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Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer

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Abstract

Dietary phytosterol supplements are readily available to consumers since they effectively reduce plasma low-density lipoprotein cholesterol. Several studies on cell cultures and xenograft mouse models suggest that dietary phytosterols may also exert protective effects against common cancers. We examined the effects of a dietary phytosterol supplement on tumor onset and progression using the well-characterized mouse mammary tumor virus polyoma virus middle T antigen transgenic mouse model of inherited breast cancer. Both the development of mammary hyperplastic lesions (at age 4 weeks) and total tumor burden (at age 13 weeks) were reduced after dietary phytosterol supplementation in female mice fed a high-fat, high-cholesterol diet. A blind, detailed histopathologic examination of the mammary glands (at age 8 weeks) also revealed the presence of less-advanced lesions in phytosterol-fed mice. This protective effect was not observed when the mice were fed a low-fat, low-cholesterol diet. Phytosterol supplementation was effective in preventing lipoprotein oxidation in mice fed the high-fat diet, a property that may explain — at least in part — their anticancer effects since lipoprotein oxidation/inflammation has been shown to be critical for tumor growth. In summary, our study provides preclinical proof of the concept that dietary phytosterols could prevent the tumor growth associated with fat-rich diet consumption.

Keywords: Phytosterols; Breast cancer; Cholesterol; Lipoprotein; Oxidation; MMTV-PyMT mice

1. Introduction

Plant sterols or phytosterols are molecules that resemble cholesterol but are found exclusively in plants. The most common phytosterols in the human diet are β-sitosterol, campesterol and stigmasterol [1]. Phytosterols are known to lower serum low-density lipoprotein (LDL) cholesterol levels by reducing intestinal cholesterol absorption [2]. Therefore, readily available food products have been engineered to be enriched in phytosterols and marketed to help lower serum cholesterol and reduce cardiovascular risk. Further, some epidemiologic studies have suggested that dietary phytosterols may exert a protective role not only against cardiovascular diseases but also against cancer [3,4].

According to the cancer statistics published by the American Cancer Society, breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death among women in the United States (American Cancer Society, Cancer Facts and Figures 2010, http://www.cancer.org/Research/cancer-facts-and-figures-2010, last accessed May 5, 2011). Interestingly, the incidence of common cancers, such as breast, colon and prostate cancer, is relatively low in Asian countries where people consume predominantly plant-based (and, thus, phytosterol-enriched) diets. Moreover, when Asians emigrate to Western countries and consume more animal-based diets, the incidence of these cancers rises [5]. While

Abbreviations: Abc, ATP-binding cassette transporter; Fasn, fatty acid synthase; GDI, GDP dissociation inhibitor; GP, generalized polarization; HDL, high-density lipoprotein; HFHC, high-fat, high-cholesterol; Hmgcr, 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL, low-density lipoprotein; Ldlr, LDL receptor; LFLC, low fat, low cholesterol; LXR, liver X receptor; MMTV, mouse mammary tumor virus; oxLDL, oxidized LDL; PAF-AH, platelet-activated factor acetyl-hydrolase; PON1, arylesterase or paraoxonase; PyMT, polyoma virus middle T antigen; REM, relative electrophoretic mobility; Scarb1 (or SR-BI), scavenger receptor class B type I; Srebf, sterol response element binding protein; Tg, transgenic.

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these studies show a potential association between phytosterol consumption and cancer activity prevention [4], they are not demonstrative of a cause–effect relationship. However, support for such a relationship was provided by several experimental studies (reviewed in Ref. [6]) in which β-sitosterol was shown to inhibit the cell growth of several breast, colon and prostate human cancer cell lines. The mechanisms by which phytosterols offered this protection are not fully understood. Some putative mechanisms include inhibition of cancer cell growth, apoptosis promotion, decreased angiogenesis, alteration in sterol metabolism and liver X receptor (LXR) agonism [6–8]. Recently, the existence of common gene networks among cancer, lipid metabolism and inflammation has been reported [9]. Interestingly, both oxidized LDL (oxLDL) and its receptor (OLR-1) were found to promote cellular transformation in a mechanism that may involve nuclear factor (NF) κB activation. Other authors have also related lipoprotein oxidation to the development of several types of cancer [10,11].

In vivo studies on the effect of phytosterol consumption on breast cancer progression have been limited, to date, to xenograft models. The consumption of phytosterol-enriched diets has been shown to reduce tumor growth after fully established human breast cancer cells were injected into rodent hosts [12,13]. Although these cancer models are useful, they do not reflect the complex multistage nature of human tumorigenesis. Therefore, we employed the well-established mouse mammary tumor virus (MMTV) polyoma middle T antigen (PyMT) transgenic (Tg) mouse model to analyze the role of dietary phytosterols in tumor initiation and progression. Further, considering the promoting effect of high-fat diets on cancer development, we studied the effect of a phytosterol supplement on PyMT Tg mice fed both a regular chow diet (which is low fat, low cholesterol; LFLC) and a high-fat, high-cholesterol (HCHF) diet.

2. Materials and methods

2.1. Mice and diets

MMTV-PyMT Tg mice with an FVB/N background were obtained from The Mouse Models of Human Cancers Consortium Repository (National Cancer Institute, Frederick, MD, USA). MMTV-PyMT Tg mice express high levels of the transforming oncogene polyoma virus middle T (PyMT) antigen under the control of the MMTV long terminal repeat promoter, which specifically directs expression to the mammary epithelium [14]. All female PyMT Tg mice spontaneously develop widespread multifocal adenocarcinomas in the mammary gland, with hyperplastic foci occurring as early as 3 weeks after birth [15]. The similitude between the PyMT Tg model and human breast cancer has been validated by histologic studies, which demonstrated a striking similarity between PyMT Tg tumorigenesis and various stages of human ductal adenocarcinoma progression [16,17].

All animals were kept on a 12-h light/dark cycle with access to food and water *ad libitum*. Breeding was carried out with male mice hemizygotes for the PyMT transgene and non-Tg females with the same genetic background. Genotyping was performed as indicated on The Jackson Laboratory's Web site (http://jaxmice.jax.org/). Animal protocols used in this study were approved by the Institutional Animal Care and Use Committee.

Except for the whole-mount studies, 4-week-old female PyMT Tg mice were randomized into 2 groups: those consuming and those not consuming a 2% phytosterol supplement added to the powdered food on either an LFLC diet (6.2% fat, no cholesterol, energy density 3.1 kcal/g, calories from protein, fat and carbohydrate, 24%, 18% and 58%, respectively; diet #2018; Teklad diets, Madison, WI, USA) or an HFHC diet (21.2% fat, 0.2% cholesterol, energy density 4.5 kcal/g, calories from protein, fat and carbohydrate, 15.2%, 42% and 42.7%, respectively; diet #TD.88137; Teklad diets). Composition of the diets is detailed in Table 1. Phytosterols were composed of 20% campesterol, 22% stigmasterol and 41% β-sitosterol (Lipofoods S.L., Gavà, Barcelona, Spain). Diets were prepared and mixed with phytosterols by Mucedola srl (Settimo Milanese, Milan, Italy).

Whole mounts of mammary glands were obtained from 4-week-old females whose mothers were fed the different diets during pregnancy and lactation. In all cases, mice were maintained on the diets until euthanized (at 4, 8 or 13 weeks of age). A graphic of the experimental design is shown in Supplementary Figure 1. It is important to note that in this study, experiments were planned to compare mice consuming and mice not consuming the phytosterol supplement rather than to compare the effects of an HFHC diet vs. an LFLC diet. Therefore, some experiments on LFLC and HCHF diets were performed at different timings, and thus, direct comparison of the results would not be appropriate.

2.2. Whole-mount analysis of mammary glands

Right inguinal mammary glands of 4-week-old females were excised, spread onto glass slides, fixed in ethanol/acetic acid for 2 h and stained overnight with carmine alum as previously described [18]. Whole mounts were digitally photographed beside a ruler, and total area measurements for the hyperplastic lesions were quantified using Image J software (available at http://rsbweb.nih.gov/ij/).

2.3. Total tumor burden determination

Total tumor burden was determined in 13-week-old female mice by excising and weighing all the tumor masses formed in each of the 10 mammary glands. Portions of the tumors were then frozen in liquid nitrogen or fixed in 10% neutral-buffered formalin.

2.4. Histologic analysis of mammary glands

Right inguinal mammary glands of 8-week-old female mice were excised, fixed in 10% neutral-buffered formalin for 24 h and embedded in paraffin after dehydration. Sections were cut at 5 μm, stained with hematoxylin and eosin and evaluated blindly by an experienced histopathologist (E.L.). Each section was graded as normal (N), hyperplasia (HP), adenoma (A) or carcinoma (C) as defined by the maximum lesion grade developed and in accordance

Table 1
LFLC (Teklad Global diet #2018) and HFHC (Teklad diet #TD.88137) diet composition

	LFLC diet	HFHC diet
Macronutrient composition (%)		
Protein	18.6	17.3
Fat	6.2	21.2
Carbohydrate	44.2	48.5
Cholesterol		0.2
Fatty acid (% of total fat)		
C4:0		1.2
C6:0		1.2
C8:0		0.9
C10:0		2.3
C12:0		3.0
C14:0		10.3
C14:1		0.8
C15:0		1.2
C16:0	12.5	29.4
C16:1		1.7
C17:0		0.8
C18:0	3.6	12.6
C18:1 (oleic)	21.4	20.7
C18:1 isomers		4.7
C18:2 (linoleic)	55.4	2.3
C18:2 isomers		1.0
C18:3 (linolenic)	5.4	0.6