Effect of High-Fat Diet on Colon Carcinogenesis in F344 Rats Treated with 1,2-Dimethylhydrazine, Methylazoxymethanol Acetate, or Methylnitrosourea

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SUMMARY

Male inbred F344 weanling rats were fed semipurified diets containing 20 or 5% beef fat. At 7 weeks of age, all animals except controls were given 1,2-dimethylhydrazine [DMH (150 mg/kg body weight s.c.; one dose)], methylazoxymethanol acetate [MAM acetate (35 mg/kg body weight i.p.; one dose)], or methylnitrosourea (2.5 mg/rat/week intrarectally; two doses), and autopsied 35 weeks later. No major differences were observed in the frequency (number of rats with tumor) of DMH- or MAM acetate induced colon tumors between the rats fed diets containing 20 or 5% fat. However, the animals fed the diet containing 20% fat and treated with DMH, MAM acetate, or methylnitrosourea had a higher frequency of colon tumors (number of rats with colon tumors) than did those fed a diet containing 5% fat and treated similarly. Dietary fat at the 20% level was associated with an increased incidence of MAM acetate- or methylnitrosourea-induced colon tumors (number of tumors per tumor-bearing rat), whereas in rats treated with DMH the diet containing 20% fat had slight although not statistically significant influence on the incidence of adenomas and adenocarcinomas in the colon.

INTRODUCTION

Geographical pathology and socioeconomic distribution of large bowel cancer, studies of Japanese migrants to the United States and, studies of life style and dietary habits of various population groups with a variety of risks indicate that dietary factors, particularly high dietary fat and beef and lack of dietary fiber, are of major importance in the etiology of colon cancer and that the genetic constitution plays a minor role (1, 2, 5, 6, 8, 27). DMH and AOM require metabolic activation, whereas MAM acetate, a metabolite of DMH and AOM, is a proximate carcinogen (27). Although there is no apparent relation between diet-mediated agents responsible for colon cancer in humans and the carcinogens that affect the colon in animals, studies in animal models may explain the complex sequence of events leading to colon cancer. Since high dietary intake of fat has been shown to increase DMH- or AOM-induced colon carcinogenesis in rats (16, 19, 20), the present studies were designed to investigate the effect of high-fat diet on colon tumor induction by DMH, MAM acetate or MNU. These 3 carcinogens, which differ in activation, were selected because they represent a broad spectrum of exogenous carcinogens. In this study a single dose of DMH or MAM acetate or 2 doses of MNU were used to produce colon tumors.

MATERIALS AND METHODS

Inbred weanling male F344 rats, obtained from the Charles River Breeding Laboratories (Wilmington, Mass.), were randomly divided into 12 groups, housed in plastic cages in a temperature- and humidity-controlled room, and fed ad libitum one of the semipurified diets containing 20 or 5% beef fat (Table 1). Animals in Groups 1, 3, 5, 7, 9, and 11 were fed a diet containing 20% fat, whereas animals in Groups 2, 4, 6, 8, 10, and 12 were maintained on a 5% fat diet.

At 50 days of age, all rats in Groups 1 and 2 received a single injection of DMH at a dose of 150 mg/kg body weight s.c., whereas animals in Groups 3 and 4 received a single injection of MAM acetate at a dose of 35 mg/kg body weight i.p. Animals in Groups 5 and 6 were given weekly MNU at a dose of 2.5 mg/rat i.r. for 2 weeks. Before the injection the DMH dihydronchbonide was dissolved in 0.9% NaCl solution and brought to pH 6.8. MAM acetate and MNU were dissolved in phosphate buffer (pH 6.8). At the end of 35 weeks, all animals were killed, and their organs, including the intestine, were examined grossly and histologically for the number and type of tumors. Tissues were fixed in 10% formalin and embedded in the paraffin; the sections were then stained with hematoxylin and eosin.

RESULTS

Body weights of animals fed the 2 diets and treated with

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2 The abbreviations used are: DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; MAM acetate, methylazoxymethanol acetate; MNU, methylnitrosourea; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; i.r., intrarectal.

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DMH, MAM acetate, or MNU are shown in Chart 1. Untreated as well as carcinogen-treated animals fed a diet containing 20% fat weighed slightly more than the animals fed a 5% fat diet.

Table 2 summarizes the tumor incidence in rats treated with DMH, MAM acetate, or MNU. There was no difference in the incidence of small intestinal tumors between the animals fed diets containing 20 or 5% fat and treated with DMH or MAM acetate. All tumors of the small intestine were found in the duodenum, approximately 2 to 3 cm from the pylorus. One of the animals in the 5% fat diet group and 1 in the 20% fat diet group showed DMH- and MAM acetate-induced ear duct tumors, respectively. Also, a DMH-induced kidney tumor was found in 1 animal fed the 20% fat diet.

The number of rats with colon tumors was significantly increased in rats fed a 20% fat diet and treated with DMH, MAM acetate, or MNU compared with that in animals fed a 5% fat diet and treated similarly with the respective carcinogens (Table 2). The incidence of colon tumors (total number of colon tumors per tumor-bearing rat) was significantly higher in animals fed a diet containing 20% fat and treated with MAM acetate or MNU compared with that in those fed a 5% fat diet and given the respective carcinogens (Table 2). The incidence of MNU-induced adenomas and adenocarcinomas was higher in rats fed 20% fat than it was in those fed the 5% fat diet; however, animals fed 20% fat developed more MAM acetate-induced adenomas, although not ade-

Table 1

Percentage composition of diets

<table>
<thead>
<tr>
<th>% high fat</th>
<th>% normal fat</th>
</tr>
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<tbody>
<tr>
<td>Casein (vitamin free)</td>
<td>22.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>33.2</td>
</tr>
<tr>
<td>Dextrose</td>
<td>11.0</td>
</tr>
<tr>
<td>Alphacel</td>
<td>7.0</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>20.0</td>
</tr>
<tr>
<td>Salt mixture*</td>
<td>4.5</td>
</tr>
<tr>
<td>Vitamin diet fortification mixture*</td>
<td>2.0</td>
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</tbody>
</table>

* For the detailed composition see Ref. 20.

Table 2

Tumor incidence in rats fed a 20 or 5% beef fat diet and treated with DMH, MAM acetate, or MNU

Thirty rats were placed on each diet and carcinogen treatment.

<table>
<thead>
<tr>
<th>Carcinogen treatment</th>
<th>Diet (% fat)</th>
<th>Small intestine</th>
<th>Colon</th>
<th>Total tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>DMH</td>
<td>20</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>MAM acetate</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

* Significantly different from the 5% fat diet by *x*2 test (p < 0.05 or better).

**Significantly different from the 5% fat diet by *t* test (p < 0.05 or better).
nomas invading into the submucosal and muscular layer; some were signet ring infiltrating carcinomas and were prominent in the proximal colon. The adenomas were benign neoplasms of intestinal glandular structure lining with slight or moderate atypical epithelial cells although not invasive into the submucosal layer.

Small intestinal tumors induced by DMH and MAM acetate were located mainly in the proximal part of the duodenum, approximately 2 to 3 cm from the pylorus. Duodenal tumors were sessile, elevated, and hemispherical. Microscopically, the duodenal tumors were well-differentiated adenocarcinomas invading into the submucosal, muscular, and serosal layers; some were benign adenomas. One animal had peritonitis carcinomatosa disseminated tumor cells into the serosal surface of the diaphragm, peritoneal cavity, and prostate and had lung metastasis. The ear duct tumors were keratinizing squamous cell carcinomas; kidney tumors were mesenchymal (benign).

**DISCUSSION**

The results obtained from this and our earlier studies (19, 20) demonstrate that the rats fed a diet high in fat were more susceptible to colon tumor induction by DMH, MAM acetate, or MNU than were rats fed a diet containing a normal level of fat. These results thus indicate that, irrespective of the colon carcinogens (AOM, DMH, MAM acetate, and MNU), which differ in activation) used to induce colon tumors, the animals fed a high-fat diet always had a higher incidence of colon tumors than those fed a low-fat diet. Our studies also confirm the observations of Zedek and Sternberg (29) and Martin et al. (12) who found that a single dose of MAM acetate or DMH, respectively, in rats induced tumors of the colon.

A strong association has been established between colon cancer incidence and high concentration of fecal bile acids and cholesterol metabolites in humans (9, 10, 23, 24). Biliary excretion of bile acids and fecal excretion of bile acids and cholesterol metabolites were higher in rats fed a diet high in fat than they were in rats fed diets low in fat (18). An increase in bile salts in the colon of rats induced either by feeding cholestyramine or by diverting bile surgically to the middle of small intestine enhanced AOM-induced colon tumor formation (3, 15). Deoxycholic acid, lithocholic acid, cholic acid, and chenodeoxycholic acid increased the frequency of MNNG-induced colon tumors (promoting effect) in rats (13, 21, 22). Thus, it is possible in the present study that the high incidence of colon tumors in rats fed a high-beef-fat diet may be the result of excretion of elevated amounts of bile acids, which act as colon tumor promoters.

Caloric intake has been shown to be associated with tumor incidence in several organs, especially the skin and mammary gland (4). In the present study, fat in the diet was adjusted at the expense of carbohydrate by weight rather than by calories. Although all animals were fed ad libitum, which suggests that the rats fed a 20% fat diet consumed more calories than did those animals fed a 5% fat diet, the difference in body weights between the animals fed 20 and 5% fat diets was not enough to account for differences in colon tumor incidence. This rules out the possibility that the observed effects in animals fed the 2 diets were due to differences in caloric intake and body weight.

In this study the difference in susceptibility between the rats fed diets containing high fat or normal fat may also be explained in terms of diet-dependent mucosal enzyme inducers or inhibitors that are in the diet and that modify the capacity of the animal to metabolize carcinogen (26). Norred and Wade (17) found that the content of hepatic cytochrome P-450 was decreased in rats fed a fat-free diet compared to animals fed diets with increasing fat content. However, this factor would presumably not play a role in carcinogenesis by MAM acetate or MNU because these carcinogens require no metabolic activation. With DMH, only a single dose was used, and yet the increase in colon tumor incidence observed in rats fed a 20% fat diet does not seem to be due to the altered metabolism of DMH as a major element. Thus, the dietary effects demonstrating higher carcinogenicity with 3 different colon carcinogens described herein are mediated most likely by an increased level of intestinal bile acids that exert a promoting action.

**ACKNOWLEDGMENTS**

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Dietary Fat and Colon Carcinogenesis


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