Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Nutritional Biochemistry 17 (2006) 396–401

Journal of Nutritional Biochemistry

Effects of different phytosterol analogs on colonic mucosal cell proliferation in hamsters

Xiaoming Jia[a], Naoyuki Ebine[a], Yanwen Wang[b], Atif B. Awad[c], Peter J.H. Jones[a,*]

[a]*School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, QC, Canada H9X 3V9*
[b]*Institute for Nutrisciences and Health, National Research Council of Canada, Charlottetown, PE, Canada C1A 5T1*
[c]*Department of Exercise and Nutrition Sciences, State University of New York, Buffalo, NY 14214, USA*

Received 10 May 2005; received in revised form 15 July 2005; accepted 21 August 2005

Abstract

Objective: The aim of this study was to investigate the effects of different phytosterols and their analogs on colonic mucosal cell proliferation in hamsters.
Method: Hamsters ($n=70$) were randomly assigned to seven groups after a 2-week acclimation and fed the experimental diet for 5 weeks. Diets included (i) the semipurified diet with no cholesterol (Con), (ii) the Con diet plus 0.25% cholesterol (Ch-con), or the Ch-con diet with (iii) 1% phytosterols (Ste), (iv) 1% phytostanols (Sta), (v) 1.76% sterol esters (esterified to fish oil, SteF), (vi) 0.71% stanol esters (esterified to ascorbic acid [disodium ascorbyl phytostanol phosphate, FM-VP4], 0.7% StaA) and (vii) 1.43% stanol esters (1.4% StaA), respectively. After 5 weeks on experimental diet, hamsters were sacrificed, and colons were collected. Colonic mucosal cell proliferation was measured by immunohistochemistry using monoclonal antibodies against antigen Ki-67.
Results: Colonic mucosal cell proliferation was 21.4% ($P<.01$) lower in the 0.7%, but not 1.4%, StaA relative to the Ch-con group. In addition, a lower (−13.9%) cell proliferation was observed in the SteF group in comparison to the Ch-con group; however, this difference achieved only a borderline level of statistical significance ($P=.069$). No differences were observed between Con and Ch-con, as well as among Ste, Sta, 1.4% StaA and Ch-con treatments.
Conclusion: Plant stanols esterified to ascorbic acid may possess anticarcinogenic properties in the colon by suppressing colonic mucosa cell proliferation; however, this effect was not observed with free plant sterols or stanols.

Keywords: Plant sterols; Plant stanols; Sterol esters; Stanol esters; n-3 Polyunsaturated fatty acid; Ascorbic acid; Colon cell proliferation; Ki-67

1. Introduction

The interest in studying plant sterols (PS) was initiated from findings that PS can reduce plasma total cholesterol and low-density lipoprotein cholesterol levels, and thus offer a protection against cardiovascular disease [1–7]. Recent evidence indicates that consumption of PS, apart from lowering cholesterol levels, also provides protection against certain cancers, such as colon cancer [8–10]. It has been suggested that the inhibition of colonic mucosal cell proliferation by PS is associated with a lower risk of colon cancer development [11]. Dietary supplementation of sitosterol resulted in a 39% reduction in the number of rats that developed methylnitrosourea-induced tumors and a 60% reduction of tumor cells per rat, in comparison to controls [8]. Consistently, in vitro studies have shown an inhibitory effect of β-sitosterol on the growth of HT-29 cells, a human colon cancer cell line, after 5 days of incubation in a medium containing β-sitosterol at 16 μmol/L [9]. Similarly, Awad et al. [10] reported that feeding rats with a diet containing 2% PS resulted in the normalization of cholic acid-induced hyperproliferation of colonocytes. Recent studies suggest that antioxidants, such as vitamins C and E, can enhance the chemotherapeutic efficacy of antitumor agents [12,13], raising the question of whether vitamin C can work synergistically with PS to reduce cancer development in the colon.

In addition, epidemiological and experimental evidence has demonstrated that n-3 polyunsaturated fatty acids (PUFA) can protect the colon from cancer development. Alaskan, Greenland Eskimos and West-Coast fishermen

* Corresponding author. Tel.: +1 514 398 7547; fax: +1 514 398 7739.
E-mail address: peter.jones@mcgill.ca (P.J.H. Jones).

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.08.013

consume higher levels of n-3 PUFA than other North Americans and have lower rates of colon cancer [14–16]. An inverse relationship has been reported between fish or fish oil consumption and the risk of colorectal cancer [17,18]. The inhibitory effect of n-3 PUFA on colon cancer has been confirmed by results from several laboratory studies in rodents, supplemented with either eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) [19–22]. However, it is not known whether plant sterols esterified to n-3 PUFA can provide added protection against colon cancer development.

The objectives of the present study were, therefore, to investigate the effects of dietary supplementation with different PS analogs, including plant sterols, stanols, sterols esterified to n-3 fatty acids of fish oil and stanols esterified to ascorbic acid on colonic mucosal cell proliferation in hamsters.

2. Materials and methods

2.1. Animals and diets

Seventy male golden Syrian hamsters, weighing 100–120 g (Charles River Laboratories, Montreal, QC, Canada), were housed individually in stainless steel mesh cages and subjected to a 12:12 light/dark cycle. Animals were fed a regular rodent chow with free access to diet and water. After 2 weeks of adaptation, hamsters were randomly divided into seven groups and fed the experimental diets for 5 weeks. Group 1 was given a semipurified corn starch–casein–sucrose diet with no cholesterol (Con). Group 2 was given the Con diet plus 0.25% cholesterol (Ch-con). The remaining five groups were given the Ch-con diet containing either 1% phytosterols (Ste), 1% phytostanols (Sta), 1.76% sterol esters (esterified to fish oil, SteF), 0.71% stanol esters (esterified to ascorbic acid [disodium ascorbyl phytostanol phosphate, FM-VP4], 0.7% StaA) or 1.43% stanol esters (1.4% StaA). Each diet contained 5% oil, in the form of a mixture of beef tallow and safflower oil, to provide a polyunsaturated to saturated fatty acids ratio of 0.4. Composition of the experimental diets is presented in Table 1. The SteF diet contained the same amount of plant sterols as the Ste diet. Diets containing 0.7% and 1.4% StaA provided 0.5% and 1% free plant stanols, respectively. Plant sterol analogs were mixed into the oil by heating at 55°C before blending into diets. Diets were prepared every 2 weeks and stored at −20°C.

After 5 weeks of experimental diet feeding, hamsters were anaesthetized with halothane and killed by decapitation. Colons were removed, flushed twice with phosphate-buffered saline (PBS, pH 7.4) and fixed in 10% neutral buffered formalin.

The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of McGill University and was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Measurement of colon cell proliferation

The formalin-fixed colon tissues were embedded in paraffin and cut into slices at a thickness of 5 μm and dried at 60°C for 1 h. Slides were deparaffinized with xylene and rehydrated using graded alcohols. Endogenous peroxidase was quenched with 3% H_2O_2 for 15 min and washed with PBS containing Triton (PBST). Antigens were retrieved with citrate buffer (pH 6.0) and microwaved twice with 10 min each, allowed to cool for 15 min and washed with PBST. The slides were then placed on an autostainer, washed with PBST and blocked with PBST containing 0.03% casein at room temperature for 30 min. The primary antibody Ki-67 (Novus Biologicals, Littleton, CO, USA)

Table 1
Composition (% wt/wt) of the experimental diets

Ingredients	Con[a]	Ch-con	Ste	Sta	SteF	0.7% StaA	1.4% StaA
Casein	20.0	20.0	19.8	19.8	19.6	19.8	19.7
Corn starch	28.0	28.0	27.7	27.7	27.5	27.7	27.6
Sucrose	36.3	36.0	35.6	35.6	35.3	35.6	35.5
Beef tallow/safflower oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose	5.0	5.0	4.9	4.9	4.9	4.9	4.9
DL-Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mixture	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Butylated hydroxytoluene	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Cholesterol	–	0.25	0.25	0.25	0.25	0.25	0.25
Phytosterols	–	–	1.0	–	–	–	–
Phytostanols	–	–	–	1.0	–	–	–
SteF[b]	–	–	–	–	1.76	–	–
StaA[c]	–	–	–	–	–	0.71	1.43

[a] Con: non-cholesterol control diet; Ch-con: cholesterol control diet which was the non-cholesterol control diet with 0.25% cholesterol added; Ste: cholesterol control diet with 1% plant sterols added; Sta: cholesterol control diet with 1% plant stanols added; 1.76% SteF: cholesterol control diet with 1.76% sterol esterified to fish oil added; 0.7% and 1.4% StaA: cholesterol control diet with 0.71% or 1.43% stanol esterified with ascorbate added.

[b] SteF: plant sterols esterified to fish oil; 1.76% SteF contains an equivalent amount of 1% unesterified phytosterols.

[c] StaA: plant stanols esterified to ascorbate; 0.7% and 1.4% StaA contain an equivalent amount of 0.5% and 1.0% unesterified phytostanols, respectively.