Increased oxidoreduction of deoxycholic acid in cholecystectomised patients

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SUMMARY The extent of oxidoreduction of deoxycholic acid in the enterohepatic circulation was studied in seven healthy subjects and seven patients after cholecystectomy. (12β-^{3}H) Deoxycholic acid was given orally together with (24-^{14}C) labelled bile acid. The rate of oxidoreduction of the 12α-hydroxyl group of deoxycholic acid was calculated from the decay in ratio between ^{3}H and ^{14}C. In spite of a normal proportion of deoxycholic acid and other secondary bile acids in bile, patients after cholecystectomy had more than two-fold higher degree of oxidoreduction of the 12α-hydroxyl group than healthy controls. The high extent of oxidoreduction is probably because of an increased exposure of the bile acid pool to intestinal bacteria and may have physiological implications.

Bile acids are subjected to extensive microbial biotransformations in the intestine, including deconjugation, 7 α-dehydroxylation and oxidoreduction.[1] Portal venous serum from fasting man contains about 10% of monoketonic bile acids.[2] In the liver reabsorbed bile acids are reconjugated – but not rehydroxylated – and keto groups are reduced, mainly to α-hydroxyl groups.[3 4] Consequently, human bile normally contains only traces of ketonic bile acids whereas they may represent a significant fraction of human faecal bile acids.[5-7]

Some investigations of bile acid metabolism have shown an increase of secondary bile acids, especially deoxycholic acid in the bile of cholecystectomised patients.[8-12] An increased proportion of ketonic bile acids has also been reported.[9 11] Since there are oxidoreductases both in the intestine and in the liver, it is not possible to evaluate the total extent of oxidoreduction of bile acids during the enterohepatic circulation from the above information.[3 4]

We have recently studied the extent of oxidoreduction of the hydroxyl groups in cholic acid and deoxycholic acid during the enterohepatic circulation in healthy man.[13] The degree of oxidoreduction was considerably higher for deoxycholic acid than for cholic acid. In the present work we have studied the extent of oxidoreduction of deoxycholic acid in cholecystectomised patients with use of deoxycholic acid labelled in 12β-position. The ^{3}H-labelled bile acid was given together with the corresponding 24-^{14}C-labelled bile acid. The rate of oxidoreduction was calculated from the decay in ratio between ^{3}H and ^{14}C. According to the results obtained, cholecystectomised patients have more than two times higher rate of oxidoreduction of deoxycholic acid than healthy controls.

Methods

PATIENTS

The study comprised five healthy men and two healthy women with no signs of liver or intestinal diseases and seven women who had undergone cholecystectomy for cholesterol gall stones at least six months before this study (Table 1). None of the patients had received antibiotic therapy during the last two months preceding the study and none was on treatment with any medication known to affect plasma or biliary lipid metabolism.

The ethical aspects of the study were approved by the Ethical Committee at Huddinge University Hospital.

MATERIALS

(12β-³H) Deoxycholic acid (specific radioactivity 83×10^6 cpm/mg) was synthesised as recently described.[13] (24-¹⁴C) Deoxycholic acid (specific radio-activity 220×10^6 cpm/mg) was obtained from New England Nuclear (Dreieichenhain, West-Germany). The compounds were purified as methyl esters by thin layer chromatography using system S 11 before use.[14] The free steroid was prepared from the methyl ester by alkaline hydrolysis. The ³H-labelled bile acid was mixed with the ¹⁴C-labelled bile acid to give a ³H/¹⁴C ratio of about 2·5–3·0. The amount of ³H given was about 8×10^6 cpm, the amount of ¹⁴C about 3×10^6 cpm and the weight of labelled material about 110 µg.

ADMINISTRATION OF LABELLED BILE ACIDS AND COLLECTION OF BILE

The labelled bile acid mixture was given as a sodium salt dissolved in water in the evening. Samples of duodenal bile were collected each morning for the four following days. Cholecystokinin was administered intravenously, and about 5 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube with the collecting orifice positioned fluoroscopically at the ampulla of Vater.

ANALYSIS OF BILE ACIDS

The duodenal bile samples were hydrolysed with 1 M KOH in closed steel tubes for 12 hours at 110°C. After acidification, the deconjugated bile acids were extracted with ethyl ether and methylated with diazomethane. One aliquot was subjected to thin layer chromatography using system S 11 to isolate the methyl ester of deoxycholic acid.[14] The purity of the bile acid was checked by gas liquid chromatography as the methyl ester trimethylsilyl derivative, using a 1·5% SE-30 column. Radioactivity was measured with an Intertechnique SL 30 liquid scintillation spectrometer, using Lumal gel (LUMAC, BV, The Netherlands) as scintillation fluid. Under the conditions used the counting efficiency was 49% for ³H and 71% for ¹⁴C. No corrections for quenching were found to be necessary. For determination of bile acid composition, another aliquot of the methylated bile acid extract was trimethylsilylated and analysed by gas liquid chromatography using a 1·5% SE-30 column.

STATISTICAL ANALYSIS

Data are presented as means (SEM). The statistical significance of differences was evaluated by Student's *t* test. Correlations were tested by estimating the correlation coefficient *r*.

Results

As seen in the Figure the ³H/¹⁴C ratio in deoxycholic acid decreased with time in the healthy subject as well

Table 1 *Fractional catabolic rate (FCR) of the 12α-hydroxyl group in deoxycholic acid, determined in healthy subjects and cholecystectomised patients*

| | | | Body weight | | |
Subject number	Sex	Age (yr)	kg	% of ideal*	FCR/day
Healthy subjects					
1	F	29	51	79	0·17
2	F	68	56	98	0·19
3	M	28	70	92	0·27
4	M	21	71	82	0·27
5	M	29	67	88	0·15
6	M	31	80	89	0·18
7	M	30	75	88	0·09
Mean (SEM)		34 (6)	67 (4)	88 (2)	0·19 (0·02)
Cholecystectomised patients					
8	F	67	69	114	0·41
9	F	58	65	102	0·38
10	F	36	69	101	0·60
11	F	20	60	86	0·50
12	F	38	63	90	0·26
13	F	44	82	137	0·26
14	F	72	58	100	0·50
Mean (SEM)		48 (7)	67 (3)	104 (6)	0·42 (0·05)†

*Calculated as $\dfrac{\text{body weight (kg)}}{\text{height (cm)} - 100} \times 100\%$
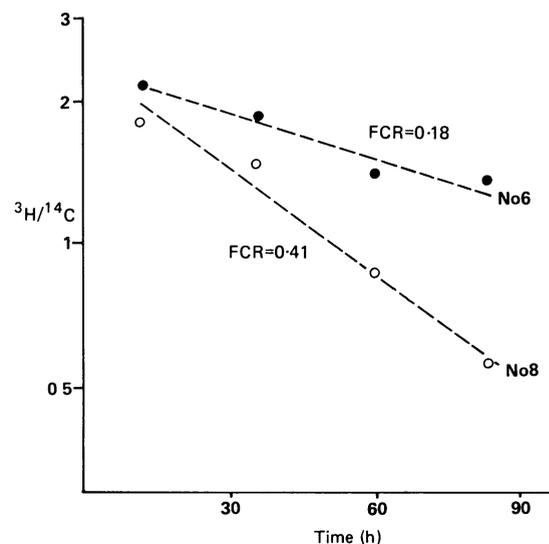
†Significantly different from healthy subjects, p<0·01.

Figure *Decay of ³H/¹⁴C ratio in deoxycholic acid isolated from duodenal bile of a healthy subject (no 6) and a cholecystectomised patient (no 8) after oral administration of (12β-³H)- and (24-¹⁴C) deoxycholic acid.*

Table 2 *Biliary bile acid composition (%) in healthy subjects and cholecystectomised patients*

Subject number	Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	Ursodeoxycholic acid	Lithocholic acid
Healthy subjects					
1	45·9	38·2	15·9	—	—
2	35·3	44·0	16·5	2·3	1·9
3	37·6	32·7	28·7	0·7	0·3
4	40·0	38·2	18·4	3·4	trace
5	47·9	21·1	30·1	—	0·9
6	45·0	30·4	22·8	1·5	0·3
7	51·1	34·8	13·0	trace	1·2
Mean (SEM)	43·2 (2·2)	34·2 (2·8)	20·8 (2·5)	1·1 (0·5)	0·7 (0·3)
Cholecystectomised patients					
8	28·8	22·5	41·4	3·8	3·5
9	48·1	32·6	18·8	trace	0·5
10	33·5	39·8	23·1	—	3·6
11	45·0	34·8	15·2	1·0	4·0
12	35·1	28·3	32·1	4·0	0·5
13	41·1	33·9	16·0	7·4	1·6
14	44·4	31·8	20·1	3·7	—
Mean (SEM)	39·4 (2·7)	32·0 (2·1)	23·8 (3·6)	2·8 (1·0)	2·0 (0·6)

as in the cholecystectomised patient. The correlation coefficient for the decay curve averaged 0·953 (0·023) in the healthy subjects and 0·995 (0·003) in the cholecystectomised patients. The fractional conversion rate (FCR) for the 12α-hydroxyl group in deoxycholic acid, calculated from the decrease in $^{3}H/^{14}C$ ratio averaged 0·19/day in the healthy subjects (Table 1). Corresponding FCR was more than two-fold higher in the cholecystectomised patients (mean value 0·42/day) (p<0·01) (Table 1). The biliary bile acid composition in the healthy subjects and the cholecystectomised patients is shown in Table 2. The percentage proportion of deoxycholic acid was not significantly increased in the cholecystectomised patients. The proportion of secondary bile acids (deoxycholic acid+ursodeoxycholic acid+lithocholic acid) tended to be higher in the cholecystectomised patients (28·6 (4·0)%) compared with the healthy subjects (21·3 (2·6)%) but the difference did not reach statistical significance (p>0·05).

Discussion

In the present study the percentage proportion of deoxycholic acid was not significantly raised in the cholecystectomised patients. This finding is contrary to some previous studies reporting 50–100% higher proportion of deoxycholic acid in cholecystectomised subjects compared with healthy controls.[8][10-12] A high level of deoxycholic acid has been ascribed to an increased bacterial 7α-dehydroxylation of cholic acid. Because dehydroxylation is a function of the colonic flora an increased bacterial transformation after cholecystectomy, may be because the bile acid

pool spends a greater part of the 24 hours in the intestine, especially the colon, being exposed to the intestinal microflora to a greater extent than normally. An increased proportion of deoxycholic acid after cholecystectomy, however, has not been found in all studies.[9][15][16] Whether this discrepancy is caused by differences in intestinal microflora and/or dietary intake in the patient groups investigated can presently not be established with certainty.

A new and important finding of the present study was the high oxidoreduction of the 12α-hydroxyl group of deoxycholic acid in cholecystectomised patients, being more than twice as high as in healthy controls. As was discussed in a recent work from our laboratory,[13] the present technique should give a minimum figure for the extent of oxidoreduction for the following reasons: first, there may be an isotope effect in the oxidation of the 12α-hydroxyl group adjacent to the 12β-³H-label. Second, a transfer of ³H may occur from the labelled bile acid to a cofactor of the 12α-hydroxysteroid dehydrogenase in the oxidative step and a subsequent utilisation of the same labelled cofactor by the hydroxysteroid dehydrogenase in the reductive step. Such a mechanism would lead to a certain retention in the bile acid in spite of an oxidoreduction. Therefore, we cannot exclude a small underestimation of the oxidoreduction in the present study.

It should be pointed out, that there are also oxidoreductases present in the liver and it might therefore be argued that part of the oxidoreduction of the 12α-hydroxyl group could occur in the liver. In a previous work we tried to evaluate the relative roles of the liver and intestine by studying the oxidoreduc-

tion of (12β-³H)deoxycholic acid in two patients with ileostomy.[13] The FCR for the oxidoreduction of the 12α-hydroxyl group was less than 20% of that obtained in healthy controls. It is thus apparent that the intestine is of major importance for the oxidoreduction of the 12α-hydroxyl group in deoxycholic acid. In cholecystectomised patients there is an increased recycling of bile acids and an increased exposure of the bile acid pool to bacterial metabolism, which may explain the high extent of oxidoreduction of deoxycholic acid, as discussed above.

12-ketolithocholic acid formed from deoxycholic acid by oxidation of the 12α-hydroxyl group may be excreted as such in faeces where it constitutes one of the major bile acids.[5-7] It may possibly also be reduced to a 3,12-dihydroxybile acid by the intestinal microflora. Absorption of 12-ketolithocholic acid from the intestine has been demonstrated indirectly by analysis of portal blood.[2] Thus, monoketonic 3,12-oxygenated bile acids constitute about 10% of deoxycholic acid in portal venous blood.[2] Most of these ketonic bile acids are probably efficiently reduced in the liver as bile normally contains only traces of ketonic bile acids. Increased amounts of monohydroxy-monoketonic bile acids in bile of cholecystectomised patients have, however, previously been obtained in some[9 11] but not in all investigations.[15] Andersén *et al*[17] recently reported an increased proportion of 12-ketolithocholic acid in bile of non-insulin-dependent diabetics and hypertriglyceridaemic patients. The results were interpreted to indicate that these patient groups may have an increased formation of secondary bile acids from cholic acid combined with a diminished capacity of the liver to reduce ketonic bile acids. None of our patients had diabetes or hypertriglyceridaemia.

The present study has shown that patients may have an abnormally high degree of oxidoreduction of a bile acid in the enterohepatic circulation in spite of a normal biliary bile acid composition. This, may have physiological implications as introduction of keto groups in a bile acid molecule may impair the degree of absorption from the intestine. An increased inflow of ketonic bile acids to the liver may possibly also influence the feed back regulation of bile acid formation and bile flow.

References

1 Hylemon PB. Metabolism of bile acids in intestinal microflora. In: Danielsson H, Sjövall J, eds. *Comprehensive biochemistry.* Amsterdam: Elsevier, 1985; **12:** 331–43.

2 Björkhem I, Angelin B, Einarsson K, Ewerth S. Fasting levels of monoketonic bile acids in human peripheral and portal circulation. *J Lipid Res* 1982; **23:** 1020–5.

3 Björkhem I, Danielsson H. Biosynthesis and metabolism of bile acids in man. In: Popper H, Schaffner F, eds. *Progress in liver diseases.* New York: Grune & Stratton, 1976; **5:** 215–31.

4 Elliott WH. Metabolism of bile acids in liver and extrahepatic tissues. In: Danielsson H, Sjövall J, eds. *Comprehensive biochemistry.* Amsterdam: Elsevier, 1985; **12:** 303–29.

5 Eneroth P, Gordon B, Ryhage R, Sjövall J. Identification of mono- and dihydroxy bile acids in human feces by gas-liquid chromatography and mass spectrometry. *J Lipid Res* 1966; **7:** 511–23.

6 Eneroth P, Hellström K, Sjövall J. A method for quantitative determination of bile acids in human feces. *Acta Chem Scand* 1968; **22:** 1729–44.

7 Hill MJ, Aries VC. Faecal steroid composition and its relationship to cancer of the large bowel. *J Pathol* 1971; **104:** 129–39.

8 Almond HR, Vlahcevic ZR, Bell CC Jr, Gregory DH, Swell L. Bile acid pools, kinetics and biliary lipid composition before and after cholecystectomy. *N Engl J Med* 1973; **289:** 1213–6.

9 Malagelada JK, Go VLW, Sumerskill WHJ, Gamble WS. Bile acid secretion and biliary bile acid composition altered by cholecystectomy. *Am J Dig Dis* 1973; **18:** 455–9.

10 Pomare EW, Heaton KW. The effect of cholecystectomy on bile salt metabolism. *Gut* 1973; **14:** 753–62.

11 Hepner GW, Hofmann AF, Malagelada JR, Szczepanik PA, Klein PD. Increased bacterial degradation of bile acids in cholecystectomized patients. *Gastroenterology* 1974; **66:** 556–64.

12 Roda E, Aldini R, Mazzella G, Roda A, Sama C, Festi D, Barbara L. Enterohepatic circulation of bile acids after cholecystectomy. *Gut* 1978; **19:** 640–9.

13 Björkhem I, Liljeqvist L, Nilsell K, Einarsson K. Oxidoreduction of different hydroxyl groups in bile acids during their enterohepatic circulation in man. *J Lipid Res* 1986; **27:** 177–82.

14 Eneroth P. Thin-layer chromatography of bile acids. *J Lipid Res* 1963; **4:** 11–4.

15 Adler RD, Metzger AL, Grundy SM. Biliary lipid secretion before and after cholecystectomy in American Indians with cholesterol gallstones. *Gastroenterology* 1976; **66:** 1212–7.

16 van den Linden W, Katzenstein B, Nakayama F. The possible carcinogenic effect of cholecystectomy. No postoperative increase in the proportion of secondary bile acids. *Cancer* 1983; **52:** 1265–8.

17 Andersén E, Karlaganis G, Sjövall J. Altered bile acid profiles in duodenal bile and urine in diabetic subjects. *Eur J Clin Invest* 1988; **18:** 166–72.