Effects of Bile Acids on Colon Carcinogenesis in Rats Treated with Carcinogens

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Abstract

Primary bile acids were studied as possible colon tumor promoters or inhibitors in a rat model of chemically induced colon cancer. Cholic acid feeding increased the number of animals with tumors, the number of tumors per animal, and the number of tumors per tumor-bearing animal. Tumor enhancement was attributed to deoxycholic acid, the bacterial metabolite of cholic acid. When chenodeoxycholic acid was fed to the rats in our model, tumor incidence was increased, but the number of tumors per animal and the number of tumors per tumor-bearing animal were similar to controls. The different fecal bile acid pattern obtained with chenodeoxycholic acid may be responsible for the differences in tumor incidence.

The methodology to characterize and identify all steroidal components of the feces requires extraction, thin-layer chromatography, gas-liquid chromatography, and gas-liquid chromatography-mass spectrometry. Newer techniques include LH-20 chromatography (for sulfated steroids) and high-pressure liquid chromatography.

Bile Acids—Effects on Colon Cancer

Large-bowel cancer is a major cause of cancer death in the United States. Among the risk factors thought to be associated with this cancer was the high dietary intake of beef and fat (1, 5). The effect of dietary fat on colon carcinogenesis may be related to changes in the composition of fecal bile acids and cholesterol metabolites as well as on the bacterial flora acting on these compounds (2, 9, 11, 13).

Certain animal models have been developed to test the tumor-promoting activity of bile acids in combination with chemical carcinogens (10). It was found that the intrarectal instillation of secondary bile acids such as lithocholic acid and taurodeoxycholic acid acted as tumor promoters in rats treated with the chemical carcinogen N-methyl-N-nitrosourea (9). There was a 2-fold increase in the number of animals with tumors as well as a 3-fold increase in the number of tumors in the bile acid-carcinogen group versus carcinogen alone. The secondary bile acids were also shown to be effective tumor promoters in germ-free rats (9). In addition, the primary bile acids cholic acid and chenodeoxycholic acid were found to increase the number of tumors as well as the number of tumors per animal in rats treated with the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (11).

Recent studies have shown that feeding the primary bile acid, cholic acid, to rats treated with the direct acting carcinogen N-methyl-N-nitrosourea significantly increased the number of animals with tumors as well as the number of tumors per animal compared to carcinogen alone (2). Histological studies revealed that the majority of tumors were adenomas, but several invasive carcinomas were detected. The pathological development of the tumors was through the adenoma-carcinoma sequence. Fecal steroid analysis was carried out on these animals including analysis of neutral sterols, plant sterols, and bile acids. The total fecal neutral sterols (cholesterol and coprostanol) were increased in the animals given carcinogen and bile acid versus those given bile acid alone. Fecal bile acid analysis revealed an increase in total fecal bile acid excretion in the bile acid-fed groups versus those groups without the bile acid supplement. We attributed the increased number of tumors to deoxycholic acid, the major bile acid present. Other bile acids identified in the feces were lithocholic acid, cholic acid, 12-ketolithocholic acid, 3α,7α-dihydroxy-12-keto-5β-cholanic acid, and α-, β-, and ω-muricholic acids. Tumor enhancement was possibly attributed to deoxycholic acid or one of its metabolites (i.e., 12-ketolithocholic acid).

When chenodeoxycholic acid was fed to rats treated with N-methyl-N-nitrosourea, there was a slight increase in the percentage of animals with tumors, but the number of tumors per animal was not altered to any significant extent. The major fecal bile acids were chenodeoxycholic, lithocholic, isolithocholic, and α-, β-, and ω-muricholic acids (13). The secondary bile acids lithocholic acid and deoxycholic acid were increased by only 1 mg/g feces over controls. The levels of α-, β-, and ω-muricholic acids were markedly enhanced. The ability of rats to 6- and 7-hydroxylate bile acids prevented a more dramatic increase in the fecal levels of lithocholic acid. Thus, the fecal bile acid pattern in the chenodeoxycholic acid feeding experiment differed from the pattern in the cholic acid feeding experiment and may be responsible for the reduced tumor incidence.

Further studies to examine the role of the individual bile acids is needed to elucidate their role in large-bowel carcinogenesis.

Methods of Analysis of Fecal Steroids

The methodology used in the fecal steroid workup is outlined in Table 1. This procedure (3, 4), a modification of certain earlier procedures (6, 8), allowed quantitation and identification of fecal neutral sterols, fecal plant sterols, and total fecal bile acids. Identification of the individual bile acid components required a different thin-layer chromatographic procedure from that described in Table 1 (4). This procedure involved separation of the fecal bile acids into various bands containing monohydroxylated, dihydroxylated, and trihydroxylated bile.
Table 1
Steps used in fecal steroid analysis (3, 4)

<table>
<thead>
<tr>
<th>Step</th>
<th>Procedure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Freeze dry and grind fresh fecal sample</td>
<td>Removal of water and preparatory for extraction</td>
</tr>
<tr>
<td>2</td>
<td>Soxhlet extraction with 95% ethanol-0.1% NH₄OH for 48 hr</td>
<td>All steroids removed from fecal sample</td>
</tr>
<tr>
<td>3</td>
<td>Save portion of fecal extract for determination of compounds which may be destroyed by subsequent analysis</td>
<td>Hydrolyze sterol esters</td>
</tr>
<tr>
<td>4</td>
<td>Mild alkaline hydrolysis of extract from Step 2</td>
<td>Remove neutral and plant steroids</td>
</tr>
<tr>
<td>5</td>
<td>Hexane extraction</td>
<td>Identification and quantitation of steroids</td>
</tr>
<tr>
<td>6</td>
<td>TLC, GLC, and GLC-MS of neutral and plant steroids (3)</td>
<td>Prevent formation of artifacts in bile acid workup</td>
</tr>
<tr>
<td>7</td>
<td>Removal of organic solvents after hexane extraction</td>
<td>Deconjugation of bile acids</td>
</tr>
<tr>
<td>8</td>
<td>Vigorous alkaline hydrolysis</td>
<td>Neutralize focal bile acids prior to organic solvent extraction</td>
</tr>
<tr>
<td>9</td>
<td>Acidification</td>
<td>Hydrolysis of bile acid sulfates</td>
</tr>
<tr>
<td>10</td>
<td>Extraction with Folch; store at 0°C for 6—12 hr</td>
<td>Remove all focal bile acids</td>
</tr>
<tr>
<td>11</td>
<td>Extraction using chloroform and combination with Folch extract</td>
<td>Derivative needed for quantitative analysis and identification</td>
</tr>
<tr>
<td>12</td>
<td>Methylation of bile acids (methanol-5% HCl)</td>
<td>Remove hydroxylated fatty acids from fecal bile acids</td>
</tr>
<tr>
<td>13</td>
<td>TLC</td>
<td>Quantitation of total focal bile acids</td>
</tr>
<tr>
<td>14</td>
<td>GLC (TMS ether derivatives on 3% SE-30)</td>
<td>Identification of all focal bile acid components</td>
</tr>
<tr>
<td>15</td>
<td>GLC-MS</td>
<td></td>
</tr>
</tbody>
</table>

References


Note: TLC, thin-layer chromatography; GLC, gas-liquid chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry; TMS, trimethylsilyl.
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