Fecal Bile Acid Excretion in Patients with Colon Cancer, Colon Polyp and Peptic Ulcer

Takashi MAKINO

Department of Internal Medicine, School of Medicine, Tokai University
(Received September 4, 1984)

Fecal bile acids were analyzed by gas chromatography in 10 patients with colon cancer, 25 patients with colon polyp and 10 patients with peptic ulcer. On admission total bile acid excretion in patients with colon cancer was significantly higher than in patients with peptic ulcer (P<0.01). Also, concentration of cholic acid, chenodeoxycholic acid and primary bile acids in patients with colon cancer were significantly higher than those in patients with peptic ulcer (P<0.05, P<0.025) and in patients with colon polyp (P<0.005). Primary bile acids in patients with colon cancer significantly decreased under ward conditions after admission (P<0.05). Total bile acids in patients with colon cancer decreased after admission though there was no significant difference. It is of considerable interest that the fecal bile acid composition of colon cancer patients was different from that of colon polyp and peptic ulcer patients. Fecal bile acids may be involved in the pathogenesis of colon cancer.

(Key Words: Fecal Bile Acids, Colon Cancer, Colon Polyp)

INTRODUCTION

The incidence of colon cancer is high in Northwest Europe, North America, but low in Asia, Africa and South America. This difference in incidence may be due to environmental factors and, in particular, to diet (5). Dietary habits may play a major role in the etiology of colon cancer and since high-risk populations ingest a large amount of animal protein and fat, cholesterol metabolites excreted in their feces may be increased (3).

Feces of healthy individuals in countries with a high incidence of colon cancer were found to have higher bile acid concentrations than feces of individuals living in low incidence countries (12, 15).

It therefore seemed worthwhile to compare the fecal bile acid excretion levels in patients with colon cancer with those in patients with other diseases. Also the correlation between fecal bile acid excretion and diet was studied.

MATERIAL AND METHOD

Patients

10 colon cancer patients aged 31–75 years (mean 59.4 yrs.), 25 colon polyp patients (tubular adenoma) aged 28–74 years (mean 57.3 yrs.) and 10 peptic ulcer patients without liver function disorder aged 35–74 years (mean 51.5 yrs.) were studied.

All patients were on hospital diets (2000–2200 cal/day, proteins 70–75g/day, lipids 50–60g/day, carbohydrates 300–330g/day).

Collection of Samples

Feces were collected under ward conditions on admission, first week and second week after admission. Feces collected were freeze-dried.

Bile Acids Analysis

Bile acids were determined by a differential solvolysis method following the method of Tazume (16, 17) according to Grundy et al. (1). A portion (200mg) of freeze-dried feces was extracted with ethanol for 5 hours at 65°C. The extract was evaporated to dryness. The sample was dissolved with 20ml water and the pH adjusted to pH 1 with 1 N-hydrochloric acid. Bile acids were extracted with 20ml ethyl ether. The ethyl ether layer was then evaporated (Fig. 1).
Directly before gas chromatography, bile acids were methylated with freshly prepared diazomethane and trifluorated with trifluoro acetic acid anhydride. 5β-cholanic acid was added as an internal standard.

Samples were re-dissolved in chloroform and injected for gas chromatography using a Hitachi instrument (type 163) with a QF-1 column. The column was maintained at 225°C during injection of the samples.

Individual free bile acid was analyzed for cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid.

Statistics
Data are given as mean (M) and standard error of the mean (SE). Differences were estimated by Student’s t-test, P value below 0.05 were considered significant.

RESULTS

Fecal bile acid excretion on admission
Fecal bile acid excretion in patients with colon cancer, colon polyp and peptic ulcer are showed in Table 1. Total bile acids are shown in the amount that are summed up cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid.

Total bile acids excretion in patients with colon cancer on admission were significantly higher than in patients with peptic ulcer (P < 0.01). Total bile acids in colon cancer were higher than in colon polyp though there was no significant difference (Fig. 2).

Cholic acid, chenodeoxycholic acid and primary bile acids (CA + CDCA) excretion in patients with colon cancer were significantly higher than in patients with peptic ulcer (P < 0.05, P < 0.025). There were no significant differences in deoxycholic acid, lithocholic acid and secondary bile acids (DCA + LCA) excretion in patients with colon cancer and in patients with peptic ulcer (Fig. 3).

There was no significant difference in primary and secondary bile acid excretion in patients with colon polyp and in patients with peptic ulcer (Fig. 4).

Cholic acid, chenodeoxycholic acid and primary bile acids excretion in patients with colon cancer were significantly higher than in patients with colon polyp (P < 0.005). Deoxycholic acid, lithocholic acid and secondary bile acids excretion in patients with colon polyp were higher than in patients with colon cancer though there was no significant difference between them (Fig. 5).

Change of fecal bile acids excretion
In patients with colon cancer, cholic acid and chenodeoxycholic acid excretion after admission were decreased though there was no significant difference. But deoxycholic acid was significantly increased (P < 0.05) and primary bile acids were significantly decreased (P < 0.05) (Fig. 6).

There was no significant change of bile acids excretion after admission in patients with colon polyp (Fig. 7).

Fecal bile acid concentration in patients with peptic ulcer was not significantly changed after admission. Primary bile acids were decreased after admission though there was no significant difference (Fig. 8).

Total bile acids in patients with colon cancer were decreased after admission though there was no significant difference. Total bile acids in patients with colon polyp and peptic ulcer were slightly decreased (Fig. 9).

DISCUSSION
Hill et al. (2, 4) and Reddy and Wynder (12) showed that metabolism by intestinal microflora in high risk populations for colon cancer, who consume western-style diets with elevated levels of animal protein and fat, was different from that in populations with other dietary habits.

Hill MJ et al. (2) reported that aerobic bacteria were recovered in greater numbers in feces from subjects residing in countries exhibiting a low incidence of colon cancer. They have postulated that bacteria producing carcinogens or co-carcinogens from bile acids might be involved in the etiology of colon cancer, and that might explain the observed correlation between fat intake and incidence of colon cancer, which might be caused by certain anaerobic bacteria, in various countries (4).

Reddy BS (14) indicates that the activity of fecal bacterial 7α-dehydroxylase which converts cholic acid and chenodeoxycholic acid to deoxycholic acid and lithocholic acid respectively, and the activity of fecal cholesterol dehydrogenase which converts cholesterol to coprostanol.
are higher in patients with colon cancer than in controls.

The primary bile acids synthesized by the human liver. Cholic acid and chenodeoxycholic acid are metabolized by the intestinal bacterial flora to deoxycholic acid and lithocholic acid by the actions of fecal bacterial 7α-dehydroxylase and this enzyme is present at higher activity in feces of colon cancer patients than in feces of control persons (7).

In studies of populations with varying incidences of colon cancer the fecal bile acid concentration correlates with incidence of colon cancer (2, 12). A significant increase in the excretion of deoxycholic acid, lithocholic acid, and total bile acids was observed in American subjects and other groups (14). Fecal bile acid concentration in patients with colon cancer was significantly higher than in other patients. The concentration of deoxycholic acid was very significantly higher in patients with colon cancer than in other patients (5). The fecal excretion of total bile acids was higher in patients with colon cancer than in normal controls (5).

The concentration of deoxycholic acid and lithocholic acid were larger in patients with colon cancer and in patients with adenomatous polyps compared with controls (5). The fecal excretion of total bile acids was significantly higher in patients with colon cancer than in patients with adenomatous polyps (5).

Animal model experiments indicate that deoxycholic acid and lithocholic acid act as colon tumor promoters to an established large bowel animal carcinogen (10, 13).

On the other hand, Mudd et al. (8) who compared the fecal bile acids levels of patients with colon cancer and colon adenomas with those of a control group, found no marked difference among the three groups. Murray et al. (9) recorded higher fecal bile acid level in their controls than in colon cancer patients. Kaibara et al. (6) showed that there was no difference in the amount of total bile acids in Japanese with colon cancer and the control group, and that the primary bile acid level, particularly that of chenodeoxycholic acid, was higher in colon cancer patients than in the controls. Perogambros A et al. (11) demonstrated that deoxycholic acid was found in lower concentrations of colon cancer patients in comparison with control subjects. These results are in disagreement with those of Hill (5), Reddy and Wynder (14).

The results obtained here showed that total bile acids excretion in patients with colon cancer was significantly higher than in patients with peptic ulcer, and primary bile acid concentration in patients with colon cancer was significantly higher than that in patients with peptic ulcer.

Therefore these results are in agreement with the results of Hill, Reddy and Wynder in the higher excretion of fecal bile acids with colon cancer patients, but does not agree with the results of Mudd, Murray, Kaibara, and Perogambros. The results are consistent with those of Kaibara's study in that the primary bile acid concentration in colon cancer patients was higher than that in controls.

Vargo D et al. (18) recorded that the cancer group and control group did not differ significantly in either total aerobic or anaerobic bacterial counts, and that the colon cancer group had a significantly lower anaerobic/aerobic ratio compared with the control group. This result is in disagreement with that of Hill's group.

It was also found that the hospital diet altered the composition and amount of fecal bile acids in patients with colon cancer. These results suggest that diet may change the intestinal bacterial flora.

These results may suggest that the concentration of fecal total and primary bile acids in patients with colon cancer may be higher than those in other disease, and that aerobes in fecal bacterial flora of patients with colon cancer may be predominant in comparison with that in other diseases.

Although a specific carcinogen for the colon has not yet been identified in the feces, an association has been established between colon cancer and fecal bile acids excretion (5).

The concentration of fecal bile acids, dietary habit and intestinal bacterial flora may be related in complex manner with carcinogenesis of the colon.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Professor Takeshi Miwa and Dr. Sohtaroh Suzuki (Dept. of Internal Medicine, Tokai University) for their helpful suggestions.
The author also wishes to express his appreciation to Dr. Seiki Tazume (Dept. of Microbiology, Tokai University) for his technical suggestions.

REFERENCES

Table 1  Fecal Bile Acid Excretion in Patients with Peptic Ulcer, Colon Cancer and Colon Polyp on Admission

<table>
<thead>
<tr>
<th></th>
<th>PEPTIC ULcer n = 10</th>
<th>COLON CANcer n = 10</th>
<th>COLON POLYP n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHOLIC ACID</strong></td>
<td>0.38 ± 0.15</td>
<td>3.04 ± 1.03</td>
<td>0.22 ± 0.11</td>
</tr>
<tr>
<td><strong>CHENODEOXYCHOLIC ACID</strong></td>
<td>0.31 ± 0.14</td>
<td>1.68 ± 0.52</td>
<td>0.25 ± 0.07</td>
</tr>
<tr>
<td><strong>DEOXOCHOLIC ACID</strong></td>
<td>0.54 ± 0.15</td>
<td>0.68 ± 0.20</td>
<td>1.33 ± 0.31</td>
</tr>
<tr>
<td><strong>LITHOCHOLIC ACID</strong></td>
<td>1.26 ± 0.40</td>
<td>1.40 ± 0.58</td>
<td>2.20 ± 0.65</td>
</tr>
<tr>
<td>CA + CDCA</td>
<td>0.69 ± 0.27</td>
<td>4.73 ± 1.43</td>
<td>0.48 ± 0.15</td>
</tr>
<tr>
<td>DCA + LCA</td>
<td>1.81 ± 0.44</td>
<td>2.08 ± 0.59</td>
<td>3.53 ± 0.77</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2.50 ± 0.46</td>
<td>6.81 ± 1.18</td>
<td>4.01 ± 0.81</td>
</tr>
</tbody>
</table>

M ± SE
Fecal Bile Acid Excretion in Patients with Colon Cancer, Colon Polyp and Peptic Ulcer—301

Dried feces
- Ethanol 100 ml refluxed for 5 hr

Filtrate
- Ethanol evaporated to dryness and dissolved with 20 ml water

Water layer
- Adjusting pH 1 with 1 N-HCl and extracted with 20 ml ethyl ether x 3

Water layer
- Ethyl ether layer evaporated to dryness

Conjugated bile acids
- Free bile acids

Gas Chromatography

Fig. 1  Procedure for extraction of bile acids from the dried feces.

Fig. 2  Total bile acid excretion in patients with peptic ulcer, colon cancer and colon polyp.
Fig. 3  Fecal bile acid excretion in patients with peptic ulcer and colon cancer on admission.

Fig. 4  Fecal bile acid excretion in patients with peptic ulcer and colon polyp on admission.
Fecal Bile Acid Excretion in Patients with Colon Cancer, Colon Polyp and Peptic Ulcer - 303

**Fig. 5** Fecal bile acid excretion in patients with colon cancer and colon polyp on admission.

**Fig. 6** Change of fecal bile acid excretion in patients with colon cancer.
Fig. 7  Change of fecal bile acid excretion in patients with colon polyp.

Fig. 8  Change of fecal bile acid excretion in patients with peptic ulcer.
Fig. 9 Change of total bile acid excretion in patients with peptic ulcer, colon cancer and colon polyp.