cholesterol reduction in twenty-three postmenopausal women by plant stanol esters with variable fat intake. It was concluded that varying the campestanol to sitostanol ratio from 1:13 to 1:2 in either margarine or butter similarly decreases cholesterol absorption and improves serum lipoprotein profile. Recently, Miettinen and Gylling (19) have suggested that plant stanol esters (which can be easily consumed in a form of fat containing foods such as margarine or spreads) may result in a better outcome as compared to free plant sterols or stanols.

However, a recent study (20) showed a lack of efficacy of dietary sitostanol (3 g/day) in reducing plasma total cholesterol, very low density lipoprotein (VLDL)- and LDL-cholesterol concentrations in 33 men with moderate hypercholesterolemia who were consuming a diet restricted to $<$200 mg cholesterol per day. Furthermore, Bhattachary and Lopez (21) found that β-sitosterol given orally to rabbits while resulting in a 60% increase in plasma cholesterol concentration did not cause increased accumulation of cholesterol in the tissues. Attempts by another group of investigators (22) to reproduce the increased plasma cholesterol concentrations observed by Bhattachary and Lopez (21), however, have not been successful.

The aim of this review is to summarize the available data on pharmacological properties of plant sterols/stanols including their unwanted side effects; plant sterols/stanols and their fatty acid esters are currently available to general population as phytosterol-supplemented food products.

Pharmacochemistry

Phytosterols which are synthesized by plants are generally extracted from by-products of either pulp and paper industry (wood-derived; "tall oil soap") or vegetable oil industry (vegetable-derived) using organic solvents (hexanes and acetone). The product is a mixture of various plant sterols which vary based on the plant source. The purified plant sterol mixture is white in color (similar to cholesterol) with extremely low solubility. To improve their solubility, at-

tempts have been made to produce plant sterols/stanols fatty acid esters. Similar to their appearance, cholesterol and phytosterols have similar chemical structures (Fig. 1). Addition of a methyl or ethyl group at carbon 24 of the cholesterol side chain leads to formation of campesterol or sitosterol, respectively. Dehydrogenation of the carbon 22–23 bond of sitosterol leads to stigmasterol which is another common phytosterol. Chemical saturation of the delta 5 double bond of each of the aforementioned plant sterols leads to the formation of 5-α-derivatives such as campestanol or sitostanol.

Pharmacokinetics

Absorption, distribution and biotransformation

Since humans are not able to synthesize phytosterols, dietary consumption is the only source of tissue and plasma phytosterols. Because of limited absorption, plasma levels are very low in healthy individuals (23). Generally, it has been suggested that in humans and other mammals only approximately 5% of ingested plant sterols are absorbed (23,24). Plasma campesterol levels may be used as a marker of cholesterol absorption in humans (25). Unlike in healthy humans, the absorption rate of plant sterols increases resulting in sitosterolemia, a rare genetic disorder (26–28).

The rates of absorption vary among the individual plant sterols. Heinemann et al. (29) compared the rate of intestinal absorption of cholesterol to that of several plant sterols in 10

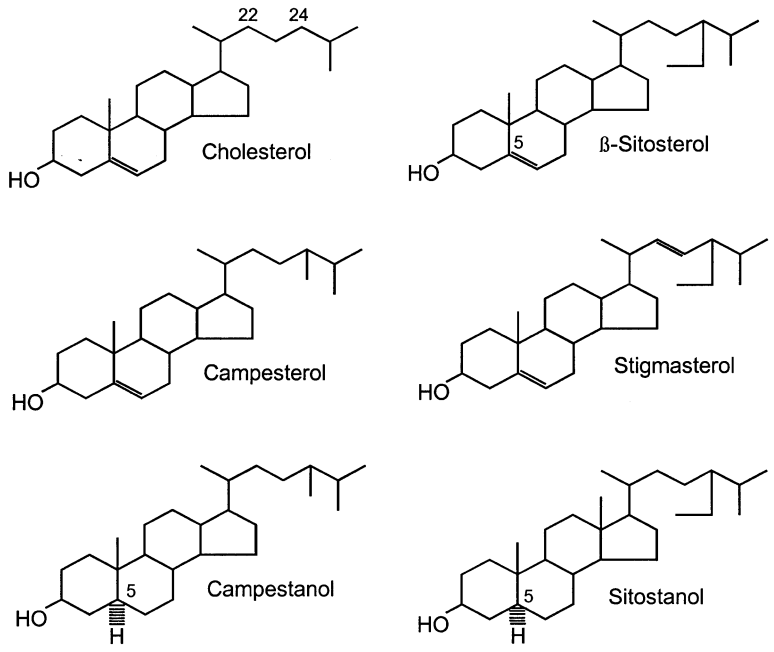

Fig. 1. Chemical structures of cholesterol, unsaturated (campesterol, sitosterol, stigmasterol) and hydrogenated (campestanol, sitostanol) plant sterols.

healthy men who underwent intestinal perfusion over a 50 cm segment of the upper jejunum. They found the highest absorption rate for cholesterol (as much as 33.0%) followed by campestanol, campesterol, stigmasterol, sitosterol and sitostanol at 12.5%, 9.6%, 4.8%, 4.2% and 0.0%, respectively. Similarly, campesterol is more easily absorbed than sitosterol in experimental animals including pigeons (30), rats (31), dogs (32) and rabbits (21). Proximal gut resection in pigs impairs cholesterol and campesterol absorption more than sitosterol absorption (33). This observation suggests that availability of jejunal villus surface area is not absolutely crucial for sitosterol absorption in pigs. Xu et al. (34) have recently studied the absorption and distribution of campestanol in New Zealand White rabbits. They have concluded that campestanol intestinal absorption is limited and can be further reduced by dietary sitostanol. The investigators also observed that campestanol was excreted rapidly, therefore, unlike cholesterol, it does not accumulate in the body.

Absorbed plant sterols circulate in plasma by lipoprotein particles in either unesterified or esterified forms. In rats, high density lipoprotein (HDL) is the major carrier of circulating plant sterols (35), while in humans it is LDL (26,27). It has been shown that lecithin:cholesterol acyltransferase catalyzes the esterification of phytosterols (36). The esterification rate was 89%, 79% and 34% for campesterol, sitosterol and stigmasterol, respectively, as compared to that of cholesterol (36). Unabsorbed phytosterols may undergo bacterial transformation by intestinal microflora to produce metabolites such as coprostanol and coprostanone (37–39). Detection of such compounds in feces of phytosterol-treated apolipoprotein E-knockout (apo E-KO) mice may also indicate this biotransformation process by gastrointestinal microflora (40).

Several mechanisms responsible for the different rates of absorption of plant sterols have been suggested: (a), micellar solubility is a major factor which affects the absorption rate (41,42). Discrimination between absorbable and non-absorbable sterols may occur during the process of their uptake into intestinal mucosa (43,44); (b) slower rate of transfer of sitosterol from the cell surface to intracellular site, compared to that of cholesterol, may also contribute to lower absorption rate for the plant sterol (45); and (c) other studies have indicated that mucosal esterification could be a possible site of discrimination in sterol absorption (46). Thus, it appears that limited absorption rate for phytosterols is due to a combination of several factors.

Total plasma plant sterol concentrations in healthy adults range from 7 μmol/L to 24 μmol/L, which accounts for less than 1% of total plasma sterol concentrations (5). Intragastric administration of radiolabeled cholesterol and sitosterol resulted in association of both sterols with the chylomicron fraction (47). While 90% of the total lymphatic cholesterol was present as cholesterol ester, only 12% of sitosterol was esterified. Unlike cholesterol, which was esterified and located in the core of chylomicron particles, sitosterol was present mainly in the unesterified form on the surface of chylomicron particles. Approximately, 70% of plasma sitosterol was esterified—a percentage similar to that of cholesterol ester (23). Animal studies have shown that plant sterols may accumulate in the liver, adrenal gland, ovary and testis (31,48–50). These observations indicate high affinity of plant sterols for steroid-synthesizing tissues, and suggest that they may be used as precursors for steroid hormones. In this regard, several investigators have reported synthesis of cortisol and sex hormones from phytosterols by human or rat endocrine tissues (51–54). More in vivo studies are needed to further characterize pharmacokinetic profile of plant sterols.

Pharmacodynamics

Anti-atherogenic effects

Anti-atherosclerotic effects of plant sterols are well documented in apo E-KO mice (12,13). These effects of plant sterols have been observed regardless of the amount of dietary cholesterol; plant sterols reduced the size of atherosclerotic lesions by 50% in the presence or absence of 0.15% (w/w) dietary cholesterol. On the other hand, anti-atherogenic effects of plant sterols are well correlated with their cholesterol-lowering effects (12). This marked reduction in the size of atherosclerotic lesions was accompanied by a significant reduction in other components of the lesions including the number of foam cells and cholesterol clefts, amounts of extracellar matrix and the extent of apparently proliferative smooth muscle cells. Anti-atherogenic effects of plant sterols may be due not only to their cholesterol-lowering activities alone, but also to other properties such as effects on coagulation system, antioxidant system, and hepatic and lipoprotein lipase activities (13).

Effects on lipoprotein metabolism

In vivo studies

Both oral and parenteral administration of plant sterols results in reduced concentrations of plasma cholesterol (9–15,55,56). This reduction in plasma cholesterol concentrations may be due not only to the inhibition of intestinal cholesterol absorption but also to other effects on hepatic/intestinal cholesterol metabolism. Laraki et al. (57) reported a significant reduction in the activity of hepatic acetyl-CoA carboxylase in rats fed a diet supplemented with a phytosterol mixture (0.5%–1% w/w) for 3 weeks. This was associated with a significant increase in the content of hepatic plant sterol in the phytosterol-fed animals.

Sitosterol also accumulated in mucosal cells of sitosterol-fed rats (both in whole homogenates and in the microsomal fraction); the sitosterol concentration reached twice that in control animals (58). This increase in sitosterol content did not affect ileac mucosal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. On the other hand, the activity of HMG-CoA reductase in ileac mucosal cells and hepatocytes of sitosterolemic subjects is significantly lower than that in control individuals (58). However, the activity of this rate limiting enzyme in de novo cholesterol synthesis pathways increased approximately 3 fold (compared to control) in the liver of apo E-KO mice fed with 2% (w/w) phytosterols for 20 weeks (40). On the other hand, cholesterol feeding (0.15% w/w) caused 60% decrease in the activity of hepatic HMG-CoA reductase (compared to controls) in apo E-KO mice (40). These observations indicate that hepatic cholesterol synthesis is influenced by intestinal cholesterol absorption: inhibition of intestinal cholesterol absorption by plant sterols stimulates de novo hepatic cholesterol synthesis, while increased cholesterol absorption suppresses it.

In rats, supplementation of diet with a phytosterol mixture (2% w/w) containing 92% sitosterol produced a 1.4-fold increase in the activity of the enzyme involved in bile acid synthesis, namely hepatic cholesterol 7 α-hydroxylase compared to controls (59). In contrast, the activities of hepatic cholesterol 7 α-hydroxylase and sterol 27-hydroxlase remained unchanged after 20-week-phytosterol feeding in apo E-KO mice (40). Since phytosterol-fed apo E-KO mice had 50% lower hepatic cholesterol content compared to controls (40)—in the face of in-

creased HMG-CoA reductase activity and unchanged bile acid synthesis enzyme activity—it seems reasonable to speculate that phytosterol treatment caused an increase in hepatic cholesterol secretion.

Decreased activity of post-heparin lipoprotein lipase and hepatic lipase was observed in phytosterol-treated apo E-KO mice (13). Reduction in hepatic lipase activity may prevent formation of small atherogenic−VLDL particles, and may also decrease their up-take by LDL-receptor related proteins. Thus, it was suggested that the observed effects of plant sterols on the activities of these two lipolytic enzymes might play a major mechanistic role in phytosterols' anti-atherogenic properties (13). Another important enzyme in cholesterol metabolism, lecithin:cholesterol acyltransferase, was also affected by β-sitosterol consumption. Supplementation with 6 g/day of β-sitosterol for 2 months significantly increased serum lecithin:cholesterol acyltransferase activity in hypercholesterolemic subjects (60).

There is good evidence that phytosterol supplementation increases LDL sitosterol content. For example, Aviram and Eias (61) showed that the sitosterol content of human LDL was elevated 2-fold in individuals consuming olive oil (50 g/day) for 2 weeks. This finding was associated with a marked reduction in LDL uptake by macrophages in vitro. Olive oil supplementation was also associated with a significant reduction in the propensity of LDL to in vitro lipid peroxidation. Several studies showed a correlation between LDL lipid composition and its uptake by various cell lines (62,63). Thus, modified LDL may have anti-atherogenic properties due to its resistance to in vitro peroxidation that results in its reduced uptake by macrophages. The effects of plant sterols on plasma enzymatic and non-enzymatic antioxidant systems have been also explored in laboratory animals (13). Although treatment with plant sterols was not associated with a strong positive effects on the antioxidant systems, a small increase was observed in the activity of glutathione peroxidase in both red blood cells and plasma of apo E-KO mice (13). This may also contribute to prevention of LDL oxidation resulting in inhibition of atherosclerotic plaque development.

In vitro studies

A significant reduction in cellular cholesterol content by plant sterols was observed in vitro when human skin fibroblasts or HepG2 cells were incubated with liposomes containing sitosterol (64). The decrease in cellular cholesterol content was accompanied by a simultaneous increase in sitosterol concentration. Incubation of CaCo-2 cells (a colon tumor cell line) with β-sitosterol decreased all of the following parameters: uptake of cholesterol from the incubation medium, cholesterol synthesis, HMG-CoA reductase activity as well as its mass and mRNA levels (65).

Other metabolic effects

Anti-xanthomatosis effect of dietary phytosterols was reported in apo E-KO mice (14). This effect of plant sterols was directly related to their cholesterol-lowering effects. In addition to their cholesterol-lowering effects, which in cholesterol-fed mice results in prevention of both cutaneous xanthomatosis and atherosclerosis (12–14), plant sterols have been shown to have a number of other metabolic effects. For example, several epidemiological and animal studies suggest that phytosterols suppress the growth of colon tumors (66). It is unclear whether this activity is related to the cholesterol-lowering effects of phytosterols or is due to

other yet unknown mechanisms. A three-month randomized placebo-controlled double-blind study of 53 men showed significant effects of plant sterols in alleviating symptoms of prostatic hyperplasia (67). Other studies (68–69) suggested that dietary phytosterols may have beneficial effects on prostate disorders.

Several studies have reported anti-coagulant effects such as reduction in platelet counts (14,70), reduced tissue plasminogen activator (71) and slightly reduced plasma fibrinogen concentrations (13) for plant sterols. These effects may also contribute to their anti-atherogenic effects. Another observation was increased resistance of red blood cells to osmotic hemolysis in phytosterol-fed apo E-KO mice as compared to controls (14). This finding may suggest alterations in red cell membrane composition due to phytosterol treatment and reduced their susceptibility to osmotic fragility. It has been shown that red cells are able to take up phytosterols under in vitro conditions (72). Thus, changes in red cell membrane compositions due to direct/indirect effects of plant sterols might play an important role in susceptibility of the cells to hemolysis. Furthermore, plant sterols prevented vacuolation in the liver and kidney of cholesterol-fed (0.15% w/w) apo E-KO mice (14). Lampe (73) has proposed that phytochemicals with vegetable and fruit source may have several biological activities including stimulation of immune system. In this regard, stimulatory effects of β-sitosterol on human lymphocyte proliferation were reported (74). The significance of the above-mentioned observations needs to be further investigated.

Adverse effects

Several studies have indicated possible undesirable side effects of phytosterols. For example, increased concentrations of phytosterols in erythrocyte membranes may result in their increased fragility; episodes of hemolysis have been reported in patients with phytosterolemia (26,75). Increased membrane rigidity was also observed in rat liver microsomes enriched with β-sitosterol and campesterol (76). Furthermore, it has been shown that high β-sitosterol levels (up to 0.7 mmol/L) can cause contraction of human umbilical vein endothelial cells in vitro (77). These observations suggest that very high plasma concentrations of β-sitosterol may have potentially cytotoxic effects and may interfere with cellular functions.

High concentrations of phytosterols in plasma of laboratory animals may have adverse effects on their reproductive organs; subcutaneous administration of 0.5 to 5 mg/kg body weight per day of β-sitosterol caused a significant reduction in both sperm count and the weight of testes in albino rats (78); application of sitosteryl ester to the vagina of rabbits significantly lowered their pregnancy rates (79). Intraperitoneal injection of β-sitosterol was associated with decreased plasma levels of testosterone and 17 β-estradiol in male and female goldfish, respectively (80). In a recent work (81), the effects of plant stanol ester on the reproductive system have been studied over two generations in rats. Consumption of a diet supplemented with 4.4% of stanol esters was associated with a significant increase in both absolute and relative weights of testes and relative weights of epididymides in F1 generation (81). Other observations were changes in spermatozoal counts in F1 generation, increased numbers of lost implantation, and increased male and female fertility indices in the treated groups as compared to controls. The above observations may be related to potential estrogenic effects of plant sterols/stanols. However, two recently published studies reported no estrogenic

activity for plant sterol/stanol esters based on the results of a series of in vitro and in vivo experiments (82,83).

Moreover, significant decreases in plasma levels of total protein, calcium, vitamins E, K and D were observed in rats treated with high dietary doses of stanol ester (5%) for 13 weeks (84). The significance of all above-mentioned observations certainly merits further investigation.

Sitosterolemia

Sitosterolemia (phytosterolemia) is a rare genetic disorder in which plasma concentrations of plant sterols, particularly sitosterol, are extremely high. It is inherited as an autosomal recessive trait (26–28). An increased dietary sitosterol absorption and a decreased elimination rate are believed to account for its accumulation in plasma and tissues of the affected individuals.

Sitosterolemic patients develop tendon xanthomas, accelerated atherosclerosis (more pronounced in young males), hemolytic episodes, arthritis and arthralgias. In several young male subjects who died of acute myocardial infarction, extensive coronary and aortic atherosclerosis was found on autopsy (85,86). Several sitosterolemic subjects had decreased activity of hepatic HMG-CoA reductase and an increased hepatic LDL-receptor binding as measured by high affinity binding to radiolabeled LDL (87). Moreover, it has been recently shown that the activities of both bile acid synthesis enzymes, namely sterol 27-hydroxylase and cholesterol 7 α-hydroxylase are inhibited in homozygous sitosterolemic patients (88). Furthermore, a study by Honda et al. (89) suggested that decreased cholesterol synthesis in sitosterolemic subjects is due to abnormal down-regulation of early, intermediate, and late enzymes in de novo cholesterol synthesis rather than a single inherited defect in HMG CoA reductase gene. The genetic make up of the disease is currently under investigation. Patel et al. (90) by studying 10 well-characterized sitosterolemic families were able to localize a genetic defect to chromosome 2p21, between microsatellite markers D2S1788 and D2S1352. Recent segregation studies have excluded the site of mutations in the genes of HMG-CoA reductase, HMG-CoA synthase, LDL-receptor, sterol regulatory element binding proteins (SREBP-1 and -2), acyl coenzyme A:cholesterol:acyltransferase (ACAT), and microsomal triglyceride transfer protein (MTP) in three affected families (91).

Conclusions

Pharmacological properties of plant sterols have not been fully studies. Inhibition of cholesterol absorption is the best understood mechanism of action of plant sterols; ingestion of 1 g of β-sitosterol may decrease cholesterol absorption by up to 42% (82). The cholesterol-lowering effects of phytosterols are associated with decreased atherogenicity in laboratory animals (12,13); however, this has not been documented in humans yet.

Recent human studies (6–10) have demonstrated similar cholesterol-lowering activities for plant stanols alone or a mixture of plant sterols and stanols. Although evidence for the efficacy of phytosterols is solid, further investigations are needed to define pharmacological characteristics of these natural substances. In particular, more pharmacokenitic data are required to better understand efficacy, potency, distribution and metabolic fate of plant sterols/stanols and their currently available fatty acid esters. Further studies are required to under-

stand why phytosterol esters have lower efficacy and potency as compared to unesterified phytosterols (92). The possibility of adverse effects of plant sterols on endocrine function and reproductive system merits further investigation (51–54,78–81). Interactions of plant sterols with other dietary or pharmaceutical agents such as lipid-lowering, anti-hypertensive, anti-epileptic, anti-inflammatory, oral hypoglycemic, and vitamins need to be defined.

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