Bile Acids in Patients Suffering from Colorectal Carcinoma—A Pilot Study

Winfried J. O. KURTZ and Ulrich LEUSCHNER

Department of Gastroenterology (Director: Prof. Dr. med. M Classen),
Center of Internal Medicine, University Clinics, Johann Wolfgang Goethe-University, Frankfurt/Main, West Germany
(Received January 7, 1983)

Bile acids are increasingly discussed in the genesis of colorectal carcinoma. In a pilot study we analysed bile acids in tumor tissue, tumor-free mucosa, serum, and feces of 6 patients with rectal and 3 patients with colonic carcinoma. 5 patients died from nongastroenterological diseases and two operated on for benign colonic stenosis served as controls for colorectal mucosa, 16 healthy persons as controls for feces and serum. Bile acids and their sulphates were determined after differential solvolysis by gas-liquid chromatography on QF-1 columns. Serum lithocholic acid was significantly (p<0.05) elevated in all carcinoma patients. The feces showed a trend towards increased secondary bile acids. Tumor-free mucosa of patients with rectal carcinoma showed elevated unsulphated bile acids, sulphated lithocholic acid (p<0.05) and sulphated chenodeoxycholic acid (p<0.025) were significantly decreased. In rectal carcinoma tissue total bile acids were elevated, cholic acid (p<0.01) and sulphated lithocholic acid decreased (p<0.05). In nonaffected colonic mucosa of patients with colonic carcinoma total bile acids were decreased, in colonic carcinoma tissue only traces of bile acids were found. Whether these changes are causes or rather consequences of colorectal tumors—e.g., tumor-induced stasis in the gut—and whether they may be useful for diagnosis remains to be elucidated in further studies.

(Key Words: Bile Acids, Intestinal Mucosa, Colorectal Carcinogenesis)

INTRODUCTION

Bile acids are increasingly discussed in the pathogenesis of colorectal carcinoma (17, 28, 35, 37, 42). Precarcinogenic, cocarcinogenic and carcinogetic mechanisms were suggested an in part proven in laboratory animals (3, 4, 31-34, 39). In investigations in humans elevated fecal bile acid concentrations in colorectal carcinoma patients often were regarded as a sign of their carcinogenicity (10, 11, 14, 15). Many results are contradictory, the mechanisms unknown (6, 8, 17, 38). Nigro and coworkers (27) found elevated bile acid concentrations in colonic mucosa during azoxymethane induction of colonic carcinoma in rats. Data on bile acids in human intestinal mucosa are lacking. We therefore conducted this pilot study analysing bile acid concentrations in tumor tissue and nonaffected intestinal mucosa of patients with colorectal carcinoma and compared them to bile acids in serum and feces.

MATERIALS AND METHODS

Patients

9 carcinoma patients aged 55—71 years were studied, 6 with rectal

Priv. Doz. Dr. med. W. KURTZ: Abt. Gastroenterologie, Zentrum der Inneren Medizin, Universitätsklinikum, Theodor Stern Kai 7, D-6000 Frankfurt/Main, West Germany
This study was supported by a grant from the Harry and Peter Fuld Foundation, Frankfurt/Main

59
and three with left colonic adenocarcinoma. Liver metastases and underlying liver disease were excluded by conventional methods. Two patients operated on for benign sigmoid stenosis (50 yrs.) and diverticulitis (47 yrs.), and five patients dead from myocardial infarction and insufficiency aged 65—70 years served as controls for colonic mucosa, 16 clinically healthy subjects as controls for serum and feces (aged 30—60 years).

Collection of Samples
Serum was drawn in the morning after an overnight fast (ca. 5ml). Feces were collected under ward conditions shortly before operation. Small portions of tumor tissue (ca. 2gm) and adjoining nonaffected mucosa were won at surgery or, in the case of 5 controls, during autopsy shortly after death.

Bile Acid Analysis
Non-sulphated and sulphated bile acids were determined by a differential solvolysis method (18) following in part the method of Grundy (9) and in part that of van Berge-Henegouwen (2). Bile acids were extracted with ethanol and washed with n-heptane/diethyl ether/water (1:1:1, v/v). After mild alkaline hydrolysis which does not destroy sulphate esters of bile acids (2) samples were halved. For determination of non-sulphated bile acids, one half was taken up in ethyl acetate, washed with water to pH 6, taken up in 70% ethanol after evaporation, then washed with petrol ether. For additional determination of bile acid sulphate esters the second half of the sample was taken up in acidified ether for solvolysis according to van Berge-Henegouwen (2), further steps followed the above protocol.

Directly before gas chromatography bile acids were methylated with freshly prepared diazomethane and trifluorinated with trifluoro acetic acid anhydride. In control experiments, bile acids were extracted with a modification of Makino's method (22).

Bile acids were analysed on a Packard gas chromatograph, series 7500-7800, with flame ionisation detector, column length 180cm, stationary phase 3% QF-1 on gaschrome Q, column temperature 237°C isothermal, carrier N2. For quantification, internal standards of lithocholic, deoxycholic, hyodeoxycholic, and 7-keto-deoxycholic acids were used in several combinations. Purity of standard bile acids (over 98%) was controlled gas chromatographically before use. For recovery experiments, bile acid sulphate esters were synthesized according to Tserng (36), they yielded single spots on thin layer chromatography. Recoveries of non-sulphated bile acids ranged from 81% to 95%, of bile acid sulphate esters from 69% to 82%.

Statistics
Data are given as mean (x) and standard error of the mean (SEM). Differences were estimated by Student's t-test, p values below 0.05 were considered significant.
RESULTS

In tumor patients, serum nonsulphated bile acids were higher than in controls (Fig. 1). Among single bile acids, the difference was significant for lithocholic acid ($p<0.05$). Deoxycholic and cholic acids and some further, nonidentified bile acids were elevated, too. There were no significant differences between rectal and colonic carcinoma patients. Serum bile acid sulphate esters (Fig. 2) were similar in all groups, highest single sulphated bile acid was sulpholithocholate.

Fecal nonsulphated bile acid concentrations (Fig. 3) were higher in tumor patients than in controls. There were no significant differences between rectal and colonic carcinoma patients. Main bile acids were lithocholic and deoxycholic acids. Interestingly, cholic acid, too, was higher than in controls; this difference was statistically significant ($p<0.05$). Among bile acid sulphate esters, in controls only sulphated deoxycholic (3.7 ± 2.6 µg/g) and cholic (0.03 ± 0.02 µg/g) acids could be identified. In tumor patients, sulphate esters of lithocholic (0.5 ± 0.4 µg/g), chenodeoxycholic (0.5 ± 0.4 µg/g), and ursodeoxycholic acids (1.3 ± 0.6 µg/g) were found apart from esters of deoxycholic (0.7 ± 0.6 µg/g) and cholic (2.7 ± 2.0 µg/g) acids. In nonaffected colonic mucosa of colon carcinoma patients (Fig. 4), concentrations of nonsulphated bile acids were low in comparison to controls, main bile acid was deoxycholic acid. Some bile acid sulphate esters were detected (sulpholithocholic acid: 1.0 ±

![Fig. 1](image)

Serum NSBA in normal subjects (N) and in colorectal carcinoma patients (C).

ABBREVIATIONS:
NSBA nonsulphated bile acids; SBA sulphated bile acids; LC lithocholic acid; DC deoxycholic acid; CDC chenodeoxycholic acid; UDC ursodeoxycholic acid; C cholic acid; R rest (= unidentified bile acids); TBA total bile acids.
0.46μg/g, sulphodeoxycholic acid: \(3.1 \pm 2.5 \mu g/g\), whereas in the colonic mucosa of controls only traces of sulphated bile acids could be found. In colonic carcinoma tissue no bile acids were detected. In nonaffected rectal mucosa of rectal carcinoma patients (Fig. 5) nonsulphated secondary bile acids —lithocholic and deoxycholic acids— were higher, primary bile acid—cholic and chenodeoxycholic acids—lower than in controls. Sulphate esters of lithocholic and chenodeoxycholic acids (Fig. 6) showed significantly lower values \((p<0.05)\). Rectal carcinoma tissue yielded similar findings: Increased secondary and low primary bile acids (Fig. 7). Bile acid sulphate esters were much lower than in controls, only sulphated lithocholic and deoxycholic acids were detectable (Fig. 8).

**Fig. 2** Serum SBA in normal subjects (N) and in colorectal carcinoma patients (C).

**Fig. 3** Fecal NSBA in normal subjects (N) and in rectal carcinoma patients (C).
Fig. 4 NSBA in colonic mucosa of normal subjects (N) and in non-affected colonic mucosa of colonic carcinoma patients (C).

Fig. 5 NSBA in rectal mucosa of normal subjects (N) and in nonaffected rectal mucosa of rectal carcinoma patients (C).
Fig. 6  SBA in rectal mucosa of normal subjects (N) and in non-affected rectal mucosa of rectal carcinoma patients (C).

Fig. 7  NSBA in rectal mucosa of normal subjects (N) and in rectal carcinoma tissue (C).
**DISCUSSION**

According to current theories (1, 7, 10–13, 17, 23, 25, 26, 31, 32, 37, 41, 42) bile acids are involved in the process of colorectal carcinogenesis. Our data in this field, however, are so far either based on epidemiological studies pointing to a larger intestinal bile acid load in colorectal carcinoma patients (10–14, 16, 24, 25, 29) or on animal experiments with combinations of known carcinogens and bile acids (21, 31–34, 38, 40). The limitations of either method are easily understandable. On the one hand, statistical correlations in epidemiological studies are hardly ever fit as proofs (5), on the other hand results in animal experiments largely depend on the choice of the main carcinogen and the bile acids and their conjugation form—there are even bile acids that prevented carcinomas in some sets of experiments (6, 38).

There are no data on what is actually happening in the human colonic and rectal mucosa during carcinogenesis. Therefore, the purpose of our pilot study was to find out hints whether there are differences in the mucosal bile acid load and pattern in colorectal carcinoma patients in comparison to controls. Strikingly enough, a number of our results fit well with results from studies correlating fecal bile acid excretion and colorectal carcinogenesis (10, 11, 14, 24, 25, 29, 30). In the serum of all tumor patients nonsulphated bile acids—which are supposedly more toxic
than bile acid sulphate esters—were elevated. Among single bile acids this difference was significant for lithochoinic acid which has been incriminated of particular toxicity and (co)carcinogenicity. For sulphated bile acids no difference was found.

Fecal nonsulphated bile acids, too, were higher in tumor patients than in controls, as could have been expected from earlier studies (10, 11, 14, 24, 25, 29, 30). Main bile acids were the two secondary bile acids lithochoinic and deoxychoinic acids which are thought to be cocarcinogenic. But, interestingly enough, choic acid, which is supposed to be relatively non-toxic, was elevated, too, and so were non-toxic sulphate esters. Some results indicate, however, that there is no strict correlation between toxicity and potential cocarcinogenicity in bile acids—Reddy et al. (31) showed that not only deoxychoinic and chenodeoxychoinic, but also cholic acid enhanced colonic carcinogenesis in germ free rats.

The results in nonaffected colonic mucosa of colonic carcinoma patients, however, are puzzling. Here, nonsulphated bile acid concentrations were much lower than in controls, with somewhat elevated bile acid sulphate esters. And in colonic carcinoma tissue, moreover, only traces of bile acids could be detected. The number of colonic carcinomas investigated in this study is very limited, so the data might change with larger collectives of patients. But preliminary data from our second study (19) confirm this trend towards lower bile acid concentrations in the colonic mucosa of colon carcinoma patients. This could be interpreted as pointing against bile acid involvement in colonic carcinogenesis. It must be noted, however, that in these patients, too, fecal bile acid concentrations were elevated.

It has to be mentioned in this context that the choice of controls poised a major problem. As surgical specimens of only two cases—with benign sigma stenos and with diverticulitis—were available to us, we had to resort to autopsy preparations. During the first few hours after death, total bile acid concentrations as well as patterns of primary and secondary bile acids do not change significantly in rats, as has been shown by as yet unpublished data from our laboratory (20). So, although quite aware of the limitation, we felt entitled to use autopsy specimens.

In contrast to colonic carcinoma patients, our findings in rectal carcinoma patients appear to conform quite well with present theories: Increased concentrations of the secondary bile acids lithochoinic and deoxychoinic acid in both nonaffected mucosa and tumor tissue and decreased bile acid sulphate esters may underline current bile acid carcinogenicity theories. These data possibly hint to a different mechanism for colonic and rectal carcinogenesis. Low concentrations of bile acids in the large intestinal mucosa of colonic carcinoma patients might point to less importance of bile acids for these tumors, while in the rectal mucosa of tumor patients high bile acid levels suggest some causative connection.

At the moment, however, it is very difficult to decide whether our findings are causes or effects of colorectal tumors. Carcinomas develop in the gut during decades, what we finally analyse is only a very late stage. What happens during these many years of tumor induction is as yet
Bile Acids in Colorectal Carcinoma Patients—67

unknown.

Some of the bile acid alterations we found may be consequences of the tumor affection. With a carcinoma producing a certain degree of stasis in the gut one might expect increased bacterial bile acid degradation. This could explain elevated secondary bile acid concentrations in rectal carcinoma patients. According to Hill (12), however, moderate changes in large bowel transit time do not have a great impact on bacterial bile acid degradation. And one would not expect an increase in cholic acid—as we found it—nor in bile acid sulphate esters under these conditions.

Then there is the question of the amount of unidentified bile acids we found in all compartments analysed. We cannot exclude that some of these might be carcinogens or promoters, but it must be underlined that none of the carcinogens or precancerous Wieland and others (3, 4, 39) synthesized from bile acids were ever identified in biological samples.

Although it may be too early to draw clear-cut consequences, our pilot study analysing for the first time bile acids in nonaffected large intestinal mucosa and tumor tissue of patients with colorectal carcinoma showed interesting trends. Contrasting bile acid levels in colonic and rectal carcinoma may point to different mechanisms of carcinogenesis in these two locations, whereas fecal bile acids were increased in both groups. Changes in serum bile acids might, if they are confirmed to be consistent, offer new diagnostic possibilities. We, therefore, feel encouraged to continue with our main study on bile acids in colorectal carcinoma patients.

REFERENCES
20) Leinweber W, Fesel A: (Unpublished results)
Bile Acids in Colorectal Carcinoma Patients—69


