Enhancing Effect of Carrageenan on the Induction of Rat Colonic Tumors by 1,2-Dimethylhydrazine and Its Relation to β-Glucuronidase Activities in Feces and Other Tissues

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Summary Since it has been demonstrated that a high level of fat is a dietary factor in the etiology of colon cancer, the effect of carrageenan, a polysaccharide extracted from the red seaweeds, on 1,2-dimethylhydrazine-induced colonic tumors in rats fed a semipurified control diet containing an ordinary level of fat was studied. Nevertheless, the enhancing effect of carrageenan on colonic tumors was observed. The rats fed a carrageenan diet had approximately twice the fecal weight compared to the rats fed a control diet. While no significant differences were found in β-glucuronidase activities in colonic mucosa, liver or plasma in the carrageenan-fed rats and controls, the activity in feces was significantly lower in the carrageenan-fed rats. At least, no β-glucuronidase activity seemed to be related to the tumor-enhancing effect of carrageenan.

Key Words colonic tumor, 1,2-dimethylhydrazine, carrageenan, β-glucuronidase

Carrageenans are high molecular weight sulfated polygalactans, with gel-forming and water-holding properties (1, 2), extracted from the red seaweeds, and they are used in the food industry. It was reported that carrageenan had an enhancing effect on colon carcinogenesis in rats treated with azoxymethane or methylnitrosourea (3). In the study, however, dietary calorie gain and growth curve were not closely comparable, and the diet contained 20% fat. It has been demonstrated that a high level of fat is a dietary factor in the etiology of colon cancer in experimental study (4). Therefore, we studied the effect of carrageenan on 1,2-dimethylhydrazine (DMH)-induced colonic tumors in rats fed a diet containing...
ordinary 6% fat.

The carcinogen DMH appears to be metabolized in the liver, and then reaches
the intestinal mucosa via both the biliary transport and the systemic circulation (5). As β-glucuronidase [EC 3.2.1.31] may be related to the final activation of DMH
metabolites (6), we examined β-glucuronidase activities of colonic mucosa, feces,
liver and plasma in rats to determine whether or not carrageenan ingestion
influenced the enzyme activity.

MATERIALS AND METHODS

Seven-week-old male F344 rats were divided into four groups. Group 1 was
given weekly subcutaneous injections of DMH (Aldrich Chemical Co., U.S.A.) at a
dosage of 20 mg/kg body weight for 16 weeks and received a carrageenan diet for 24
weeks. Group 2 received the same DMH treatments for the same period as group 1
and fed a control diet for the same period as group 1. Group 3 received a
carrageenan diet without carcinogen treatment. Group 4 received a control diet.
The control diet contained 22% casein, 10% sucrose, 2% cellulose, 6% corn oil, 2%
vitamin mixture (Clea Japan Inc., Tokyo), 7% mineral mixture (Clea Japan Inc.,
Tokyo), and corn starch up to 100%. The carrageenan diet contained carrageenan
(CS-47, κ-type, San-ei Chemical Ind., Co., Osaka) substituted with corn starch at a
6% level. Rats had free access to food and water in a temperature and humidity
controlled room. Body weights and food intake of rats were estimated weekly.
Weights of the dried fecal collection from the rats in each group were estimated for 3
days. For the assay of β-glucuronidase activity, feces collected from the colon were
processed and measured by the method of Reddy et al. (6) with some modifications
of 0.1 M phosphate buffer, pH 7.5, containing p-nitrophenyl β-D-glucuronide as a
substrate. Blood was obtained from the abdominal aorta of the rat to prepare
EDTA-plasma. Colonic mucosa was scraped from the colon as described by
Freeman et al. (7), and the liver was also removed. The three samples were processed
and measured by the method of Mian and Cowen (8). The protein was determined
by the method of Lowry et al. (9).

RESULTS

The growth, food intake and dried fecal weight are shown in Table 1. No
significant differences were found in the body weight or the food intake between
groups 1 and 2, and the diets were quite comparable. The daily intake of
carrageenan per rat in both groups 1 and 3 is presumed to be approximately 0.8 g.
The rats fed the carrageenan diets excreted more feces than those fed the control
diets. As shown in Table 2, the number of tumors per rat was significantly higher in
group 1 given DMH and the carrageenan diet compared to group 2 given DMH
and the control diet. There were increased numbers of tumors in proximal colon
compared to distal colon in group 1, whereas the opposite distribution was shown in

Table 1. Growth, food intake and fecal weight.

<table>
<thead>
<tr>
<th>Group (No. of rats)</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Food intake (g/day/rat)</th>
<th>Fecal weight (g/day/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>1 (20)</td>
<td>DMH + carrageenan</td>
<td>149.7 ± 1.6^a</td>
<td>374.6 ± 3.2</td>
<td>13.6 ± 0.2</td>
</tr>
<tr>
<td>2 (20)</td>
<td>DMH</td>
<td>148.3 ± 1.8</td>
<td>383.8 ± 5.8</td>
<td>13.2 ± 0.2</td>
</tr>
<tr>
<td>3 (15)</td>
<td>Carrageenan</td>
<td>149.5 ± 1.8</td>
<td>391.2 ± 5.8</td>
<td>13.4 ± 0.2</td>
</tr>
<tr>
<td>4 (15)</td>
<td>—</td>
<td>145.3 ± 1.9</td>
<td>401.5 ± 4.6</td>
<td>12.9 ± 0.4</td>
</tr>
</tbody>
</table>

^a Mean ± SE.

Table 2. Number and distribution of colonic tumors.

<table>
<thead>
<tr>
<th>Group (No. of rats)</th>
<th>Treatment</th>
<th>No. of rats with tumors</th>
<th>No. of tumors/rat</th>
<th>Colonic site</th>
<th>Size distribution^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>1 (20)</td>
<td>DMH +</td>
<td>15 (75)^b</td>
<td>1.00 ± 0.16^cd</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>carrageenan</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
</tr>
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<td></td>
<td></td>
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<td>10</td>
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<td></td>
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<td>1</td>
</tr>
<tr>
<td>2 (20)</td>
<td>DMH</td>
<td>8 (40)</td>
<td>0.55 ± 0.18</td>
<td>3</td>
<td>8</td>
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<td>8</td>
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<td></td>
<td>1</td>
</tr>
<tr>
<td>3 (15)</td>
<td>Carrageenan</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
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<td>4 (15)</td>
<td>—</td>
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</table>

^a Greatest diameter. ^b Numbers in parentheses, percentage. ^c Mean ± SE. ^d p < 0.05 between groups 1 and 2 by Student's t test.

group 2. The size of most tumors in group 2 was smaller than 5 mm, while about one-half in group 1 was larger than 5 mm. Visible ulceration was not present in the tumor-free colonic mucosa of rats in group 1 or in the colon of rats in group 3.

DISCUSSION

Studies on the effects of dietary fiber on carcinogen-induced rat colonic tumors had frequently provided contradictory results. For instance, a 15% pectin and 20% fat diet was protective (10), a 6.5% pectin and 18% fat diet had an enhancing effect (11), and a 4.5% pectin and 7.6% fat diet was not effective (12). It is likely that these results are explained, in part, by the difference of contents of fiber and fat in the diets. In the previous study on carrageenan (3), a diet containing 15% carrageenan and 20% fat was used. Because the development of tumors is greatly influenced by the quantity of fat (4), we used a diet containing ordinary fat dose as a control diet to elucidate clearly the effect of carrageenan. Nevertheless, the observed result indicated the enhancing effect of carrageenan on colonic tumors. It has been
reported that dietary fiber is protective against the development of colonic tumors with increased fecal bulk and reduced transit time (13). However, increased fecal bulk in this study did not show protective effect, suggesting that the enhancing effect of carrageenan may be related to the physicochemical properties of carrageenan. It has been indicated that carrageenan influenced bile acid metabolism, increasing the excretion of fecal secondary bile acids through adsorption process (14). Another experiment shows that degradation of carrageenan, extent of which was obscure, occurred during passage through the gut (15). The products, as well as carrageenan, would alter the luminal physicochemical environment. The findings on the action and the fate of carrageenan in the alimentary tract are of particular interest in determining the reason for the tumor-enhancing effect, which will be investigated by the authors.

As shown in Table 3, there were no significant differences in β-glucuronidase activities in the samples obtained from groups 1 and 2, except for feces in which the enzyme activity was significantly lower in group 1 compared to group 2, and this tendency was also observed in groups 3 and 4. Alteration of bacterial flora by carrageenan administration might influence the enzyme activity in feces. Although Bauer et al. (11) found the significant elevation of fecal β-glucuronidase activity in pectin-fed rats having a higher incidence of colonic tumors, the relation was not observed in this study. At least, no β-glucuronidase activity seemed to be related to the tumor-enhancing effect of carrageenan.

To evaluate the above results, further investigation will be required on some remaining problems, namely, purity of the carrageenan preparation, histological changes of colonic tumors and tumor-free mucosa, and alterations in the composition and amount of fecal bile acids.

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REFERENCES


