Antral Mucosal Bile Acids in Two Types of Chronic Atrophic Gastritis

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Bile acids may damage the gastric mucosa, and they are cocarcinogenic in experimental colonic and gastric cancer. Chronic atrophic gastritis (CAG) and chronic atrophic gastritis with intestinal metaplasia (CAGIM) are associated with gastric carcinoma. We, therefore, analysed bile acids in the antral mucosa in controls (n = 10), in patients with CAG (n = 12) and CAGIM (n = 20). In both forms of chronic antral gastritis, total mucosal bile acid concentrations drop, caused mainly by lower primary bile acids. The proportions of secondary bile acids rise, in particular of toxic lithocholic acid. This is probably caused by bacterial activity in the stomach. Whether secondary bile acids, especially lithocholic acid, alone or in combination with other bacterial degradation products, influence gastric carcinogenesis remains to be elucidated in further studies.

(Key Words: Bile acids, Antral mucosa, Chronic atrophic gastritis, Gastric carcinogenesis)

INTRODUCTION

Chronic atrophic gastritis and chronic atrophic gastritis with intestinal metaplasia have repeatedly been considered as precursors of gastric carcinoma (2,3,17,26,34,35). In particular, the intestinal type of gastric carcinoma has been correlated with chronic atrophic gastritis with intestinal metaplasia (3,15,33,34,35). Antral chronic atrophic gastritis is considered to be caused by duodenogastric reflux of bile acids and lysolecithin (21,29). Most gastric carcinomas are localized in the antrum (11,18,39). Experimentally, bile acids are (co)carcinogenic not only in the colon, but also in the stomach (22-24,28,29). Duodenogastric reflux after partial gastric resections is considered a causative factor in gastric stump carcinoma (4,24,33). (Co)carcinogenic properties have in particular been described for the secondary bile acids lithocholic and deoxycholic acid, but also for other bile acids (19,28).

Little is known on bile acid concentrations at the actual site of carcinogenesis, the gastric mucosa. We, therefore, determined bile acids with a newly developed microanalytical method in the antral mucosa of controls and in chronic atrophic antral gastritis without and with intestinal metaplasia.

MATERIAL AND METHODS

Patients. Biopsies.

Antral mucosal biopsies were taken endoscopically in 10 persons with histologically normal antral mucosa, in 12 patients with chronic atrophic gastritis, and in 20 patients with chronic atrophic gastritis with intestinal metaplasia (cf. Table 1). Two biopsies were taken endoscopically from the gastric antrum at the greater curvature about 5 cm from the pylorus. One biopsy was taken up in formaldehyde for histology, one biopsy was taken up in saline, then dried from adhering moisture on filter paper, weighed and stored at −22°C until bile acid analysis.

Bile Acid Analysis

After tissue homogenisation, bile acids were
extracted with boiling ethanol and washed with water/n-heptane/diethyl ether (cf. Fig. 1). Nonsulphated and sulphated bile acids were separated on a Sephadex LH20 column according to Makino (25). After acid solvolysis of sulphate esters and alkaline hydrolysis, the fractions were washed with petrol ether and dried.

Directly before gas-liquid chromatography, bile acids were methylated with freshly prepared diazo methane and trimethylsilyl ethers were formed with hexamethyl disalazane/trimethyl chlorosilane (3 : 2, v/v) in dry pyridine. Glass-capillary gas-liquid chromatography was carried out on a Carlo Erba LT 430 chromatograph with a 22m OV-1 column, internal diameter 0.28-0.35 mm, with on-column injection and flame-ionization detection. The temperature program was: injection temperature 110°C, then ballistically till 180°C and at 2.5°C/min. till 280°C. The carrier gas was hydrogen, pressure 0.6 bar. A murine bile acid (β-muricholic acid) was used as internal standard. Recoveries ranged from 79.7-87.2% for nonsulphated bile acids and from 80.7-81.0% for bile acid sulphate esters which had been synthesized according to Tserng et al.(37) and were 95-98% pure by thin layer chromatography.

Statistics
Data are given as mean ± SEM. For statistical analysis, the Wilcoxon test(31) was performed, p-values below 0.05 were regarded as significant.

Abbreviations:
CAG = chronic atrophic gastritis
CAGIM = chronic atrophic gastritis with intestinal metaplasia
LCA = lithocholic acid
DCA = deoxycholic acid
CDCA = chenodeoxycholic acid
CA = cholic acid
HDCA = hyodeoxycholic acid
UDCA = ursodeoxycholic acid
HCA = hyocholic acid

RESULTS
The results are given in Table 2 and Fig. 2. Below, only significant differences are mentioned.

Absolute Concentrations of Mucosal Bile Acids
In chronic atrophic antral gastritis (CAG) as well as in chronic atrophic gastritis with intestinal metaplasia (CAGIM), total mucosal concentrations of nonsulphated and sulphated bile acids are lower than in controls. In CAG, among nonsulphated bile acids, DCA, CDCA, CA, and HCA are reduced, only UDCA is elevated in comparison to controls. Among bile acid sulphate esters, reductions in DCA, CDCA, CA, and HCA are significant.

In CAGIM, mucosal concentrations of nonsulphated LCA, DCA, CDCA, CA, and HCA as well as concentrations of sulphated DCA, CDCA, CA, and HCA are reduced, only nonsulphated and sulphated UDCA is higher than in controls. In CAGIM, mucosal nonsulphated DCA, and sulphated DCA and UDCA, and total bile acids sulphate esters are higher than in CAG.

Relative Proportions of Mucosal Bile Acids
In CAG, relative proportions of nonsulphated LCA and UDCA and of sulphated LCA are higher than in controls, percentages of nonsulphated and sulphated DCA, CDCA, and HCA are lower.

In CAGIM, relative proportions of nonsulphated UDCA and sulphated LCA and UDCA are higher than in controls, percentages of nonsulphated CDCA, and sulphated CDCA and CA are lower.

In CAGIM, percentages of nonsulphated and sulphated LCA are lower, of nonsulphated DCA and sulphated DCA and UDCA are higher than in CAG.
BIOPSY SPECIMEN
HOMOGENISATION
EXTRACTION WITH BOILING ETHANOL
WASHING WITH WATER/N-HEPTANE/DIETHYL ETHER
SEPHADEX LH20 COLUMN
NONSULPHATED BILE ACIDS
SULPHATED BILE ACIDS
SOLVOLYSIS
ALKALINE HYDROLYSIS
WASHING WITH PETROL ETHER
METHYLATION
TRIMETHYLSILYLATION
GAS-LIQUID CHROMATOGRAPHY

Fig. 1  Bile acid analysis—Prechromatographic procedure

Table 2  Concentrations of nonsulphated (nsBA) and sulphated bile acids (sBA) in the antral mucosa of controls and patients with CAG and CAGIM (μg/g wet weight). Mean, SEM.

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<td>HDCA</td>
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<td>HCA</td>
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<td>–</td>
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<td>–</td>
<td>4.87†</td>
<td>2.79</td>
<td>10.10†</td>
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* significant in comparison to controls
† significant in comparison to CAG
DISCUSSION

In both types of chronic atrophic gastritis the mucosal concentrations of all single bile acids with the exception of UDCA are lower than in controls. In particular, the decrease in both nonsulphated and sulphated CA, CDCA, and DCA causes the drop in total bile acids. The concentration of LCA is less reduced and thus shows a relative increase in CAG and, less pronouncedly so, in CAGIM. The bile acid concentration still is about 10 times higher than in the serum indicating that the bile acids reached the mucosa through duodenogastric reflux (5).

Thomas et al (36) demonstrated a positive correlation between the degree of reflux and the damage of gastric mucosa with a higher reflux rate in chronic gastritis.

The lower mucosal bile acid concentrations in our patients with chronic atrophic gastritis may be explained by the dissociation properties of the bile acids and the pH in the stomach. Because of their relatively high pKa-values bile acids are protonated in case of normal gastric acid production and low gastric pH. Wherever there is no active transport system for bile acids, bile acid absorption takes place mainly in the form of passive nonionic diffusion, i.e. protonated bile acids are absorbed (6,7,38). Bile acid absorption of the gastric mucosa depends from the intragastric hydrochloric acid concentration (10,30,32).

When hydrochloric acid production is substantially reduced due to mucosal atrophy in chronic gastritis, a higher pH causes the bile acids to remain partly dissociated in the

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Fig. 2 Relative proportions of nonsulphated bile acids (open bars) and sulphated bile acids (hatched bars) in the antral mucosa of controls and patients with CAG, and CAGIM. Mean, SEM.

* significant in comparison to controls
+ significant in comparison to CAG
stomach lumen and be less absorbed. Thus, lower mucosal bile acid concentrations in CAG and CAGIM are probably not caused by reduced reflux or reduced amounts of bile acids in the stomach, but by reduced bile acid absorption caused by a higher gastric pH.

In superficial antral gastritis, on the other hand, with hydrochloric acid secretion still intact and gastric pH low, we have found increased mucosal bile acid concentrations(20). Evidently, low total mucosal bile acid concentrations are only found in a late stage in the development of chronic gastritis.

There is, however, a striking difference in the bile acid pattern between controls and both forms of chronic gastritis. The marked decrease of primary bile acids and the increase in the proportion of the secondary bile acid lithocholic acid suggests bacterial activity.

Reduction of gastric acid production in chronic atrophic gastritis predisposes to an overgrowth of metabolically active bacteria in the stomach(8,9,12,16,27). These bacteria are similar to the colonic flora and they are capable of dehydroxylating bile acids. Thus, cholic acid is transformed to deoxycholic acid, and chenodeoxycholic acid to lithocholic acid(8). This fits well with our results for lithocholic acid: while the percentage of lithocholic acid rises, the proportion of chenodeoxycholic acid drops. The primary bile acid cholic acid, too, is reduced in both CAG and CAGIM, its metabolic product, deoxycholic acid, however, is not elevated. This may in part be due to the lower passive permeability constant of deoxycholic acid in comparison to lithocholic acid. But in comparison to CAG, mucosal deoxycholic acid concentrations are higher in CAGIM.

The absolute concentrations of sulphate esters of lithocholic acid are unchanged in both forms of chronic atrophic gastritis, the percentages rise. Sulphoglucolithocholic acid has been shown to be hepatotoxic in rats(14), but in this study we did not differentiate between the two amidation forms of bile acids, so the meaning of this finding remains to be assessed. The sulphate esters of most other bile acids are low in CAG and CAGIM with the exception of sulphated UDCA in CAGIM.

Fisher et al(14) and Yamanaka et al(40) have demonstrated that the hepatotoxicity of bile acids does not only depend on their absolute concentrations, but also from their relative proportions in comparison to other bile acids. Higher proportions of cholic acid may e.g. protect from the toxic effects of lithocholic acid(14). In health, higher concentrations of primary bile acids may be a protective factor against the detrimental effects of secondary bile acids. Whether the (co)carcinogenicity of lithocholic acid is increased in the absence of other bile acids is still an open question.

An interesting finding is the rise in ursodeoxycholic acid in the mucosa in both forms of chronic gastritis. Intestinal bacteria may transform chenodeoxycholic acid directly to ursodeoxycholic acid(13), or an intermediate product, 7-keto-lithocholic acid, may be formed(1,13), but it is not known whether the reduction of the 7-keto-group may take place in the gastric mucosa. We also determined small amounts of two bile acids rarely found in humans, hyodeoxycholic acid and hyocholic acid, which tend to be lower in chronically inflamed mucosa.

In chronic atrophic antral gastritis without and with intestinal metaplasia mucosal bile acid concentrations are reduced with a significant change in the bile acid pattern towards secondary bile acids, in particular towards toxic and (co)carcinogenic lithocholic acid. Whether bile acid degradation alone or possibly in combination with other bacterial degradation products plays a role in gastric carcinogenesis remains to be elucidated in further studies.

REFERENCES
6) Dietzsch JD: Mechanisms for the intestinal absorption