Protective Effect of Plant Sterols against Chemically Induced Colon Tumors in Rats

Robert F. Raicht, Bertram I. Cohen, Eugene P. Fazzini, Amar N. Sarwal, and Makoto Takahashi


ABSTRACT

Diets rich in vegetables are associated with a low incidence of colon cancer. Since plant sterols are plentiful in vegetarian diets, we studied the effect of \( \beta \)-sitosterol on colon tumor formation in rats treated with the carcinogen \( N \)-methyl-\( N \)-nitroso­urea. We demonstrated that \( \beta \)-sitosterol nullified in part the effect of this direct-acting carcinogen on the colon. We suggest that plant sterols may have a protective dietary action to retard colon tumor formation. The beneficial effects of vegetarian diets may be enhanced because of the presence of these compounds.

INTRODUCTION

Carcinoma of the colon is one of the most important cancers in developed countries (6). Recent studies have linked diet to large bowel cancer. The specific dietary risk factors have not been identified, but there appear to be both high risk and protective components in the diet (10). For example, the frequent ingestion of animal fat and beef is associated with a high risk of colon cancer (7, 22). Others claim that diets rich in vegetables, and thus high in fiber, protect against the development of colon cancer (1).

Diets rich in vegetables are also rich in plant sterols (20). These compounds are structurally similar to cholesterol, possessing a cyclopentenophenanthrene ring, a \( \Delta 6 \) double bond, and a \( \Delta 2 \)-hydroxyl group. In the side chain of the molecule, the plant sterols contain one or two more carbons than does cholesterol (in the form of methyl or ethyl groups), and some of the plant sterols have unsaturation at C-22. Although a variety of plant sterols have been identified, \( \beta \)-sitosterol, campesterol, and stigmasterol are most common and average 20% of sterols in our diets (8). In view of the abundance of these compounds in the diets of individuals with a low incidence of colon cancer, we determined if plant sterols could retard tumor development in an animal model of colon cancer.

MATERIALS AND METHODS

Male Fischer CD rats obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) at the age of 28 days were divided into 4 experimental groups: Group 1, control chow and intracolonial 0.9% NaCl solution (10 rats); Group 2, control chow plus \( \beta \)-sitosterol (0.2%) and intracolonial 0.9% NaCl solution (10 rats); Group 3, control chow and intracolonial MNU (71 rats); Group 4, control chow plus \( \beta \)-sitosterol (0.2%) and intracolonial MNU (48 rats). Thirty of the rats in Group 3 were run concurrently with the other rats in this experiment, and 41 were treated identically but were studied at a different time. The results in both groups were similar, and they were combined for statistical purposes. The \( \beta \)-sitosterol was kindly supplied by E. R. Diller from Eli Lilly & Co., (Indianapolis, Ind.); it was 95% \( \beta \)-sitosterol. The minor plant sterols were campesterol (4%) and stigmasterol (1%). All chow was prepared by the Ralston Purina Co. (Richmond, Ind.) from one batch and stored in a dry, cool environment until needed. At 6 weeks of age, MNU was administered into the colon utilizing a 7.5-cm-long, 18-g cannula needle (inserted via the anus). MNU was administered in 0.5 ml of sterile 0.9% NaCl solution (2 mg/dose/animal) on Days 1, 4, 7, and 10 of the experiment (total, 8 mg/animal). The dose of MNU was chosen to produce about 50% incidence of colon tumors. The animals were fed the experimental diet concurrently with the first MNU instillation, and the diets were continued over the entire 28-week experimental period. Before sacrifice, the animals were transferred to metabolic cages where 2-day fecal samples were obtained for analyses of fecal neutral and acidic steroids. At Week 28, each intestine was opened from cecum to anus, and the number of tumors was recorded. Sections of all abnormal-appearing tissue were removed for histological examinations. The criteria used for adenomas and carcinomas are as described by Morson (14).

Fecal samples were freeze dried, and an aliquot was extracted and analyzed for fecal bile acids and neutral steroids as previously described (2, 3).

RESULTS

There were no adverse effects of \( \beta \)-sitosterol feeding at the 0.2% level, and no deaths occurred in any group during the 28-week experiment. The average initial and final weights of the animals in each group showed no significant differences. The average food intakes and fecal outputs determined in metabolic cages were also similar.

Table 1 shows the incidence of colonic tumors. No tumors were present in either Group 1 or 2. In the rats given control...
chow and MNU (Group 3), tumors occurred in 54% with 1.1 tumors/animal and 2.1 tumors/tumor-bearing animal. In diets supplemented with $\beta$-sitosterol and MNU (Group 4), tumors occurred in 33%, with 0.44 tumor/animal and 1.3 tumors/tumor-bearing animal. $\beta$-Sitosterol feeding significantly decreased the proportion of tumor-bearing animals ($p < 0.05$ by $\chi^2$ test). The Mantel-Haenszel test confirmed the $\beta$-sitosterol group to have fewer tumors than do the MNU controls (Group 3) ($p < 0.01$). There were 78 tumors in Group 3 and only 21 tumors in Group 4. Microscopically, most lesions were adenomatous polyps. Five adenomas in Group 3 rats and 7 in Group 4 rats demonstrated areas of invasive carcinoma. In these lesions, we observed a transition from normal colon mucosa to an adenomatous growth pattern with irregular glands infiltrating the muscularis mucosa.

The fecal neutral and acidic steroids were analyzed by combined thin-layer chromatography, gas-liquid chromatography, and gas-liquid chromatography-mass spectrometry, and the results are shown in Table 2. Total fecal bile acid concentrations were similar in all groups. The partition of the fecal bile acids was unchanged by $\beta$-sitosterol feeding. There was a significant elevation in the fecal concentration of neutral sterols (cholesterol and its bacterial metabolites) in Group 3 compared to controls (Group 1); however, there were no dramatic differences in neutral sterol concentrations in the various groups. As expected, the concentration of plant sterols in the $\beta$-sitosterol-fed rats was elevated 7- to 8-fold compared to rats given control chow.

**DISCUSSION**

Environmental factors are thought to be responsible for the large majority of large bowel cancers. Studies demonstrating geographic variation in the incidence of colon cancer (18) and changing patterns of disease among migrant populations support an environmental etiology (19, 23). Diet is considered to be a major etiological factor. The possibility that the diet may contain protective components was postulated by Burkitt et al. (1) on the basis of epidemiological studies. They claimed that a diet rich in vegetables with a high fiber content would produce a large stool mass and protect against the development of colon cancer.

Plant sterols are habitually consumed by humans in their usual diet. It has been estimated that a typical American diet contains up to 250 mg of these sterols per day (20). Enrichment of the diet with vegetable products will increase plant sterol intake. These plant sterols pass through the intestinal tract almost completely unabsorbed and are usually recovered quantitatively in the feces. The intestinal absorption in humans is estimated at less than 5% of the amount in the diet (9). In the United States, the vegetarian diet consumed by Seventh Day Adventists has been credited with lowering the incidence of colon cancer in this group (16). Vegetarian diets are abundant with vegetables, fruits, whole grains, and nuts, and thus contain large amounts of plant sterols (20). Vegetarians have an increased concentration of $\beta$-sitosterol in their feces (13). In view of the high concentrations of plant sterols in the feces of vegetarians, a group with a low incidence of colon cancer, we studied the possible protective role of these sterols in an animal model of colon cancer.

The present experiment documented a decrease in colonic tumor formation when the plant sterol $\beta$-sitosterol was added to the diet. Although the total number of tumors was dramatically decreased, the number of invasive carcinomas was similar in both groups. $\beta$-Sitosterol may prevent adenoma formation, but may not affect the transition from adenoma to invasive carcinoma. Since dietary manipulations can alter the metabolic activity of the host as well as the intestinal flora, we selected a direct-acting carcinogen, MNU, to initiate tumor development. Studies using direct-acting carcinogens have been carried out by other investigators (15, 21). Thus, any change in tumor incidence would not be due to altered carcinogen metabolism. A low dose of MNU over a short time span was utilized to create an experimental setting where any protective effects of $\beta$-sitosterol could be detected. The transition from adenoma to carcinoma documented in this animal model has many similarities to human colon cancer (11). The plant sterols were supplemented in a chemical form to separate their action from the action of other vegetable constituents, e.g., fiber. Since plant sterols pass essentially unabsorbed into the colon, we studied the effect of high colonic concentration of these sterols on tumor formation.

Cholesterol and bile acids have been incriminated as colon tumor promoters (4, 17). In our experiment, plant sterols did not alter the fecal concentrations of either cholesterol or bile acids (see Table 2). The mechanism by which plant sterols retard colonic tumor formation is not known. Since their chemical structure is almost identical to cholesterol, one might

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>No. of Animals</th>
<th>Carcino-</th>
<th>Tumors with Tumors</th>
<th>Animalsbearing animal</th>
<th>Tumorscarcinogen</th>
<th>Tumorsbears</th>
<th>Total bile acids</th>
<th>Total neutral sterols</th>
<th>Total plant sterols</th>
<th>Total bile acids</th>
<th>Total neutral sterols</th>
<th>Total plant sterols</th>
<th>Total bile acids</th>
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<tr>
<td>1</td>
<td>Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1.22 ± 0.63</td>
<td>1.00 ± 0.45</td>
<td>3.83 ± 1.10</td>
<td>1.00 ± 0.45</td>
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<tr>
<td>2</td>
<td>$\beta$-Sitosterol</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1.43 ± 0.33</td>
<td>8.05 ± 0.87</td>
<td>3.24 ± 1.32</td>
<td>1.43 ± 0.33</td>
<td>8.05 ± 0.87</td>
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<td>3</td>
<td>Control</td>
<td>7</td>
<td>38</td>
<td>54</td>
<td>1.1</td>
<td>2.1</td>
<td>0.44</td>
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<td>1.3</td>
<td>1.79 ± 0.19</td>
<td>1.33 ± 0.89</td>
<td>4.18 ± 1.21</td>
<td>1.79 ± 0.19</td>
<td>1.33 ± 0.89</td>
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<td>$\beta$-Sitosterol</td>
<td>7</td>
<td>16</td>
<td>33</td>
<td>1.3</td>
<td>1.43</td>
<td>0.33</td>
<td></td>
<td>1.1</td>
<td>2.11 ± 0.72</td>
<td>7.20 ± 1.10</td>
<td>4.91 ± 2.71</td>
<td>2.11 ± 0.72</td>
<td>7.20 ± 1.10</td>
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<td>a</td>
<td>Mean ± S.D.</td>
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<td>b</td>
<td>Differs from Groups 1 and 3; p &lt; 0.01.</td>
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<td>c</td>
<td>Differs from Group 1; p &lt; 0.05.</td>
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speculate that they could affect colonic epithelial cell membranes and subsequently alter cellular metabolism or kinetics. Plant sterols have been isolated from certain human tumor tissues (5, 12). Their source and possible role are unknown.

These preliminary studies are of interest since they identify another possible protective component of our diet. Studies with other dietary levels as well as experiments aimed at the mechanism of action are needed.

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REFERENCES

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