Effect of Dietary Carbohydrates on the Growth of Dysplastic Crypt Foci in the Colon of Rats Treated with 1,2-Dimethylhydrazine

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ABSTRACT

The effect of dietary starch and sucrose on the growth of foci of dysplastic crypts in the colon (FDC) was studied in female Sprague Dawley rats treated twice p.o. with 25 mg/kg of 1,2-dimethylhydrazine (DMH). After DMH administration, the animals were fed high-fat (23% corn oil, w/w)/low-calcium (0.1%, w/w)/low-cellulose (2%, w/w) diets in which carbohydrates were represented by corn starch (starch diet) or sucrose (sucrose diet) (46%, w/w). The animals were fed for either 30 or 105 days with the experimental diets. The number of FDC was not significantly affected by diet. However, after 30 days the percentage of small FDC (formed by 1–2 dysplastic crypts) was higher in the animals fed the starch diet compared to the animals fed the sucrose diet (90.3 ± 1.1% (SE) and 82.6 ± 3.1%, respectively; P < 0.05). In contrast, foci formed by 3–4 dysplastic crypts were decreased by the starch diet (P < 0.05). After 105 days of feeding, the starch diet induced a number of dysplastic crypts/focus lower than that induced by the sucrose diet (2.6 ± 0.1 and 2.9 ± 0.1, respectively; P < 0.05). The percentage of small FDC was also higher in the animals fed the starch diet compared to animals fed the sucrose diet (P < 0.01). After 30 days of feeding, DMH treatment increased colon proliferative activity in both dietary groups (P < 0.05). But after 105 days of feeding, proliferation was similar in both treatments. The number and dimensions of the foci of dysplastic crypts in the colon of mice fed starch or sucrose diets were 10.4 ± 0.8 and 4.4 ± 0.5 in the sucrose and starch diets, respectively; P < 0.05). The percentage of small FDC was also higher in the animals fed the starch diet compared to animals fed the sucrose diet (46%, w/w). The animals were fed for either 30 or 105 days with the experimental diets. The number of FDC was not significantly affected by diet. However, after 30 days the percentage of small FDC (formed by 1–2 dysplastic crypts) was higher in the animals fed the starch diet compared to the animals fed the sucrose diet (90.3 ± 1.1% (SE) and 82.6 ± 3.1%, respectively; P < 0.05). In contrast, foci formed by 3–4 dysplastic crypts were decreased by the starch diet (P < 0.05). After 105 days of feeding, the starch diet induced a number of dysplastic crypts/focus lower than that induced by the sucrose diet (2.6 ± 0.1 and 2.9 ± 0.1, respectively; P < 0.05). The percentage of small FDC was also higher in the animals fed the starch diet compared to animals fed the sucrose diet (P < 0.01). After 30 days of feeding, DMH treatment increased colon proliferative activity in both dietary groups (P < 0.05). But after 105 days of feeding, proliferation was similar in both treatments. The number and dimensions of the foci of dysplastic crypts in the colon of mice fed starch or sucrose diets were 10.4 ± 0.8 and 4.4 ± 0.5 in the sucrose and starch diets, respectively; P < 0.05). The overall results suggest that starch in high-fat/low-calcium/low-cellulose diets has a protective role against DMH-colon carcinogenesis in the rat.

INTRODUCTION

Dietary habits have been linked to the development of colon cancer in experimental and epidemiological studies (1–3). While the consumption of diets containing high fat, low calcium, or high protein has been shown to increase the risk of colon cancer (1, 3), some epidemiological studies have suggested that dietary starch might be a protective factor (4, 5).

Recently, we demonstrated in mice that high-fat/low-starch/low-cellulose diets increased the proliferative activity of colonic mucosa (6). We have also shown low proliferative activity in the colon of mice fed either high- or low-fat diets, in which carbohydrates were supplied by corn starch or by other starchy foods such as pasta, bread, and rice (7, 8). Since proliferative activity in the colon is positively correlated with the risk of developing cancer in this organ (9, 10), the previous results on carbohydrates and proliferation suggest that starch might be a protective factor against colon carcinogenesis.

To test this hypothesis, we tried to determine whether dietary starch affects the number and growth of DMH-induced FDC in the colon of rats.

MATERIALS AND METHODS

Animals. We started our experiment with 8–9-week-old female Sprague-Dawley rats (Morini, Inc., Reggio Emilia, Italy). Rats were housed in plastic cages with wire tops and bottoms. The weights of the animals were monitored weekly for the duration of the experiment.

Dietary and Carcinogen Treatments. All the diets used in the experiments (Table 1) were based on the composition of AIN-76 semisynthetic diet (19), modified to contain a high level of fat (23% corn oil, w/w) and a low level of calcium (0.1%, w/w) and cellulose (2%, w/w). We wanted, in fact, to reproduce dietary conditions similar to the high-fat/low-calcium/low-cellulose diet of western populations with an elevated incidence of colon cancer (16–18).

The experimental diets were fed to the animals for either 30 or 105 days; at the end of these periods we determined the number and dimensions of the foci of dysplastic crypts in the colon. Moreover, given the correlation between proliferative activity and promotion of colon carcinogenesis, we also determined the colon proliferation in DMH-treated and control animals in both dietary groups.

These histological abnormalities are considered to be early events in the development of experimental colon cancer (11). Recently it has been reported that FDC induced by carcinogen treatments can easily be observed and quantified in unsectioned colons (12); it has also been proposed that the number and dimensions of these dysplastic foci might be related to the risk of developing colon cancer (12–15).

In the present study rats were initiated with DMH and then assigned to isocaloric diets containing either starch or sucrose as a source of carbohydrates. The diets used were based on the AIN-76 semisynthetic diet, modified by us to contain a relatively high level of fat (23.1% corn oil, w/w) and a low level of calcium (0.1%, w/w) and cellulose (2%, w/w). We wanted, in fact, to reproduce dietary conditions similar to the high-fat/low-calcium/low-cellulose diet of western populations with an elevated incidence of colon cancer (16–18).

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Received 12/14/90; accepted 4/30/91.

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1 The abbreviations used are: DMH, 1,2-dimethylhydrazine; FDC, foci of dysplastic crypts.
containing either 46% sucrose or starch as a source of carbohydrates (Table 1); these diets were fed for either 30 or 105 days. In both dietary groups the number of animals used was 4 controls and 8 DMH-treated animals in the 30-day study and 7 controls and 8 DMH-treated animals in the 105-day study.

Dietary components were from Piccioni Laboratories (Gessate, Italy).

**Determination of Colon Proliferative Activity and the Number and Dimensions of FDC.** After 30 or 105 days from the beginning of the two experimental diets animals were sacrificed by decapitation. The colon was removed, and two small samples of tissue, close to the rectal end of the colon (about 2 mm² each), were taken for the determination of proliferative activity, as described below. The two colon samples were transferred to a 20-ml sterile vial fitted with a sleeve-style rubber stopper (Wheaton, Mays Landing, NJ), containing 1 ml of Eagle’s α-minimal essential medium (Gibco, Paisley, England) and 5 μl of [³H]thymidine (specific activity, 41 Ci/mmol; final concentration, 5 μCi/ml; Amersham, Amersham, England). Oxygen (25 ml) was injected into the vials, which were then incubated for 90 min at 37°C in a Dubnoff incubator. At the end of the incubation the pressure was released with a syringe, and the specimens were washed 10 times with saline. The specimens were then oriented (mucosa side up), set in a plastic frame, and fixed in this orientation with a 2% agar solution at 4°C. The colon was also processed for the determination of FDC as described below.

**RESULTS**

The weights of the animals at the beginning and during the various phases of the experiment are reported in Table 2. We observed a significant reduction in body weight after DMH treatment; this reduction appeared 5 days after the treatment, but persisted even 30 days after the beginning of the two experimental diets. In contrast, 105 days after the beginning of the experimental diets DMH had no further effect on the weight of the animals. Rats fed the starch diets were heavier than those fed the sucrose diet.

![Fig. 1](cancerres.aacrjournals.org) **Fig. 1.** Number of FDC per colon (left) and number of dysplastic crypts per focus (right) in animals treated with DMH and later fed diets containing sucrose (C) or starch (B) as a source of carbohydrates for either 30 days (top) or 105 days (bottom). Columns, mean; bars, SE. n = 8.

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**Table 1 Composition of experimental diets**

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial diet (g/100 g)</th>
<th>Sucrose diet (g/100 g)</th>
<th>Starch diet (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>23.1</td>
<td>23.1</td>
<td>23.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>23</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Corn starch</td>
<td>23</td>
<td>23.1</td>
<td>23.1</td>
</tr>
<tr>
<td>Casein</td>
<td>23.1</td>
<td>23.1</td>
<td>23.1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mineral mix*</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Energy (kcal/g)</td>
<td>4.86</td>
<td>4.81</td>
<td>4.90</td>
</tr>
</tbody>
</table>

*AIN-76 mineral mix (19) modified to provide 0.1% calcium in the diet (20).

*AIN-76 vitamin mix (19).

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**Table 2 Weight of animals during experiment (g)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DMH-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beginning of the experiment</strong></td>
<td></td>
<td>191 ± 2 (54)</td>
</tr>
<tr>
<td>5 days after DMH treatment</td>
<td>219 ± 4 (22)</td>
<td>207 ± 3 (32)*</td>
</tr>
<tr>
<td><strong>30 days after</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose diet</td>
<td>271 ± 5 (11)</td>
<td>252 ± 5 (16)*</td>
</tr>
<tr>
<td>Starch diet</td>
<td>274 ± 8 (11)</td>
<td>270 ± 6 (16)*</td>
</tr>
<tr>
<td><strong>105 days after</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose diet</td>
<td>312 ± 13 (7)</td>
<td>303 ± 9 (8)</td>
</tr>
<tr>
<td>Starch diet</td>
<td>320 ± 9 (7)*</td>
<td>338 ± 9 (8)*</td>
</tr>
</tbody>
</table>

* Mean ± SE. Numbers in parentheses, number of animals.

* P < 0.01 versus control animals.

* P < 0.05 versus control animals.

* P < 0.05 versus the animals fed the sucrose diet.
of dysplastic crypts per focus in the two dietary groups (Fig. 1, top right), we found that the starch diet caused a slight decrease in the dimension of FDC (number of dysplastic crypts forming a focus) that did not attain statistical significance.

The number of FDC in the colon was also not varied after 105 days of feeding (Fig. 1, bottom left), confirming the 30-day observations. It is interesting to note that the number of foci per colon did not vary significantly between 30 and 105 days.

In contrast (Fig. 1, bottom right), the starch diet caused a significant reduction in the dimensions of the dysplastic foci when compared to the sucrase diet.

We also analyzed the distribution of FDC according to their dimensions. The results of this analysis (Fig. 2, top) showed that after 30 days of feeding, the percentage of small foci (formed by 1–2 dysplastic crypts) was significantly higher in the animals fed the starch diet, while the percentage of larger foci (formed by 3–4 dysplastic crypts) was significantly lower in this dietary group.

The results after 105 days of feeding (Fig. 2, bottom) indicated that in the animals fed the starch diet there was a significantly higher percentage of foci formed by 1–2 crypts and a significantly lower percentage of foci formed by 3–4 and 5–6 dysplastic crypts. We also observed that larger foci, which constitute a small percentage of the dysplastic lesions in the colon, were not significantly changed by the starch diet.

Our results also demonstrated that dysplastic foci grew progressively in dimension but not in number after the treatment; in fact in both dietary groups FDC are larger after 105 than after 30 days.

We also determined the proliferative activity in the colonic epithelium of controls and DMH-treated animals fed the two different diets. These results, analyzed by multifactorial analysis of variance, indicated that after 30 days of feeding, there was a significant increase in proliferation due to the treatment with DMH (Fig. 3, top). It should be noted that as shown in Fig. 3 (top), control animals fed starch had a marked reduction in proliferative activity; however, because of the small number of observations (4 animals on the sucrase diet and 3 on the starch diet), this difference did not attain statistical significance (P = 0.07).

The proliferative activity of colonic mucosa was also determined after 105 days of feeding. The results (Fig. 3, bottom) indicated that after this period the starch diet caused a marked and significant decrease in cell proliferation in both control and DMH-treated animals. The results as illustrated in Fig. 3 show that long after DMH administration, the proliferative activity was similar in treated and control animals in both dietary groups.

DISCUSSION

The early evolution of colon carcinogenesis is not completely understood. However, dysplastic crypts have been observed and described in rodents treated with colon carcinogens and are interpreted as early lesions leading to colonic cancer (11, 21). Dysplastic crypts have also been described in the colonic mucosa of patients with ulcerative colitis and familial polyposis, diseases characterized by a high risk of colon cancer (22).
Recently it has been reported that foci of dysplastic crypts can be easily quantified in the unsectioned colon of experimental animals treated with carcinogens (12–15). It has also been demonstrated that the growth of these dysplastic lesions is enhanced by certain diets (such as high-fat diets) that are believed to promote colon carcinogenesis (14).

The results obtained in the present paper demonstrate that after 105 days of high-fat/low-calcium/low-cellulose diets containing starch as a source of carbohydrates, a significant decrease in the dimensions of DMH-induced dysplastic foci in the colon was observed when compared to similar high-fat diets in which carbohydrates were supplied by sucrose. Moreover, we found that after 30 and 105 days of feeding with the same experimental diets, the number of small foci (formed by 1–2 dysplastic crypts) was higher in the animals fed the diet containing starch, while the percentage of larger foci (3–4 or 5–6 dysplastic crypts) was decreased. Finally, we showed that the number of dysplastic foci induced by DMH was not significantly affected by diet.

Altogether these results suggest that in high-fat/low-calcium/low-cellulose diets, starch as a source of carbohydrates does not influence the initiation process but reduces the promotion of the growth of preneoplastic lesions of the colon.

The diets we used were all based on the AIN-76 semisynthetic diet modified to contain a relatively high level of fat (23.1% corn oil, w/w) and a low level of calcium (0.1%, w/w) and cellulose (2%, w/w). These conditions were chosen to produce an experimental dietary regimen similar to the high-fat/low-calcium/low-cellulose diet of those western populations with a high incidence of colon cancer (16–18). Similar “nutritional stress diets” (18) have been used in other studies on the effect of diet on experimental colon carcinogenesis in order to magnify the effects of a given dietary component (6, 18, 20). In our previous studies in mice we have demonstrated that starch caused a decrease in the proliferative activity of colonic mucosa compared to sucrose, this effect being more evident when the animals were fed high-fat/low-calcium/low-cellulose diets (6). For the present study, we selected the diet we thought most appropriate to evaluate the effects of starch on preneoplastic lesion in the colon.

We also observed a significant increase in mucosal proliferation 30 days after the beginning of the experimental diets, following DMH treatment. Proliferative activity in response to DMH treatment has been well studied in rodents, especially in mice, in which multiple injections with DMH increase colon proliferation (23, 24). A significant increase in colonic proliferation has also been observed in rats treated with single or multiple doses of DMH, although some authors reported a nonsignificant effect on colonic proliferation when DMH was chronically administered (24–27). In our study we found that DMH administrated twice, 4 days apart, for a total dose of 50 mg/kg, caused a significant increase in colon proliferation. We also demonstrated that at a later time (110 days) after the treatment with DMH the treated animals had a proliferative activity similar to that of controls in both dietary groups.

In our experiments the starch diet caused a marked decrease in mucosal cell proliferation, especially evident after 105 days of feeding. An effect on cell proliferation induced by the starch diet was also observed in control animals after 30 days of feeding, but because of the small number of animals, this effect was not statistically significant. These last results confirm our previous observations in mice using similar diets (6–8), indicating a common effect of starch in high-fat diets in two different species.

High cell proliferation rates in the colon have been reported to increase the incidence of preneoplastic lesions induced by DMH in mice (9). Other studies performed in experimental animals and epidemiological studies have indicated that increased proliferative activity in the colonic mucosa is correlated with an increased risk of developing colon cancer (10). Given this correlation between proliferation and carcinogenesis, it is interesting to note that we found statistically significant correlations between individual values of proliferative activity in the colon and the distribution of dysplastic foci according to their dimensions. In fact, we found a positive correlation \( (r = 0.60; P = 0.013) \) between the number of labeled cells per crypt and the percentage of foci formed by 3–4 dysplastic crypts, while we found a negative correlation that bordered on statistical significance \( (r = 0.46; P = 0.06) \) between the number of labeled cells per crypt and the percentage of foci formed by 1–2 dysplastic crypts.

Therefore, our results suggest that the reduced growth of the dysplastic lesions observed in the colon of rats fed a starch diet might be connected to a diminished proliferative activity of the colonic mucosa induced by starch consumption.

The mechanisms underlying the effect of starch on colonic mucosa are not known. Starch consumption has been suggested to alter the colonic environment (28). In a parallel study conducted by us on the same animals we found that starch diets increased the production of short-chain fatty acids and increased the percentage of butyrate in the cecum (29). Butyrate is known to induce differentiation of colon cancer lines, as well as other types of cancer cells in vitro (30). Moreover, in the same study, we found that animals fed starch diets had a significantly lower cecal pH than those fed sucrose diets (mean, 7.16 ± 0.03 and 7.37 ± 0.04, respectively; \( P < 0.01 \)). Fecal pH was also slightly lower in the starch diet, but this effect was not statistically significant. A reduction in colonic pH has been suggested to be a protective factor against the development of colon cancer (2, 31).

It is not known at present how variations in short-chain fatty acids and colonic pH are related to the results obtained regarding the growth of dysplastic foci, and other studies will be required to understand the mechanism of the inhibitory effect of starch on the promotion of the growth of dysplastic foci.

It must be noted also that the effect of starch on the growth of the dysplastic foci, although significant, is not striking. This might be due to the experimental conditions used, in which initiation by DMH produces a very high number of dysplastic crypts and may overwhelm the modulating effect of starch on the growth of dysplastic foci. As pointed out above, our results have been obtained using diets containing some of the supposed risk factors for colon cancer such as high fat, low calcium, and low cellulose (18, 24). It is not known at present how each of these dietary variables is responsible for the results obtained and whether starch will have the same effect with a low level of dietary fat or with different cellulose or calcium levels.

However, since some epidemiological studies have suggested that starch is a protective factor in the development of colon cancer in humans (4, 5, 32), our results, although obtained in the DMH-rat model, indicate a possible mechanism for the explanation of the correlation between starch consumption and colon cancer.
REFERENCES

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