Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition

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Oligofructose and inulin, naturally-occurring fermentable chicory fructans, have been shown to stimulate the growth of bifidobacteria which are regarded as beneficial strains in the colon and inhibit colon carcinogenesis in the laboratory animal models. The present study was designed to determine the effect of oligofructose and inulin on the azoxymethane (AOM)-induced preneoplastic lesions such as aberrant crypt foci (ACF) formation in the colon of male F344 rats. At 5 weeks of age, groups of animals were fed the AIN-76A (control) and the experimental diets containing 10% oligofructose or inulin. At 7 weeks of age, all animals received s.c. injection of AOM dissolved in normal saline at a dose rate of 15 mg/kg body wt., once weekly for 2 weeks. The animals were necropsied 7 weeks after the last AOM injection, and the ACF were visualized under light microscopy in the formalin-fixed, unsectioned methylene blue-stained colons. They were distinguished by their increased size, more prominent epithelial cells and pericryptal space. The feeding of oligofructose or inulin significantly inhibited the ACF formation and the crypt multiplicity in the colon. The degree of ACF inhibition was more pronounced in animals fed inulin than in those fed oligofructose. The findings suggest that chicory fructan supplements inhibit ACF formation, an early preneoplastic marker of malignant potential in the process of colon carcinogenesis.

Introduction

Cancer of the colon is one of the leading causes of cancer deaths in both men and women in the Western countries including North America (1). Epidemiological studies have demonstrated that increased consumption of fruits and vegetables and high intake of total dietary fiber reduce the risk of colon cancer (2,3). Human metabolic epidemiological studies indicate that beneficial effects of dietary fiber in relation to modulation of colon cancer development depends on composition and physical properties of fiber (4). Animal model studies also suggest that colon tumor-inhibitory properties of dietary fiber depends on its composition (5).

Among the types of dietary fiber, inulin and oligofructose are β(2-1)β fructans which are not hydrolysed by mammalian enzymes in the gut, but are fermented by colonic microflora and behave as soluble fiber (6–8). Oligofructose and inulin occur in common food stuffs such as chicory, leeks, garlic, onion, artichoke, and asparagus at high levels (9). Chicory fructans selectively stimulate the growth of bifidobacteria at the expense of bacteriodes, clostridia, or coliforms which are maintained at low levels (6,10,11). These food ingredients behave like dietary fiber and modulate lipid metabolism (11). Since oligofructose and inulin selectively stimulate the growth and/or activity of certain types of bacteria in gut that are beneficial to the host, these ingredients are classified as prebiotics (6). Bacterial fermentation of chicory fructans and other oligofructoses produces short-chain fatty acids (SCFA*) in the colon, including a small amount of butyric acid (6,12) which has been shown to increase apoptosis in the colon (13). Furthermore, there are studies to demonstrate that cultures of bifidobacteria increase the host’s immune response (14), and inhibit 2-amino-3-methyl-imidazo[4,5-f]quinoline-induced colon, liver, and mammary carcinogenesis (15) and azoxymethane-induced colon carcinogenesis (16,17) in rats. These observations raise the possibility that selective fermentable non-digestible oligosaccharides that enhance the growth of bifidobacteria in the gut could potentially inhibit colon carcinogenesis. A recent study demonstrates that dietary administration of oligofructose or inulin significantly suppressed the growth of transplantable tumors, TLT and EMT in mice (18).

Aberrant crypt foci (ACF), which are recognized as early preneoplastic lesions in the colon, have consistently been observed in experimentally induced colon carcinogenesis in laboratory animals (19,20). Pretlow et al. (21) have also shown that these lesions are present in the colonic mucosa of patients with colon cancer, and have suggested that aberrant crypts are putative precursor lesions from which adenomas and carcinomas may develop in the colon. ACF express mutations in the *apc* gene and *ras* oncogene that appear to be biomarkers of colon cancer development (22). There is some evidence that several inhibitors of ACF formation reduce the incidence of colon tumors in laboratory animals (20) suggesting that ACF induction can be used to evaluate novel agents for their potential chemopreventive properties against colon cancer. The purpose of this study was to determine the effect of oligofructose and inulin on carcinogen-induced colonic ACF formation in the rat.

Materials and methods

*Abbreviations:* SCFA, short-chain fatty acids; ACF, aberrant crypt foci; AOM, azoxymethane; DP, degree of polymerization; HP, high performance.

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Table I. Body weights of animals fed the control diet and experimental diets containing oligofructose and inulin

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body wt (g) on experimental diets a week</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AOM-treated</td>
<td></td>
</tr>
<tr>
<td>1. Control diet</td>
<td>119 ± 5.9(^a)</td>
</tr>
<tr>
<td>2. Oligofructose, 10%</td>
<td>119 ± 6.6</td>
</tr>
<tr>
<td>3. Inulin, 10%</td>
<td>120 ± 7.1</td>
</tr>
<tr>
<td>Saline-treated</td>
<td></td>
</tr>
<tr>
<td>4. Control diet</td>
<td>117 ± 8.5</td>
</tr>
<tr>
<td>5. Oligofructose, 10%</td>
<td>120 ± 5.9</td>
</tr>
<tr>
<td>6. Inulin, 10%</td>
<td>119 ± 5.7</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD.

Table II. Effect of dietary oligofructose and inulin on colonic ACF formation in male F344 rats

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total ACF/colon</th>
<th>Foci containing number of aberrant crypts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 crypt/focus</td>
</tr>
<tr>
<td>Control diet</td>
<td>120 ± 28</td>
<td>19.5 ± 7.3</td>
</tr>
<tr>
<td>Oligofructose, 10%</td>
<td>92 ± 28(^b)</td>
<td>15.4 ± 7.5</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.024)</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>Inulin, 10%</td>
<td>78 ± 37(^b)</td>
<td>15.7 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.006)</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD.

\(^b\)Significantly different from the control diet. The level of significance is shown in parenthesis.

AIN-76A semipurified control diet. They were randomly distributed by weight into various dietary groups and were transferred to an animal holding room where they were housed in plastic cages, three rats/cage, under controlled conditions of a 12 h light/12 h dark cycle, 50% relative humidity, and 21°C room temperature. Raftilose and Raftiline were added to the control diet at 10% level at the expense of starch.

Experimental procedure

Beginning at 5 weeks of age, groups of animals were fed the control or experimental diets. All animals except the vehicle-treated rats received AOM s.c. once weekly at 7 and 8 weeks of age at a dose rate of 15 mg/kg body wt/week. Animals intended for vehicle treatment were given an equal volume of normal saline. The rats were continued on control or experimental diets until the termination of the study, when they were 16 weeks of age. All animals were killed by CO₂ euthanasia. The colons were removed, flushed with Krebs-Ringer solution, opened from cecum to anus, and fixed flat between two pieces of filter paper in 10% buffered formalin. After a minimum of 24 h in buffered formalin, the colons were cut into 2-cm segments, placed in a Petri dish containing 0.2% methylene blue in Krebs-Ringer solution and left for 5–10 min. They were then placed, mucosal side up, on a microscope slide and observed through a light microscope. ACF were recorded according to standard procedures (19). Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, significantly increased distance from lamina to basal surface of cells and the easily discernible pericytoplasmic zone. Crypt multiplicity was determined as the number of crypts in each focus and categorized as those containing up to three, four or more aberrant crypts/focus. All colons were scored by one observer without knowing the identity of agents under study; scores were checked at random by a second observer.

Statistical analysis

All results were expressed as the means ± SD and were analysed by one-tailed Student’s t-test. Differences were considered statistically significant at P < 0.05.

Results

General observations

The body weights of AOM- and vehicle-treated animals fed the control and experimental diets containing 10% inulin or oligofructose were comparable throughout the study (Table I). There were no signs of any adverse effects in liver, kidney, stomach, intestine, or lungs of animals fed inulin or oligofructose.

Aberrant crypt foci

Table II summarizes the AOM-induced ACF in the colon of rats fed the control diet, AOM treatment induced animals were killed by CO₂ euthanasia. The colons were removed, flushed with saline (vehicle), and fed the control and experimental diets containing inulin or oligofructose showed no evidence of ACF formation in the colon (data not shown). In the animals fed the control diet, AOM treatment induced on the average ~120 ACF/colon. ACF were predominantly observed in the distal colons. Efficacy end points used in this study were inhibition of the total number of ACF/colon as well as the reduction of the number of multicrypt clusters (≥2) of aberrant crypts/focus. Administration of oligofructose or inulin in the diet significantly suppressed the total number of ACF/colon as compared to the control diet; the degree of inhibition was more pronounced in the animals fed inulin (P < 0.0006) than in those fed oligofructose (P < 0.02). Crypt multiplicity in terms of 2 or 3 aberrant crypts/focus were also significantly inhibited in animals fed inulin (P < 0.02–0.0001) and oligofructose (P < 0.04–0.01).

Discussion

The present study was undertaken to evaluate the selective chicory fructans for their potential inhibitory properties against ACF formation in the colon, which are putative neoplastic lesions. Because multiplicity of aberrant crypts has been a probable predictor of colon tumor outcome (24), the present study used this criterion to evaluate oligofructose and high
demonstrated that dietary administration of oligofructose or inulin inhibits AOM-induced colonic ACF formation in rats suggesting the potential colon tumor inhibitory properties of chiosry fructans. Although the precise mechanisms by which oligofructose and inulin inhibit preneoplastic lesions of colon are not fully known, it is likely that the effect of these agents proceeds through the modulation of microflora (6,11) and production of SCFA (6,25) in the colon. In vitro studies showed that incubation of fecal bacterial cultures with oligofructose and inulin selectively stimulated the growth of bifidobacteria while maintaining the E.coli or clostidia at low levels (10,11). In diet intervention studies Gibson et al. (11) demonstrated that dietary administration of oligofructose or inulin significantly increased fecal bifidobacteria, whereas bacteriodes, clostridia and fusobacteria and/or gram-positive cocci were decreased on total fecal bacterial count. A recent study by Bouhnik et al. (26) demonstrated that dietary administration of fructo-oligosaccharide for 12 days had no significant effect on total anaerobes, but increased the bifidobacterial counts. In this study, individual strains of anaerobes were not analysed. The colonizing cells of bifidobacteria produce lactic acid, thereby lowering the intestinal pH to create a bacteriocidal environment for putative enteropathogens such as E.coli and C.perfringens, thus developing a favorable micro-environment in the gut. This favorable micro-environment may also involve the modulation of bacterial enzymes such as β-glucuronidase that can convert procarcinogens to proximate carcinogens (17). It is also possible that the metabolites produced by bifidobacteria may affect the mixed-function oxidases, especially cytochrome P450s which are believed to be involved in the conversion of AOM from proximate to ultimate carcinogen (27). Also, these bifidobacteria, colonizing at the expense of enteropathogens as reported earlier (6,11), may bind the ultimate carcinogen methylazoxymethanol, which is released into the intestinal lumen, thereby minimizing its absorption into circulation by physically removing it via feces. In this context, it is noteworthy that dietary administration of lyophilized cultures of Bifidobacterium longum has been shown to inhibit AOM-induced colonic ACF (17) and tumors (16) in laboratory animal models. In addition to selective modulation of bifidobacteria, clostridia and E.coli, oligofructose and inulin increase the production of SCFA especially butyrate in the colon by microbial fermentaition (6,25). Although the production of butyrate is around 5% of total SCFA, it is of particular interest because it inhibits proliferation of a number of cell types in vitro and induces a more differentiated phenotype including colorectal tumor cells (28). Butyrate analogs are currently being evaluated as potential antineoplastic agents (29). The reduction of colorectal cell proliferation and induction of differentiation in colonic epithelial cells have led to clinical trials of butyrate enemas in the treatment of ulcerative colitis (30). Also, sodium butyrate induces apoptosis in human colorectal tumor cell lines in p53-independent pathway: implications for possible role of dietary fiber in the prevention of large bowel cancer. Int. J. Cancer, 55, 498–505.

References
9. Van Loo,J., Coussement,P., De Leenheer,L., Hoebregs,H. and Smits,G. (1993) Sodium butyrate induces apoptosis in human colonic adenoma and adenocarcinoma cell lines (13). Although this is by no means the only mechanism of ACF inhibition by oligofructose and inulin, this could, in part, explain why these agents appear to be protective against ACF formation.

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