Possible Mechanisms by Which Pro- and Prebiotics Influence Colon Carcinogenesis and Tumor Growth

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ABSTRACT Oligofructose and inulin, selective fermentable chicory fructans, have been shown to stimulate the growth of bifidobacteria, which are regarded as beneficial strains in the colon. Studies were designed to evaluate inulin (Raftiline) and oligofructose (Raffilose) for their potential inhibitory properties against the development of colonic aberrant crypt foci (ACF) in rats. ACF are putative preneoplastic lesions from which adenomas and carcinomas may develop in the colon. The results of this study indicate that dietary administration of oligofructose and inulin inhibits the development of ACF in the colon, suggesting the potential colon tumor inhibitory properties of chicory fructans. The degree of ACF inhibition was more pronounced in animals given inulin than in those fed oligofructose. Because these prebiotics selectively stimulate the growth of bifidobacteria, ornithine decarboxylase (ODC) activities, ras-p21 oncoprotein expressions and tumor inhibitory activity of lyophilized cultures of Bifidobacterium longum against chemically induced colon and mammary carcinogenesis and against colonic tumor cell proliferation were examined. Dietary administration of lyophilized cultures of B. longum strongly suppressed colon and mammary tumor development and tumor burden. Inhibition of colon carcinogenesis was associated with a decrease in colonic mucosal cell proliferation and activities of colon mucosal and tumor ornithine decarboxylase and ras-p21. Human clinical trials are likely to broaden our insight into the importance of the pre- and probiotics in health and disease. J. Nutr. 129: 1478S–1482S, 1999.

KEY WORDS: colon cancer • oligofructose • inulin • Bifidobacterium longum

Cancer of the colon is one of the leading causes of cancer morbidity and mortality among men and women in the Western countries, including the United States (Parker et al. 1997). Epidemiologic studies suggest that increased consumption of fruits and vegetables and high total dietary fiber reduce the risk of development of colon cancer (Howe et al. 1992, Steinmetz and Porter 1991). Human metabolic and laboratory animal model studies indicate that beneficial effects of dietary fiber in relation to colon cancer development depend on the composition and physical properties of the fiber (Reddy et al. 1992, Reddy, 1995).

Among the types of dietary fiber, inulin and oligofructose, which occur in common food stuffs such as chicory, leeks, garlic, onion, artichoke and asparagus at high levels, are β (2→1)1 fructans. They are fermented by colonic microflora and behave as soluble fibers (Gibson and Roberfroid 1995). It is of great interest that they selectively stimulate the growth of bifidobacteria at the expense of bacteriodes, clostridia or coliforms, which are maintained at low levels (Gibson and Roberfroid 1995, Gibson et al. 1995). Bacterial fermentation of these prebiotics produces short-chain fatty acids (SCFA)2 in the colon, including a small amount of butyric acid (Campbell et al. 1997, Gibson and Roberfroid 1995), which has been shown to increase apoptosis in the colon (Hague et al. 1993). Of special interest are the beneficial effects of certain lactic acid–producing enterobacterial food supplements, probiotics, in the prevention of cancer (Hitchins and McDonough 1989, Le et al. 1986). The lactic cultures, which are primarily used for fermentation of milk and other dairy products, have also been shown to possess antimutagenic and anticarcinogenic properties (Bodana and Rao 1990, Goldin and Gorbach 1980, Lidbeck et al. 1992). Furthermore, there are studies to demonstrate that cultures of bifidobacteria increase the host’s immune response (Sekine et al. 1995). These observations raise the possibility that selective fermentable, nondigestible oligosaccharides that enhance the growth of bifidobacteria in the gut and cultures of lactic acid–producing bacteria could potentially inhibit colon carcinogenesis. It was, therefore, of interest to evaluate the inhibitory properties and modes of action of prebiotics such as oligofructose and inulin, and probiotics, including bifidobacteria, against colon carcinogenesis.

Inhibitory activity of oligofructose and inulin against colon carcinogenesis

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 and in the colonic mucosa of patients with colon cancer (McLellan et al. 1991, Pretlow et al. 1992). ACF also express mutations in the APC gene and ras oncogene that are involved in colon cancer development (Vivona et al. 1991). Studies were designed to evaluate the potential tumor inhibitory properties of chicory fructans against colon carcinogenesis using inulin (Raftiline) and oligofructose (Raffilose) in rats, which were selected as potential prebiotics for their inhibitory properties against the development of colonic aberrant crypt foci (ACF) in rats. ACF are putative preneoplastic lesions from which adenomas and carcinomas may develop in the colon. The results of this study indicate that dietary administration of oligofructose and inulin inhibits the development of ACF in the colon, suggesting the potential colon tumor inhibitory properties of chicory fructans. The degree of ACF inhibition was more pronounced in animals given inulin than in those fed oligofructose. Because these prebiotics selectively stimulate the growth of bifidobacteria, ornithine decarboxylase (ODC) activities, ras-p21 oncoprotein expressions and tumor inhibitory activity of lyophilized cultures of Bifidobacterium longum against chemically induced colon and mammary carcinogenesis and against colonic tumor cell proliferation were examined. Dietary administration of lyophilized cultures of B. longum strongly suppressed colon and mammary tumor development and tumor burden. Inhibition of colon carcinogenesis was associated with a decrease in colonic mucosal cell proliferation and activities of colon mucosal and tumor ornithine decarboxylase and ras-p21. Human clinical trials are likely to broaden our insight into the importance of the pre- and probiotics in health and disease. J. Nutr. 129: 1478S–1482S, 1999.
MECHANISMS OF COLON CANCER INHIBITION BY PRE- AND PROBIOTICS

1479S

TABLE 1

Effect of dietary oligofructose and inulin on colonic aberrant crypt foci (ACF) formation in male F344 rats.\(^1,2\)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total ACF/colon</th>
<th>2 crypts/focus</th>
<th>3 crypts/focus</th>
<th>4 or more crypts/focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet</td>
<td>120 ± 28</td>
<td>43.7 ± 7.6</td>
<td>28.2 ± 7.5</td>
<td>28.3 ± 8.2</td>
</tr>
<tr>
<td>Oligofructose, 10%</td>
<td>92 ± 29*(^*)</td>
<td>31.2 ± 13*(^*)</td>
<td>21.3 ± 7.8*</td>
<td>23.9 ± 8.2</td>
</tr>
<tr>
<td>Inulin, 10%</td>
<td>78 ± 37*(^*)</td>
<td>24 ± 12*</td>
<td>16.6 ± 7.2*</td>
<td>21.8 ± 14.2</td>
</tr>
</tbody>
</table>

\(^1\) Reddy et al. (1997).
\(^2\) Values are means ± SD.
\(^*\) Significantly different from the control diet. The level of significance is shown in parentheses.

1993). Aberrant crypts are putative precursor lesions from which adenomas and carcinomas may develop in the colon. Several inhibitors of ACF formation have been shown to reduce the incidence of colon tumors in laboratory animals (Wargovich et al. 1996), suggesting that ACF induction can be used to evaluate novel agents for their potential chemopreventive properties against colon cancer.

Studies were conducted in our laboratory to determine the potential inhibitory properties of oligofructose (Raftilose) and inulin (Raftiline) on azoxymethane (AOM)-induced colonic ACF in male F344 rats feeding in the process of colon carcinogenesis, suggesting that dietary AOM administration of lyophilized cultures of \(B.\) longum at 1.5 and 3.0% levels significantly inhibited the total ACF formation and crypt multiplicity (two, three, or four or more crypts per focus). The results of this study provide evidence for potential colon tumor-inhibitory properties of \(B.\) longum.

**Effect on AOM-induced colon tumorigenesis.** Because the lyophilized cultures of \(B.\) longum inhibited the neoplastic lesions in the colon, studies were conducted to determine the colon tumor inhibitory properties of \(B.\) longum (Singh et al. 1997). Male F344 rats were fed the AOM-76A diet containing 0 or 2% lyophilized cultures of \(B.\) longum and subcutaneously administered AOM dissolved in normal saline at a dose rate of 15 mg/kg body weight, once weekly, for 2 wk. Vehicle controls received subcutaneously an equal volume of normal saline. Animals were maintained on control or experimental diets until termination of the study at 40 wk after last AOM treatment. Table 2 summarizes the AOM-induced tumors in the colon in terms of tumor incidence (percentage of animals with tumors) and colon tumor multiplicity (number of tumors per animal). Dietary administration of \(B.\) longum cultures significantly inhibited the incidence of adenocarcinomas (\(P < 0.05\)) and colon tumor multiplicity in terms of tumors per animal (\(P < 0.001\)) and tumors per tumor-bearing animal (\(P < 0.01\)).

**Effect of \(B.\) longum in 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colon carcinogenesis.** The formation of mutagens upon broiling fish and meat was first discovered by Sugimura et al. (1977). IQ, a heterocyclic aromatic amine produced from food pyrolysis, was first isolated from broiled fish. Subsequently, it was isolated from a variety of broiled or cooked fish and meat (Kasai et al. 1980). IQ has a multitarget organospecificity with specific cancer induction in the Zymbal gland, skin, colon, oral cavity and mammary gland of rodents (Sugimura et al. 1991). Although it is not clear whether these heterocyclic amines may contribute to human cancer development, it is certain that these compounds are present in cooked foods and pose a credible risk to humans.

Because IQ induces colon tumors in male and female rats and mammary tumors in female rats, and bacterial cultures that ferment milk possess anticarcinogenic properties, the possibility exists that these bacterial cultures may prevent IQ-induced carcinogenesis. Accordingly the inhibitory effect of lyophilized cultures of \(B.\) longum on IQ-induced carcinogenesis was investigated in male and female F344 rats (Reddy and Rivenson 1993). Beginning at 5 wk of age, male and female...
rinsed with warm saline, and then counted in a hemocytometer. The results indicated that the percentage of animals with tumors was significantly (P < 0.05) inhibited in female rats fed the diet containing B. longum. The mammary tumor multiplicity (tumors per animal) in female rats was significantly (P < 0.05) inhibited compared with the control diet (Table 2). Biasco et al. (1993) demonstrated that dietary bifidobacterium supplements inhibited IQ-induced colon tumor development in F344 rats.

Possible mechanisms of colon cancer inhibition by prebiotics

Although the precise mechanisms by which oligofructose and inulin inhibit preneoplastic lesions of the colon are not completely understood, it is likely that the effects of these agents may involve the modulation of microflora (Gibson and Roberfroid 1995, Gibson et al. 1995) in the colon. In vitro studies showed that incubation of fecal bacterial cultures with oligofructose and inulin selectively stimulated the growth of bifidobacteria and/or clos- tridia at low levels (Wang and Gibson 1993). In diet intervention studies, Gibson et al. (1995) 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. These bifidobacteria, colonizing at the expense of enteropathogens, may bind the ultimate carcinogen by physically removing it via feces. The colonizing cells of bifidobacteria also produce lactic acid, thereby lowering the intestinal pH to create a bacteriocidal environment for pathogens. Ornithine decarboxylase (ODC; EC 4.1.1.17) is the first and rate-limiting enzyme of this crucial polyamine biosynthetic pathway. Elevated levels of ODC activity have been reported in normal human colons vs. normal-appearing colon mucosa (Porter et al. 1987, Singh et al. 1992), in dysplastic polyps vs. nondysplastic polyps (Luk and Baylin 1984) and also in noninvolved mucosa from polyposis patients vs. noninvolved mucosa from normal individuals (Luk et al. 1989). Similarly, ODC activity has been found to be consistently higher in colon adenocarcinomas compared with the adjacent mucosa. Evidence that enhanced ODC activity may play an important role in colon tumor development is provided by the observation that diuroxymethylornithine, a highly specific and irreversible inhibitor of ODC, suppressed colon tumor development in a time-dependent manner in carcinogen-treated rodents (Singh et al. 1992). Studies conducted in our laboratory demonstrate that the colon tumor inhibitory property of lyophilized cultures of B. longum was associated with the inhibition of colonic mucosal cell proliferation and with suppression of ODC activity in the colonic mucosa and tumors compared with that in control diet (Table 2). Biasco et al. (1991) observed a significant decrease in mucosal cell proliferation in upper colonic crypts of patients with colon adeno-

### Table 2

<table>
<thead>
<tr>
<th>Dietary regimen</th>
<th>AOM-induced colon tumorigenesis&lt;sup&gt;1&lt;/sup&gt;</th>
<th>IQ-induced colon tumorigenesis&lt;sup&gt;2&lt;/sup&gt;</th>
<th>IQ-induced mammary carcinogenesis&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Multiplicity&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Incidence&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>77</td>
<td>1.8 ± 1.27&lt;sup&gt;5&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>2% B. longum</td>
<td>53</td>
<td>0.83 ± 0.98&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>0.5% B. longum</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>1</sup> Singh et al. (1997).
<sup>2</sup> Reddy and Rivenson (1993).
<sup>3</sup> Percentage of animals with tumors.
<sup>4</sup> Tumors/animal.
<sup>5</sup> Means ± SD.
<sup>6</sup> Significantly different from its respective control diet in the same gender, P < 0.05.
activity. The results summarized in ras colon tumorigenesis is associated with the modulation of on ras. We also analyzed the modifying effects of tenacious malignant behavior (Mukhopadhyay et al. 1991). indicating that activated preexisting tumor phenotype reverts to a more normal form, 
effect of and tumors compared with the control diet. This inhibitory expression of total and mutated ras expression is strongly correlated with colon tumor outcome. As regards the B. longum that dietary cultures is not clear, it is likely that these effects may proceed through diverse physiologic and metabolic alterations.

Ras activation represents one of the earliest and most frequently occurring genetic alterations associated with human cancers, especially the cancer of the colon (Barbacid 1990). Elevated levels of ras-p21 have been correlated with increased cell proliferation, histologic grade, nuclear anaplasia and degree of undifferentiation (Kotsinas et al. 1993). In experiments in which mutated ras genes are selectively inactivated, the preexisting tumor phenotype reverts to a more normal form, indicating that activated ras may be necessary for the maintenance of malignant behavior (Mukhopadhyay et al. 1991). We also analyzed the modifying effects of B. longum cultures on ras-p21 expression to determine whether the inhibition of colon tumorigenesis is associated with the modulation of ras-p21 activity. The results summarized in Table 3 demonstrate that dietary B. longum cultures significantly suppressed the expression of total and mutated ras-p21 in the colon mucosa and tumors compared with the control diet. This inhibitory effect of B. longum cultures on ras-p21 expression was again strongly correlated with colon tumor outcome. As regards the mechanism of inhibition of ras activation afforded by B. longum cultures, it is likely that bifidobacterial cells, as a biological response modifier, modulate the induction of the methylguanine repair protein, O6-methylguanine DNA methytransferase, which acts as a suicide enzyme that stoichiometrically accepts a methyl group onto itself, restoring the original guanine in DNA by in situ demethylation (Pegg and Dolan 1989). To our knowledge, no other data exist pertaining to the modulation of ras function by lactic cultures. It is clear from these results that B. longum–augmented suppression of AOM-induced ras activity may interfere with the progression of events leading to colon tumor development.

An additional mechanism of tumor suppression may involve a role for B. longum as an immunomodulator and biological response modifier (Okawa et al. 1993, Sekine et al. 1995). For example, the administration of viable or nonviable intestinal bacteria to germ-free mice has been shown to enhance intestinal production of immunoglobulin A plasmacytes (George 1994). Kohwi et al. (1978) demonstrated that repeated intraliteral injections of Bifidobacterium inhibited the growth of Meth-A tumor cells transplanted subcutaneously into syngeneic BALB/c mice. Furthermore, Sekine et al. (1995) and Okawa et al. (1993) demonstrated that a water-soluble cell fraction, WPG, of bifidobacteria induces an antitumor effect and plays an important role as an immunomodulator in the intestines of humans and animals.

### CONCLUSION

In summary, the results demonstrate that dietary administration of prebiotics such as oligofructose, inulin and lyophylized cultures of B. longum inhibits the formation of preneoplastic lesions in the colon. In addition, dietary administration of lyophylized cultures of B. longum suppressed colon and mammary carcinogenesis in the laboratory animal models. Inhibition of colon carcinogenesis by lyophylized cultures of B. longum is associated with the modulation of colonic cell proliferation and colonic mucosal and tumor ODC and ras p21. Further studies are required to investigate the efficacy of prebiotics in combination with probiotics on the inhibition of colon tumors. Although pre- and probiotics comprise a diverse group with different modes of action, their ability to inhibit colon carcinogenesis may be important to the development of potential nutritional and related food supplements against colon cancer.

### LITERATURE CITED


