Distribution of Tissue Bile Acids in the Human Alimentary Tract and Colon Polyps

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To determine the characteristic distribution of tissue-bound bile acids in the human alimentary tract and colon polyps, we measured the concentration of bile acids in the mucosal tissues of the alimentary tract obtained at autopsy and polyps obtained by endoscopic polypectomy, using enzymatic fluorimetry and gas-liquid chromatography. The concentration of tissue-bound bile acid, especially chenodeoxycholic acid, was significantly higher in the ileum or ascending colon than in the other portions of the alimentary tract. The bile acid level of polyps was also higher in the ascending colon than in the other portions of the colon. These results suggest that the high concentration of tissue-bound bile acids is obtained at the site of absorption of bile acids in the alimentary tract.

Key words: Tissue-bound bile acid, Autopsy, Alimentary tract, Endoscopic polypectomy

As bile acids have a so-called detergent effect, it is assumed that they are able to injure the gastrointestinal organs which are closely associated with the enterohepatic circulation of bile acids. Several studies (1-4) have revealed the implication of bile acids in digestive diseases such as esophagitis, gastritis, gastric ulcer, colon polyps and colon cancer. In these studies, the bile acids in the intestinal contents, especially gastric juice and feces, were analyzed. However, on the assumption that there is considerable difference in the strength of each bile acid for binding to the tissue or the affinity of bile acids to each portion of the alimentary tract, the tissue-bound bile acids should be analyzed quantitatively before the pathogenic relationships between bile acids and alimentary tract diseases can be discussed. In this study, to determine the distribution of bile acids in the human alimentary tract, we analyzed the tissue-bound bile acids in the samples of alimentary tract obtained at autopsy and adenomatous polyps endoscopically removed by snare cautery.

MATERIALS AND METHODS

PATIENTS AND MATERIALS

Specimens of the alimentary tract, from the cardia of the stomach to the sigmoid colon, were obtained from six patients at autopsy: patient YK, a 73-year-old male, died of perforation of a gastric ulcer; patient TI, a 45-year-old male, of bleeding from a gastric ulcer; patient OS, a 48-year-old male, in a traffic accident; patient MN, a 61-year-old female, of pneumonia; patient NT, a 50-year-old male, of hepatocellular carcinoma and patient IT, a 82-year-old female, of pancreatic carcinoma. Three patients (YK, TI and OS) had been healthy and had no medication which influences bile acid metabolism, because they died suddenly of circulatory insufficiency or mortal wounds. Patient MN was treated with antibiotics for about three

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weeks. Patients NT and IT were administered various anti-cancer drugs and treated by intravenous hyperalimentation for about three months, but they had no bile duct obstruction that interrupts the enterohepatic circulation of bile acids. All patients were autopsied within four hours after death and the specimens were stored at \(-20^\circ\text{C}\) until use.

Specimens of colon polyps with a diameter from 6 to 15 mm were obtained from 20 patients (seven females and 13 males, 46 - 73 years old) by endoscopic polypectomy. Histological examination of the polyps revealed tubular adenoma with no malignancy.

**BILE ACID ANALYSIS**

After the autopsy specimens were warmed in water, the mucous membranes (inner walls) of the stomach, duodenum and intestines were scratched off with a razor blade. Two samples were obtained separately from each portion of the alimentary tract. The samples from the alimentary tract and polyps were washed three times with saline to remove contamination. After being dried under hot air, the tissues were weighed. The alimentary tract samples weighed 70 - 191 mg (mean 142 mg) and the polyp samples, 6 - 20 mg (mean 11 mg). The dried tissue was dissolved completely in 1 ml of a 5% aqueous solution of sodium hydroxide at 80\(^\circ\text{C}\) (5) and diluted with 9 ml of distilled water. The solution obtained was applied to a Bond Elut C\(_{18}\) column. After the column was washed with water, the bile acids were eluted from the resin with 90% ethanol. The total bile acid level of the eluate was determined by enzymatic fluorimetry with 3\(\alpha\)-hydroxysteroid dehydrogenase (Neo-sterognost 3\(\alpha\)-kit, Nyegaard & Co. A/S, Oslo, Norway). To determine the bile acid composition, the bile acid conjugates were further hydrolyzed with cholylglycine hydrolase (Sigma Chemical Co., St. Louis, Mo, USA) (6). The hydrated mixture adjusted to pH 1.0 was extracted with ethyl ether. The bile acids obtained were methylated with freshly prepared diazomethane and acetylated by heating with acetic anhydride at 140\(^\circ\text{C}\) for 4 hrs (7). The sample was dissolved with 0.1 ml of acetone containing 2 \(\mu\)g of cholesteryl caproate as an internal standard and subjected to gas-liquid chromatography (GLC) using a 1.5% silicone AN-600 column (8, 9). The peak area ratio (the peak area of each bile acid vs. that of the internal standard) on the chromatogram was used for quantitation of each bile acid.

In order to determine the reproducibility of the results, authentic bile acid mixtures which consisted of 1 mg of deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA) and cholic acid (CA) were added to 100 mg of colon tissue and their recovery rates were determined by GLC.

In two cases (YK, IT), to determine the bile acid composition of gallbladder bile, the bile (0.5 ml) was diluted in 4.5 ml of physiological saline (pH 11.0) and the solution was applied to a Bond Elut C\(_{18}\) column as described above.

**RESULTS**

**EVALUATION OF THE ANALYTICAL PROCEDURE**

The overall recoveries of authentic DCA, CDCA, UDCA and CA added to colon tissue were approximately 98\%, 85\%, 94\% and 95\%, respectively.

Furthermore, to determine whether the present procedure can exclude intraluminal bile acids from the alimentary tract tissues, the bile acid pattern of the duodenal tissue was compared with that of gallbladder bile in two cases (YK, IT) by using the present analytical procedure. The profile of bile acids of the duodenal tissue was found to be quite different from that of bile in each case; DCA, CDCA, UDCA and CA accounted for 37\%, 47\%, 8\% and 8\% (mean value obtained from the two patients) of the total bile acid level in the duodenum, and 18\%, 42\%, 5\% and 35\% in bile, respectively.

**TISSUE BILE ACIDS OF THE ALIMENTARY TRACT**

The total bile acid concentration in each portion of the alimentary tract is shown in Fig. 1. The total bile acid level ranged from 0.02 to 0.87 nmol/mg dry weight. Although there was considerable variation in the levels among the individual cases, the ileum showed the highest level of bile acids in four of the six cases. The mean \(\pm\) S.E. (number) values of total bile acid level (nmol/mg dry weight) in the alimentary tract tissues were as follows: cardia, 0.17 \(\pm\) 0.06 (4); antrum, 0.11 \(\pm\) 0.04 (4); duodenum, 0.19 \(\pm\) 0.05 (5); jejunum, 0.20 \(\pm\) 0.06 (3); ileum,
Fig. 1. Distribution of tissue-bound bile acids in the human alimentary tract obtained at autopsy.

Each point represents the mean value obtained from two separate materials in each portion of alimentary tract of individual case. The details of presented cases were shown in the text.

0.43 ± 0.11 (6); caecum, 0.26 (2); ascending colon, 0.29 ± 0.07 (3); transverse colon, 0.26 ± 0.07 (4); descending colon, 0.17 ± 0.03 (4) and sigmoid colon, 0.24 ± 0.08 (3). The level was significantly higher in the ileum than in the cardia or the antrum (P < 0.02).

In addition, the bile acid composition in several different portions of the alimentary tract was determined in four cases. As shown in Fig. 2, CDCA accounted for the greater part of the tissue bile acids in the ileum or caecum in all cases.

TISSUE BILE ACIDS OF COLON POLYPs

The total bile acid levels of 20 colon polyps including five of the ascending colon, four of the transverse colon, four of the descending colon and seven of the sigmoid colon were measured (Fig. 3). The bile acid level of the polyps was significantly higher in the ascending colon than in the sigmoid colon (P < 0.02). In each portion of the colon, the bile acid level of the polyps was almost equal to that of colon tissues obtained at autopsy as shown in Fig. 1.

DISCUSSION

In analyzing tissue-bound bile acids, it must be taken into consideration that the sample has been contaminated by biliary bile or intraluminal contents. At the beginning of this study, therefore, we determined whether the contamination was present in the pretreated sample. Considering the difference of the bile acid pattern of the duodenum from that of the gallbladder bile in two cases, the present clean-up procedure appeared to be able to exclude the majority of water-soluble bile acids from the tissue. Furthermore, the good recoveries of the authentic bile acids added to the tissue revealed that the present GLC method is applicable to the quantitative assay of tissue bile acids of the alimentary tract.

Because it is considered that some preparative procedures, e.g. using a laxative or an enema, wash
away the intraluminal bile acids, and that treatment with antibiotics prevents the formation of secondary bile acids, the management or medication before the patient's death might affect the concentration and composition of the tissue-bound bile acids. In the present study, although the levels of tissue-bound bile acids of the alimentary tract varied considerably among different cases, tissue-bound bile acid concentration was characteristically highest in the ileum or ascending colon in all cases. Of the individual bile acids, CDCA was at the highest level in the ileum in four cases. There was no definite tendency in the distribution of the other bile acids. These results show clearly that the characteristic distribution of alimentary tract-bound bile acids is not influenced by a difference in the underlying diseases or treatments before the death. Therefore, the present findings may be also applicable to alimentary tract of healthy humans. The exact reason why tissue-bound bile acids, especially CDCA, showed the highest level in the ileum is still unclear. However, considering the facts that the ileum is the major site of absorption of bile acids and that a dihydroxy bile acid such as CDCA is less water-soluble than a trihydroxy bile acid such as CA, CDCA may bind to the tissues and remain in the epithelial cells for a long period.

Recent studies (10, 11) indicated an increased risk of developing right-sided colonic cancer and adenomatous polyps after cholecystectomy in women. Vernick et al. (12) state that the right side of the colon is most likely to be affected by the potential carcinogenic effects of bile acids. The present data suggest that the high concentration of the tissue-bound bile acids may be one of the predisposing factors in the development of cancers and adenomas in the right colon. In animal experiments, DCA induced activity of colonic epithelial ornithine decarboxylase, a marker of promoter, more strongly than primary bile acids such as CA and CDCA (13). The present results, however, suggest the importance of tissue-bound CDCA in the pathogenesis of human colonic diseases.

As an adenomatous polyp is generally thought to be a precursor of colon cancer (14), we further determined the bile acid level of polyp tissues obtained by colonoscopic polypectomy. Although the bile acid level of polyps in the ascending colon was significantly higher than in the sigmoid colon, the present data did not indicate a higher concentration of bile acids in polyps than in colon tissues. Previously, no significant difference in tissue bile acids was found in colorectal carcinomas, polyps or adjacent normal tissues (15). Specific receptors to DCA were, however, reported to be detected in approximately 30 percent of colorectal cancers (16). These bile acid receptors are likely to be involved only in the late stage of carcinogenesis, because they were not detected in normal colorectal mucosa. At this time, although the role of tissue-bound bile acids in the growth of colorectal cancers and polyps is controversial, we conclude that the high level of bile acids in colon tissues may be one of the predisposing factors for carcinogenesis in the ascending colon.

REFERENCES

Bile Acids of Alimentary Tracts and Polyps


