Intestinal involvement in progressive systemic sclerosis detected by increased unconjugated serum bile acids

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SUMMARY   In patients with progressive systemic sclerosis, impaired motor function of the small intestine may lead to bacterial overgrowth causing diarrhoea, steatorrhoea and malabsorption. As unconjugated serum bile acids have been proposed as markers for small bowel bacterial overgrowth, we studied individual unconjugated serum bile acids in 36 patients with progressive systemic sclerosis. These patients had significantly higher serum concentrations of unconjugated cholic acid (median 0·18; range 0·05–30·75 v 0·09; 0·01–0·19 μmol/l, p<0·001) and chenodeoxycholic acid (0·10; 0·01–6·83 v 0·04; 0·01–0·39 μmol/l, p<0·025) than healthy controls (n=16). This difference was mainly due to patients with diarrhoea (n=10), who had significantly higher concentrations of unconjugated serum bile acids than patients with normal bowel habit (cholic acid median 0·55 v 0·16 μmol/l, p<0·001; chenodeoxycholic acid 0·75 v 0·07 μmol/l; p<0·005). All patients with raised unconjugated serum bile acids had oesophageal motility disorders. These results confirm a relationship between motility disorders and bacterial overgrowth in patients with progressive systemic sclerosis.

Progressive systemic sclerosis is a multisystem disorder with frequent involvement of the gastro-intestinal tract. The oesophagus is affected with or without symptoms in over 50% of the patients.[1-4] Radiological small bowel abnormalities have been reported in about half of the patients with progressive systemic sclerosis.[1,5] The main findings are hypomotility, dilatation of the duodenum, segmental dilatation of other bowel loops and pseudo-diverticula.[1,5-7] In patients with radiological evidence of small bowel involvement an abnormal fasting motor activity has been shown.[8] Segmental dilatation and impaired motor function of the small intestine may lead to bacterial overgrowth causing diarrhoea, steatorrhoea, and malabsorption in patients with progressive systemic sclerosis.[9-12]

Enteric bacteria rapidly hydrolyse glycine and taurine conjugates of cholic acid, chenodeoxycholic acid, and deoxycholic acid.[13-15] Deconjugated bile acids are rapidly absorbed from the small intestine and spill over from the enterohepatic circulation into the blood stream.[16-18] Thus, a sensitive method for the determination of unconjugated serum bile acids should permit detection of patients with bacterial overgrowth and progressive systemic sclerosis. We are not aware of a systematic evaluation of serum bile acids in patients with progressive systemic sclerosis. Therefore, we determined conjugated and unconjugated serum bile acids in patients with progressive systemic sclerosis. The findings in patients with and without history of diarrhoea as well as with and without advanced oesophageal involvement assessed by functional scintigraphy were compared.

Methods

PATIENTS

Serum of 36 consecutive patients with progressive systemic sclerosis and normal liver tests (alkaline phosphatase, aminotransferases, bilirubin, and total serum conjugated bile acids) was analysed. The group consisted of nine men and 27 women with a mean age of 51 years. All patients had a history of Raynaud's phenomenon and evidence of cutaneous involvement with scleroderma (sclerosis, sclerodactyly and digital pitting scars). The patients had a history of disease between one year and 19 years (mean eight±SD five years). All but one patient revealed antinuclear antibodies. None of the patients received antibiotics at least three weeks before blood sampling. Ten of the patients had diarrhoea defined as more than four loose stools per day over a period of the last three months. In 14 patients radiological studies of the upper intestinal tract were carried out. Eight of those patients showed dilated small bowel loops and/or a markedly decreased peristalsis. Sixteen healthy volunteers (six men, 10 women comparable in age, mean 55 years) served as controls. Blood was drawn two to three hours after breakfast in all subjects and centrifuged. Serum was stored at −20°C. In addition, the progressive systemic sclerosis patients underwent oesophageal functional scintigraphy within six months before blood sampling.

DETERMINATION OF SERUM BILE ACIDS

Two millilitres of serum were extracted on Bond Elut C_{18} cartridges.[19 20] The bile acids were eluted with 6 ml 75% methanol, passed through a SP-Sephadex column (H^+-form, 4·5 cm×8 mm) and bound to a DEAP-LH-20 column (4·5 cm×8 mm). According to Setchell *et al*[21] the unconjugated bile acids were eluted with 6·5 ml 0·1 mol/l acetic acid in 72% ethanol (pH 4·0), the conjugated fraction with 6·5 ml 0·15 mol/l acetic acid in 72% ethanol (pH 6·4) and the sulfated conjugated fraction with 10 ml 0·3 mol/l acetic acid in 72% ethanol (pH 9·6). The last two fractions were pooled. After cleaning the unconjugated fraction and the pooled conjugated fractions by extracting the eluate with three volumes of n-hexane after acidification to pH 4, the solutions were evaporated to dryness. The unconjugated fraction was directly methylated and trimethylsilylated.[20] The pooled conjugated fraction was solvolysed[22] and deconjugated enzymatically.[23] Thereafter the deconjugated bile acids were extracted on Lipidex 1000,[21] methylated and trimethylsilylated. The methyl ester TMS ether derivatives were dissolved in 25 µl isooctane and 1–2 µl were analysed by capillary gas liquid chromatography/electron impact mass spectrometry, and selected ion monitoring. A 25 m×0·32 mm fused silica capillary OV-1701 column[20 24] (CP Sil 19 CB, Chrompack, Middelburg, The Netherlands) was directly inserted into a Finnigan 4000 mass spectrometer. Samples were introduced by cold on-column injection and temperature programming.[20] Quantification was carried out using 11,12-$^2H_2$-chenodeoxycholic acid as an internal standard.[25]

OESOPHAGEAL FUNCTIONAL SCINTIGRAPHY

Oesophageal transit was measured as described previously.[26] After the patient had swallowed 10 MBq[99m] Tc-DTPA in 15 ml water in supine position, counts were stored in 1 sec frames and processed with a region of interest including the whole oesophagus. The time activity histogram was subsequently transformed into a clearance curve. The value established for healthy subjects in our department was 91±4·8% of the maximal oesophageal radioactivity cleared within 10 seconds.

STATISTICAL ANALYSIS

The data for the different groups of patients and the controls are expressed as median and range. For statistical evaluation of differences between the medians, the Mann-Whitney rank test for unequal group sizes was used. The upper limit of the reference values was defined as mean+2 standard deviations of the healthy controls. A p value less than 0·05 (one tailed) was considered statistically significant.

Results

Whereas serum concentrations of conjugated bile acids were similar in progressive systemic sclerosis patients (2·16; 0·50–4·26 mol/l) and controls (1·47; 0·45–5·22 µmol/l), the unconjugated bile acids were significantly raised in progressive systemic sclerosis patients (Table). This rise was mainly caused by unconjugated cholic acid and unconjugated chenodeoxycholic acid. Unconjugated deoxycholic acid was not significantly raised.

Raised unconjugated serum bile acids were only found in patients with impaired oesophageal clear-

Table   *Unconjugated serum bile acids (µmol/l) in PSS patients and controls (median and range)*

	PSS patients (n=36)	Controls (n=16)	
Cholic acid	0·18 (0·05–30·75)	0·09 (0·01–0·19)	p<0·001
Chenodeoxy-cholic acid	0·10 (0·01–6·83)	0·04 (0·01–0·39)	p<0·025
Deoxycholic acid	0·34 (0·10–6·37)	0·28 (0·01–1·16)	ns
Total	0·74 (0·23–42·81)	0·39 (0·03–1·36)	p<0·005

ance (values below 80%) as shown for unconjugated cholic acid in Figure 1. Rises of unconjugated serum bile acids were most pronounced in patients with low oesophageal clearance.

In patients with diarrhoea, the levels of unconjugated serum bile acids were significantly higher (p<0·001) than in patients without diarrhoea (1·68 v 0·51 µmol/l; p<0·001). Again, this difference was caused mainly by unconjugated cholic acid (0·55 v 0·16 µmol/l; p<0·001) and chenodeoxycholic acid (0·75 v 0·07 µmol/l; p<0·05). Of the 10 patients with diarrhoea, eight had raised unconjugated cholic acid (>0·23 µmol/l) and six had raised concentrations of unconjugated chenodeoxycholic acid (>0·28 µmol/l) (Fig. 2).

Of the 26 patients without diarrhoea, only five had raised concentrations of unconjugated cholic acid or chenodeoxycholic acid (Fig. 2). Of the eight patients in whom dilated loops and/or a decreased peristalsis were detected radiologically, seven had raised serum concentrations of unconjugated cholic acid. All six
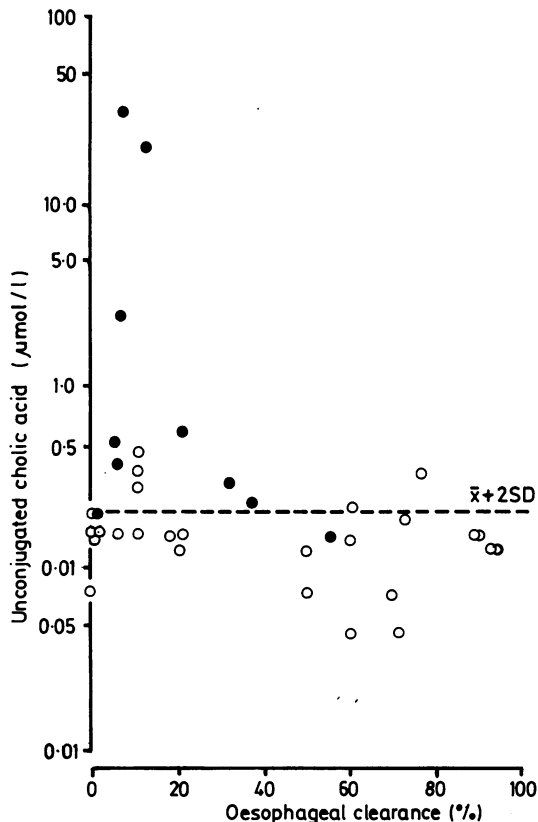
patients who showed no radiological evidence of an altered intestinal function, had normal concentrations of unconjugated cholic acid in the serum.

Discussion

In patients with progressive systemic sclerosis, defects of small bowel motility may lead to stasis of intestinal contents and may favour bacterial overgrowth of the upper small intestine by anaerobic bacteria such as *Bacteroides fragilis* or bifido bacterium. It has been shown that these bacteria are able to deconjugate bile acids.[27] Deconjugated bile acids can easily pass into the portal blood by passive diffusion. Because serum bile acids reflect the bile acid pattern of the portal blood,[28] it may be expected that bacterial overgrowth leads to rises in unconjugated bile acids in peripheral blood.[29] Indeed, the rise in unconjugated bile acids in the serum of our patients with progressive systemic sclerosis may be due to this mechanism.

Further analysis of the data showed that a rise in unconjugated bile acids in serum was mainly restricted to patients with signs of motility disorders such as impaired oesophageal clearance and diarrhoea. Diarrhoea in these patients may not only be related to the motility disorder but also to the properties of deconjugated bile acids. Deconjugated bile acids are poor micelle formers[12] and in addition may exert toxic effects on the enterocytes.[30-32] Our findings confirm earlier observations[10 11 33] stressing deconjugation of bile acids by bacterial overgrowth as an important pathogenetic factor for malnutrition and diarrhoea in patients with progressive systemic sclerosis.

Further prospective studies are warranted to investigate the clinical value of serum bile acid determinations in the evaluation of patients with progressive systemic sclerosis. Especially, a comparison of unconjugated serum bile acids with the 1-¹⁴C-glycocholate breath test[34] appears desirable. In a separate study (in preparation) of patients with bacterial overgrowth of the small intestine not caused by progressive systemic sclerosis, we have found agreement between a pathological breath test and raised concentrations of unconjugated primary bile acids in serum.

Our results suggest a relationship between motility disorders and bacterial overgrowth in patients with progressive systemic sclerosis. Unconjugated bile acids in serum were raised in about one third of patients with impaired oesophageal clearance and in most of those with diarrhoea.

Fig. 1   *Serum concentrations of unconjugated cholic acid and oesophageal clearance of 10 MBq⁹⁹ᵐ Tc-DTPA in progressive systemic sclerosis (PSS) patients with (●) and without (○) diarrhoea.*

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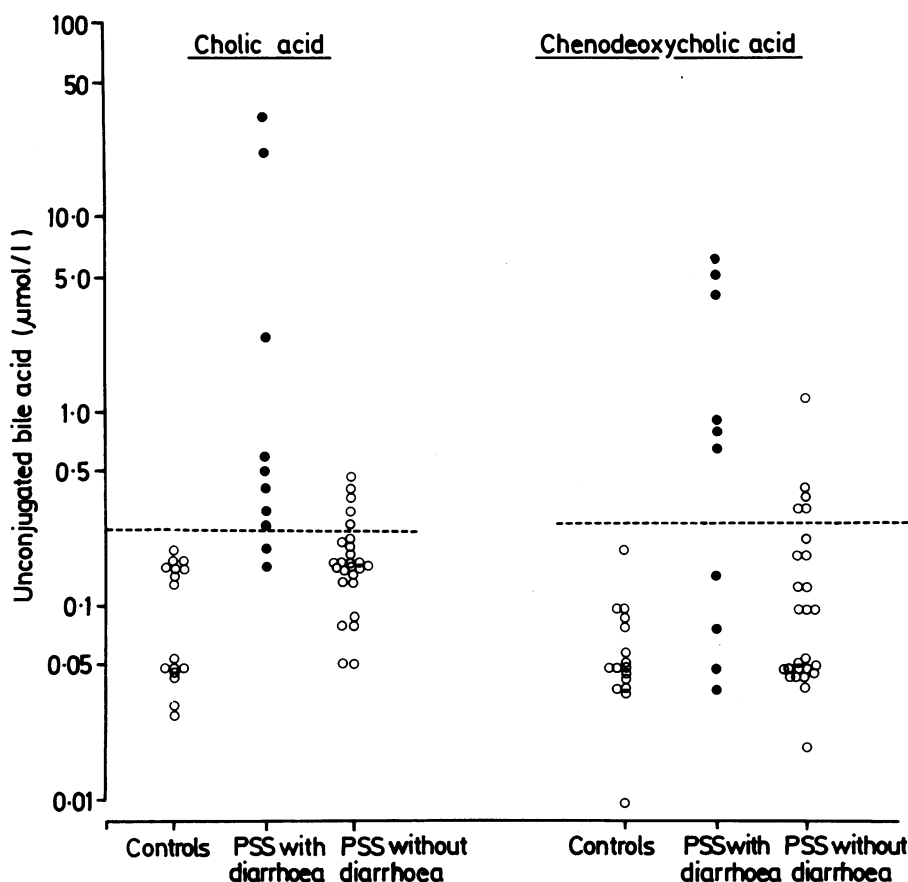1 Poirier TJ, Rankin GB. Gastrointestinal manifestations

Fig. 2   *Serum levels of unconjugated cholic acid and chenodeoxycholic acid in healthy controls and PSS patients with (●) and without (○) diarrhoea. The dotted lines represent the mean ±2 SD of healthy controls.*

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