Further Leads on Metabolic Epidemiology of Large Bowel Cancer

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Summary

Studies in metabolic epidemiology have shown that the dietary intake of high fat affects the composition of the intestinal bacteria and their metabolic activity as well as the levels of certain neutral sterols and bile acids that may act as tumor promoters for the colon.

A strong association has also been established between microbiologically modified bile acids and cholesterol metabolites and the risk of colon cancer among different populations. The patients with colon cancer had high concentrations of fecal bile acids and cholesterol metabolites compared with the controls. It remains to be shown whether this established association is causative in nature.

Introduction

Largely on the basis of geographic and socioeconomic distribution of colorectal cancer, migrant population studies, retrospective studies of United States and Japanese patients with large bowel cancer, and dietary habits of different risk populations, it is generally accepted that diet, particularly high intake of dietary fat and beef, is a major etiological factor, although not the sole factor, in colon cancer (5, 7, 8, 12, 20–23, 25, 26). Wynder et al. (22, 26) first proposed that colon cancer incidence is mainly associated with dietary fat and further suggested that dietary fat influences the composition of fecal flora and thus is involved in the pathogenesis of cancer of the colon. Diets high in fat and animal protein are consumed by populations in high-risk areas. People in low-risk areas eat diets low in fat content but rich in vegetable protein or fish protein. Beef is higher in saturated fat than other sources of animal protein, such as pork, chicken, or fish. A major portion of the dietary fat comes from meat, and beef contributes significantly to the overall fat intake. It therefore must be determined whether beef contains carcinogenic compounds as such or increases the incidence of colon cancer by contributing significantly to the overall fat intake of a population, a concept that we prefer.

Etiological Factors

It is logical to consider that intraluminal constituents of the large bowel with tumorigenic activity could relate to colon carcinogenesis and could arise, at least in part, from high intake of dietary fat. Important endogenous compounds that are related to dietary fat and secreted into the gut include bile acids and neutral sterols. The intake of high dietary fat not only changes the composition of bile acids and neutral sterols but also modifies the large bowel bacteria quantitatively, which in turn may produce tumorigenic substances from bile acids and neutral sterols (11, 19, 23). The importance of microflora and of bacterially modified substrates in colon carcinogenesis is supported by the fact that (a) the incidence of small bowel cancer is low and (b) this part of the gut has a low total bacterial count and a low total anaerobic count compared to that of the large bowel.

Several investigators (2–4, 6, 9, 13) have become interested in potential carcinogenic activity of certain bile acids and neutral sterols due to: (a) they are sterically similar to carcinogenic polycyclic aromatic hydrocarbons; (b) full aromatization of bile acid nucleus would potentially yield a carcinogen metabolite based on cyclopentaphenanthrene; (c) human gut flora, especially a group of Clostridia, have been shown to achieve partial aromatization of the sterol ring; and (d) the fecal concentrations of bile acids and bacteria are related to diet. Such microflora-mediated reactions are unlikely to yield polycyclic hydrocarbons but are much more likely to give products that act as colon tumor promoters or accelerators rather than complete carcinogens.

The evidence for bile acids as colon tumor promoters has been obtained from animal experiments. Narisawa et al. (15) reported that the development of adenomas significantly increased among those rats given intrarectally an initiating dose of N-methyl-N’-nitro-N-nitrosoguanidine and receiving intrarectal taurodeoxycholic acid or lithocholic acid as promoters compared to the group given only the N-methyl-N’-nitro-N-nitrosoguanidine although bile acids themselves produced no tumors. Taurodeoxycholic acid given intrarectally is deconjugated into deoxycholic acid by bacterial enzymic hydrolysis in the large intestine. Thus, in this animal model, these bile acids, present in high concentration in human stools, act as colon tumor promoters to an established large bowel animal carcinogen. Nigro et al. (16) and Chomchai et al. (1) observed that the carcinogenic effect of azoxymethane in rats was enhanced by the increase of bile salts in the colon, induced either by feeding cholestyramine or by diverting bile to the middle of the small intestine.

Overall, current concepts point out that qualitative and quantitative differences in luminal compounds such as bile acids and cholesterol metabolites play an enhancing role in...
colon carcinogenesis, and these compounds are primarily derived from dietary factors and subsequently modified by the gut bacteria. It was of course an item of 1st priority to establish in man whether changes in the composition of diet would alter the concentration of fecal bile acids, cholesterol metabolites, and fecal bacteria and whether differences in these intraluminal constituents occur between high-risk and low-risk populations for colon cancer as well as patients with colon cancer, polyps, ulcerative colitis, and familial polyposis. The patients with polyps, ulcerative colitis, and familial polyposis constitute a considerably high-risk group for the development of colon cancer.

Effect of Diet on Neutral Sterol and Bile Acid Excretion and on the Composition of Fecal Microflora

Bile acids, themselves end products of cholesterol catabolism, act to regulate virtually every step in cholesterol metabolism, including dietary absorption, endogenous synthesis, excretion, and bile acid formation. The influence of the intestine on bile acid and neutral sterol metabolism is determined by several factors, including variations in hepatic synthesis and biliary excretion, the enterohepatic circulation, and transformation in the intestinal lumen by the gut bacteria. It is apparent that both the quantity and quality of diet affect bile acid and neutral sterol production and excretion.

Hill (10) reviewed, in this symposium, the effect of dietary fat, fiber, and lactulose on bile acids and neutral sterol excretion in man. During this discussion, we elaborated our studies, which demonstrate the effect of diet on bile acids and neutral sterol excretion in man. Our studies include the analysis of individual 24-hr stool samples collected daily on consecutive days for 2 to 4 days from each volunteer.

Reddy and Wynder (19) showed that the daily fecal excretion of cholesterol metabolites, coprostanol and coprostanone, was higher in Americans consuming a mixed Western diet than in American Seventh-Day Adventists eating a mixed Western diet without meat but with vegetable protein and in American vegetarians, Japanese, and Chinese. A significant increase in the excretion of total bile acids, deoxycholic acid, lithocholic acid, 12-ketolithocholic acid, 3β, 12α-dihydroxy-5β-cholanic acid, 12α-hydroxy-3-keto-5β-cholanic acid, and 3-keto-5β-cholanic acid, was found in Americans eating a mixed Western diet. The fecal bacteria of Americans consuming a mixed Western diet also contained a higher β-glucuronidase activity (19). Many exogenous and endogenous compounds, including important carcinogen metabolites, are excreted via bile as glucuronide conjugates. This suggests that the intestinal bacteria of Americans eating a mixed Western diet are more active in hydrolyzing glucuronide conjugates than are those of other groups.

Thus far, most studies compared different individuals with different dietary habits. We also investigated the effect on the composition of fecal microflora, acid and neutral sterols, and β-glucuronidase activity of a high-fat, high-meat mixed Western diet (providing 23% protein, 45% fat, and 32% carbohydrate) in the same individuals (17, 18, 24). The test subjects, eating a high-fat, high-meat mixed Western diet, were transferred to a nonmeat diet and fecal samples were collected 4 weeks later. The total anaerobic microflora as well as the counts of Bacteroides, Bifidobacteria, Peptococcus, and anaerobic Lactobacillus were higher during the period of consumption of high-fat, high-meat mixed Western diet compared to the nonmeat diet consumption period (18). Fecal excretion of secondary bile acids (deoxycholic acid, 3,12-diketo-5β-cholanic acid, 12-ketolithocholic acid, and 3-keto-5β-cholanic acid) and cholesterol metabolites (coprostanol and coprostanone) and activity of fecal bacterial β-glucuronidase were decreased when subjects eating a high-meat diet transferred to a nonmeat diet (17, 18).

In a similar study in which the meat content was changed without altering the fat content, there were no major changes in the bacterial flora; but the fecal acid and neutral sterol levels fell during the period of no meat consumption (14). Reduction in the amount of dietary fat from 100 to less than 30 g/day resulted in a great decrease in the fecal acid and neutral steroid levels (10).

The results of experiments thus far in our laboratory have led to the following conclusions. Dietary intake of high fat affects the composition of the intestinal microflora and their metabolic activity as well as the levels of certain steroids that may act as tumor promoters for the colon. Diet may also control the secretory and functional ability of the liver to yield many metabolites that are subsequently modified by the intestinal bacteria. The populations on a high-fat mixed Western diet, among whom the rate of large bowel cancer is high, excreted cholesterol and bile acid metabolites to a greater degree than did those people among whom the rate of colon cancer is comparatively low.

Fecal Bile Acids and Cholesterol Metabolites and Microflora in Patients with Colon Cancer, Ulcerative Colitis, Polyps, and Familial Polyposis

It is generally agreed that patients with ulcerative colitis, polyps, and familial polyposis are at increased risk of developing carcinoma of the colon. Comparisons of fecal constituents in terms of bacteria, cholesterol, and bile acid metabolites and bacterial enzymes were carried out on patients with colon cancer, polyps, familial polyposis, and ulcerative colitis. The fecal excretion of cholesterol metabolites and bile acids (mainly deoxycholic acid, lithocholic acid, and other secondary bile acids) was higher in patients with colon cancer compared to controls (Table 1). In contrast, total fecal neutral sterol and bile acid excretion was similar in patients with familial polyposis and controls. The pattern of cholesterol excretion was unique. The patients with familial polyposis showed an increase in cholesterol excretion and a decrease in the excretion of coprostanol and coprostanone. The fecal excretion of deoxycholic acid and lithocholic acid was decreased in patients with familial polyposis. This observation suggests a decrease in microbial conversion of cholesterol and primary bile acids in these patients. This may be due to either physiological conditions in the colon unfavorable for the bacteria to act on cholesterol and bile acids or low concentration of cholesterol and bile acid-degrading bacte-
ria in the colonic contents. The reasons for this unique difference are under study.

The activity of fecal 7α-dehydroxylase, which converts cholic and chenodeoxycholic acid to deoxycholic acid and lithocholic acid, respectively, was higher in patients with colon cancer than in controls (Table 2). These data support the concept that the patients with colon cancer are more able to convert primary bile acids into secondary forms in colonic contents than are the controls.

The bacteriological data at present are too preliminary to delineate distinction at the generic level. Speciation is incomplete and, while more subtle differences may become apparent, the early data produced thus far preclude any simplistic generalization.

Future studies should concentrate on identifying chemical and/or bacterial indicators that will reflect high and low-risk populations as well as patients with colon cancer. Identification of such specific indicators would be more realistic than time-consuming, expensive, and perhaps unnecessary identification and quantification of bacteria and intraluminal compounds. Fecal bacterial β-glucuronidase and 7α-dehydroxylase activities, fecal *clostridia*, lithocholic acid, and deoxycholic acid could be mentioned in this respect.

Table 1

<table>
<thead>
<tr>
<th>Neutral sterols</th>
<th>Colon cancer (12)*</th>
<th>Control (15)</th>
<th>Familial polyposis (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>13.8 ± 4.6±c</td>
<td>3.8 ± 1.0</td>
<td>10.8 ± 2.3±</td>
</tr>
<tr>
<td>Coprostanol</td>
<td>23.0 ± 5.1±c</td>
<td>12.6 ± 2.6</td>
<td>7.2 ± 2.1±</td>
</tr>
<tr>
<td>Coprostanone</td>
<td>1.6 ± 0.6</td>
<td>1.4 ± 0.3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Total</td>
<td>38.4 ± 4.4±c</td>
<td>17.8 ± 2.5</td>
<td>15.2 ± 2.3±</td>
</tr>
<tr>
<td>Bile acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholic</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Chenoxycholic</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>7.2 ± 1.5±c</td>
<td>4.2 ± 0.3</td>
<td>2.0 ± 0.5±</td>
</tr>
<tr>
<td>Lithocholic</td>
<td>5.7 ± 1.0±c</td>
<td>3.4 ± 0.2</td>
<td>0.9 ± 0.4±</td>
</tr>
<tr>
<td>Other bile acids</td>
<td>8.1 ± 2.0</td>
<td>4.4 ± 0.3</td>
<td>5.3 ± 1.8</td>
</tr>
<tr>
<td>Total bile acids</td>
<td>22.0 ± 4.3</td>
<td>13.2 ± 0.9</td>
<td>10.3 ± 3.8±</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of subjects.
* Mean ± S.E.
* Significantly different from control subjects (p < 0.05)

Table 2

<table>
<thead>
<tr>
<th>Fecal bacterial 7α-dehydroxylase activity in patients with colon cancer</th>
<th>% conversion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer (7)*</td>
<td>63.4 ± 6.2±c</td>
</tr>
<tr>
<td>Control (12)</td>
<td>42.0 ± 2.8</td>
</tr>
</tbody>
</table>

* Activity is expressed as percentage of [14C]cholic acid converted to secondary bile acids when incubated with 0.5 g fecal sample for 2 hr.
* Mean ± S.E.; significantly different from control subjects (p < 0.01).
Diet and Colon Cancer


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