Reduction of N-Methyl-N-nitrosourea-induced Colon Tumors in the Rat by Cholesterol

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ABSTRACT

Patient populations with a propensity to develop colon cancer have increased amounts of fecal cholesterol (and/or cholesterol metabolites). In this study, we report the effect of increased colonic concentrations of cholesterol and its metabolites on colon tumor promotion. The chemical carcinogen N-methyl-N-nitrosourea was instilled intrarectally into rats to initiate colon tumor formation. Following initiation, a cholesterol-supplemented diet was given. Despite a 2-fold elevation of fecal cholesterol, the number of colon tumors found was significantly reduced. These studies suggest that under certain conditions cholesterol may inhibit colon carcinogenesis.

INTRODUCTION

Large bowel cancer is a major cause of death from cancer in the United States (1). Evaluation of populations with a high risk for colon cancer suggests that dietary factors, particularly high intake of dietary fat and beef and lack of dietary fiber, have a strong association with this disease (3, 11, 15, 23, 24). Metabolic epidemiological studies have shown that high-risk populations have increased bile acid concentrations of bile acids and cholesterol (or its metabolites) in the feces (14, 19, 20). Current concepts are that bile acids and cholesterol (or its metabolites) facilitate the development and growth of large bowel tumors (i.e., behave as cocarcinogens) and that these compounds, either directly or indirectly, are derived from dietary factors (4, 14, 17, 18, 20).

Studies in animal models of colon cancer have supported the role of bile acids as tumor promoters (18, 20). There have been few studies designed to evaluate the role of neutral sterols. In this report, an animal model of colon cancer is utilized to examine the effect of dietary cholesterol on tumor development. This experiment showed that dietary cholesterol retarded colon carcinogenesis.

MATERIALS AND METHODS

Treatment of Animals. Male cesarean section-derived F344 rats were obtained from Charles River Breeding Laboratories, Inc. (North Wilmington, Mass.) when they were 28 days old. They were divided into 4 groups: Group 1, 30 rats fed control chow; Group 2, 30 rats fed control chow plus 0.2% cholesterol; Group 3, 91 rats fed control chow and treated with 8 mg MNU; and Group 4, 74 rats fed control chow plus 0.2% cholesterol and treated with 8 mg MNU. Twenty-four rats in Group 3 were tested concurrently while 67 were treated identically but studied at earlier times. All rats were housed in plastic cages with stainless steel tops and filters covering the entire cage. The rats had access to food and water ad libitum. The food for the experiment was prepared by the Ralston Purina Co. (Richmond, Ind.). The chow pellets were ground, the dietary supplement was added, and the food was repelleted. The food was prepared at the same time from a single lot. All food was stored in a cool, dry environment until needed.

The animals were quarantined in plastic cages (3 rats/cage) 2 weeks prior to administration of the carcinogen. MNU, was dissolved in sterile 0.9% NaCl solution at room temperature and administered intrarectally using an 18-g (7.5-cm) cannula syringe (Becton-Dickinson & Co.) (4, 13). The MNU was given in 4 equal doses (2 mg/dose) on Days 1, 4, 7, and 10 of the experiment. The experimental diets were begun 5 days after the last MNU dose. The dose of MNU was chosen to produce a tumor incidence of 50%. At various time periods (Weeks 9 and 16 and the week prior to sacrifice), the animals were placed into individual metabolic cages. The food intake and fecal output for the animals were measured a week prior to sacrifice is shown in Table 1. Two days were allowed for adaptation to the environment. The measurements of the food intakes and fecal outputs were made during the next 2 days (Days 3 and 4). Feces were collected and analyzed for neutral sterols and bile acids using procedures already described (5–7).

At Week 28, each intestine was opened from cecum to anus and the position of every tumor was noted, scored, and recorded. Notations were made of the location of each tumor in terms of its distance from the anal verge and its maximum dimensions. Sections of abnormal appearing tissue were removed for pathological and histological examination. The animals were eviscerated, and all organs were inspected for the presence of tumor. The organs were fixed in 10% Millonig’s buffered formalin at pH 7.5, and sections were cut 6 µm thick and stained with hematoxylin and eosin.

Compounds. Cholesterol was obtained from Sigma Chemical Co. (St. Louis, Mo.) and was found to be 97% pure by TLC on Florisil TLC plates (Floridin Co., Berkeley Springs, W. V.) using ethyl ethenheptane (55:45, v/v). The bile acids were separated as described previously (7).

Analytical and Statistical Methods. All TLC separations were performed by procedures already described (5, 6). The neutral sterols (cholesterol, coprostanol) and plant sterols (β-sitosterol, coprostanol) were plated on Florisil, and developed in ethyl ether-heptane (55:45, v/v). The bile acids were separated as described previously (7).

All gas-liquid chromatography analyses were performed on a Hewlett Packard 5830A gas chromatograph (7). The neutral and acidic steroids were analyzed as their trimethylsilyl ether derivatives on 3% SE-30 with 5a-cholestanol as an internal standard.

The neutral sterols and bile acids were identified with the use of a Hewlett Packard 5992A mass spectrometer using a 3-ft (2-mm-inside diameter) column packed with 1% SE-30.

All radioactivity measurements were made using new scintillation
vials on a LS-7500 liquid scintillation system (Beckman Instruments, Inc., Fullerton, Calif.). Each sample was corrected for background and quenching using appropriate standards.

Methods used to determine statistical significance were Student's t test, χ² test, or 2-way analysis of variance (7).

RESULTS

Rats were fed cholesterol (0.2% in the diet) for 26 weeks after being treated with MNU (total dose, 8 mg). There were no adverse effects of the cholesterol and no deaths occurred during the experiment. There was also no diarrhea or histological evidence of liver damage. Table 1 shows the physiological data for the animals. The weight gain, food intake, and fecal outputs for all groups of animals were similar.

Pathology. Table 2 shows the tumor incidence for the animals sacrificed at Week 28. No tumors were observed in the animals given control chow or chow supplemented with 0.2% cholesterol. In the rats given MNU and fed control Purina chow, there were 43 animals with tumors (47%), 1.00 tumor/animal, and 2.11 tumors/tumor-bearing animal. The animals used in 4 previous studies which were given only MNU and treated identically to the 24 rats given only MNU in this experiment were combined and the data were averaged. Our statistician could find no statistical differences in the MNU animals in the 5 experiments. In the group given MNU and fed cholesterol, 22 of 74 animals had tumors (30%, p < 0.05 by χ²), there were 0.39 tumor/animal (p < 0.02 by t test), and 1.32 tumors/tumor-bearing animal (p < 0.02 by t test). All tumors were discrete polyplid excrescences. Most of the tumors were adenomas (85 in Group 3; average size, 0.42; and 27 in Group 4; average size, 0.38), but several invasive carcinomas were also detected (6 in Group 3; average size, 0.50; and 2 in Group 4; average size, 0.55). In these lesions, we observed a transition from normal colonic mucosa to an adenomatous growth pattern with irregular glands infiltrating the muscularis mucosa. Location of tumors in Groups 3 and 4 were similar.

Chemical Analyses. Fecal neutral sterols and bile acids were analyzed during the experiment. The data obtained at Week 28 for animals in the various groups are shown in Table 3. Fecal neutral sterols (cholesterol, coprostanol) were elevated 2-fold in the cholesterol-fed groups [Group 2, 4.06 ± 0.39 (S.D.) mg/g, and Group 4, 4.12 ± 0.56 mg/g, versus Group 1, 1.85 ± 0.35 mg/g, and Group 3, 1.98 ± 0.21 mg/g; p < 0.01]. The degradation of cholesterol to coprostanol was similar in all groups indicating similar bacterial metabolic activity. The bacterial modification of β-sitosterol to coprosterol was also similar. β-Sitosterol recovery is used to correct for losses of fecal neutral steroids. In all cases, recovery was greater than 85%. Total fecal bile acids were higher in the
groups given cholesterol, but these increased levels did not reach statistical significance. The amount of deoxycholic acid was similar in all groups. This suggested that cholesterol did not affect the bacterial modification of bile acids.

**DISCUSSION**

In this animal model of colon cancer, the ingestion of dietary cholesterol to elevate fecal neutral sterol concentration 2-fold resulted in a biologically and statistically significant reduction in colon tumor formation. This was an unexpected finding since metabolic studies in humans have demonstrated high concentrations of fecal neutral sterols (cholesterol and metabolites) in: (a) high-risk populations for colon cancer (14); (b) patients with colorectal cancer (20); (c) patients with adenomatous polyps (20); and (d) patients with a propensity to form colorectal cancer (14). However, in most situations where increased fecal neutral sterols were demonstrated, the fecal concentrations of bile acids were also increased. Bile acids have been implicated as colon tumor promoters in studies in animal models (4, 16, 17). Their mode of action may be either to alter the kinetic behavior of colonic epithelial cells (4) or to increase the permeability of cell membranes to carcinogens (21). Cholesterol has been touted as a cocarcinogen for human colon cancer (9). However, the epidemiological evidence is not very strong (12, 22).

The role of cholesterol in colon carcinogenesis has been studied by other investigators (2, 8, 16, 17). They found that cholesterol either increased (2, 8) or had no effect upon colon tumor formation (16). Our studies show for the first time that cholesterol may decrease tumor development. In the earlier studies, the animal model, the carcinogen, the diet, or the method of administering the cholesterol was different. Thus, it may be difficult to compare the results among these experiments. In our experiment, we studied the postinitiation effects of cholesterol. A direct-acting carcinogen was given intrarectally and produced tumors only in the area of the colon exposed to the carcinogen. The dose of carcinogen was carefully chosen so that tumors would be produced in approximately one-half of the rats. At this dose level, the ability of a compound to enhance or retard carcinogenesis can be detected. Cholesterol was added to the diet 5 days after the last dose of carcinogen to elevate fecal neutral sterol concentration 2-fold and produced tumors only in the area of the colon exposed to the carcinogen. Cholesterol and its metabolites interacted with the colonic epithelial cells at a physiological concentration and in a physiological state, i.e., admixed with feces.

Under the conditions of this experiment, we have demonstrated that cholesterol can inhibit tumor formation. Cholesterol appears to retard the action of an already-initiated carcinogenic process. In previous studies, we have demonstrated that a plant sterol, β-sitosterol, with a structure very similar to cholesterol reduced colon carcinogenesis (13). Cellular kinetic studies demonstrated that β-sitosterol slowed epithelial cell proliferation, and this was thought to explain the reduced expression of neoplastic transformation (10). Cholesterol may act by a similar mechanism. Cholesterol is essential for the structure of all cell biological membranes and is a precursor of all steroid hormones. Obviously, there are many mechanisms by which cholesterol may interact with the carcinogenic process. These processes require further study.

Studies in animal models suggest possible interactions between cholesterol and carcinogenesis. This experiment raises the possibility that cholesterol may somehow retard carcinogenesis. This notion is supported by epidemiological studies that have shown an inverse association between serum cholesterol and mortality from malignant neoplasms (12, 22). Although further studies of the interaction of cholesterol and colon cancer are required, we would suggest that dietary recommendations to drastically reduce cholesterol intake should be reevaluated.

**REFERENCES**

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