Effect of small doses of deoxycholic acid on bile cholesterol saturation in patients with liver cirrhosis

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SUMMARY To test the hypothesis that the detergent power of each individual bile acid – that is, its separate capacity to solubilise cholesterol and to induce biliary cholesterol secretion, present in the biliary bile acid mixture might be one of the determinant factors of biliary cholesterol saturation, we studied the effect of feeding small doses of deoxycholic acid on biliary cholesterol saturation in patients with liver cirrhosis and low deoxycholic acid pool. Eleven hospitalised patients with cirrhosis of various degree of severity were put on a standard solid diet. Fasting bile rich duodenal fluid was obtained at the beginning of the study, after a three to four weeks treatment with deoxycholic acid (3 mg/kg/day, in two doses) and one month after discontinuing bile acid ingestion. Before treatment the fraction of deoxycholic acid was $5.3 \pm 4.9\%$ (mean $\pm$ SD); after treatment the fraction rose to 43.9 ± 12.0 of total bile acids, but returned to the basal values after stopping bile acids. Bile cholesterol saturation increased significantly from a mean of 0.92 ± 0.26 (before treatment) to a mean of 1.34 ± 0.34 after deoxycholic acid feeding ($p < 0.005$). One month after treatment, bile saturation was not significantly different from the basal values (0.91 ± 0.44). We conclude that feeding low doses of deoxycholic acid to patients with liver cirrhosis induces a significant increase of the fraction of this bile acid in the total pool and this is followed by a sharp increase of bile cholesterol saturation. These data are compatible with the hypothesis that the detergent capacity of individual bile acids is one of the main determinants of bile cholesterol saturation.

Despite numerous studies over the last two decades little is known about the intimate mechanisms which lead to the formation of cholesterol gall stones. Although recent studies emphasise the importance of factors stimulating the nucleation of cholesterol or the lack of factors inhibiting this process, it is still assumed that cholesterol supersaturation of bile is necessary for the formation of cholesterol gall stone, at least in man.¹ Evidence of this comes from the observation that many drugs – such as oral contraceptives or some hypolipidemic agents – which increase bile saturation also predispose to gall stone formation,² and that drugs like chenodeoxycholic acid (CDCA) or ursodeoxycholic acid (UDCA) reduce bile cholesterol saturation and dissolve gall stones.³⁻⁵

Various studies have recently suggested that the

physical chemical properties of each of the individual bile acids might play a relevant role in the regulation of biliary lipid secretion.^{6,7} Thus, the acute duodenal infusion of bile acids with a poor detergent power – such as UDCA – leads to a marked reduction of biliary cholesterol secretion and saturation, whereas the infusion of an equivalent dose of a bile acid of a strong detergent nature – such as deoxycholic acid (DCA) – leads to the secretion of bile which is supersaturated with cholesterol. On the basis of this and other evidence we suggest that bile saturation depends on the balance of the different detergent effects of each individual bile acid present in the total pool.⁸

It would appear logical, therefore, that the abundance of DCA in the total pool might be related to bile supersaturation, either directly because of its strong capacity to solubilise cholesterol, or indirectly because of its inhibition of the synthesis of other less detergent bile acids – that is, CDCA

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Received for publication 28 May 1985

and cholic acid. The administration of DCA to human subjects has produced controversial effects on bile saturation, probably dependent on the different length of treatment, the clinical features of the investigated groups, and on the dose of DCA which was used.⁹⁻¹¹ It is worth mentioning that in the sole study where low doses of DCA were given (100–150mg/day) a consistent increase of bile saturation was observed.¹²

The present study was designed to further investigate the relation between changes in the bile acid pool composition and bile cholesterol saturation. We fed small ('physiological' as they were close to the amount daily produced by the intestinal degradation of cholic acid) doses of DCA to patients with liver cirrhosis, in whom the fraction of DCA in the total pool was markedly lower than in normal subjects and often reduced to trace amounts.¹³⁻¹⁵ This was to ascertain if the administration of these physiological doses of DCA would induce changes of bile lipid composition compatible with the above mentioned hypothesis.

Methods

PATIENTS AND EXPERIMENTAL DESIGN

Eleven male patients with liver cirrhosis entered the study. The age, major liver function tests, and clinical features (Child-Turcotte classification) for each patient are shown in Table 1.¹⁶

Informed consent was obtained from each patient in accordance with the Declaration of Helsinki. All were inpatients and given from the time of admission a standard solid diet providing 30 Kcal/kg and approximately 400 mg cholesterol per day. Most patients were receiving lactulose, diuretics, and vitamins, and these drugs were maintained throughout the length of the study at the same dose.

Seven to 10 days from the admission the patients, after an overnight fast, were intubated with a

nose-duodenal catheter under fluoroscopic control. Gall bladder contraction was induced by an intravenous injection of cerulein (40 µg per 100 ml saline, given over a 10 minute period). Bile rich duodenal fluid (10–20 ml) was collected by siphonage and immediately stored for analysis. From the following day the patients received DCA (3 mg/kg/day – that is, 180–250 mg) in two separate doses at 8 am and at 8 pm for three to four weeks. At the end of treatment the patients were reintubated and a second bile sample was collected. The patients were then discharged but encouraged to continue with the same diet at home. After one month the patients returned for a third duodenal intubation.

Liver function tests (SGOT, SGPT, bilirubin, γGT and alkaline phosphatase) were obtained after one week and at the end of the DCA treatment period.

Deoxycholic acid, 3α-hydroxysteroid-dehydrogenase and cholyl-glycine-hydrolase were supplied by Sigma Chemicals Co (St Louis, MO, USA), SP-2401 by Supelco Inc (Bellefonte, PA, USA) and cerulein (Caeruleide TAKUS) was a gift of Farmitalia (Milano, Italy).

Bile lipid composition was studied with standard spectrophotometric techniques as already described.^{8, 17} Total bile acid concentration was estimated with the 3α-hydroxysteroid-dehydrogenase method of Talalay;¹⁸ total biliary cholesterol was measured according to Abell's and coworkers' method,¹⁹ and phospholipids as inorganic phosphorus according to Bartlett's method.²⁰ Saturation index of bile was calculated according to the 'critical tables' of Carey and assumed an average total solid concentration of 5 g/dl.²¹

The relative proportion of the individual bile acids was assessed by gas-liquid chromatography. After enzymatic hydrolysis (cholylglycine-hydrolase) of the conjugated bile acid and the extraction of the free bile acids,¹⁷ the hexafluoroisopropyl esters

Table 1 Clinical data

Patients (initials)	Age	SGOT	Albumin g/dl	Bilirubin mg/dl	Prothrombin time %	Child-Turcotte classification
DCD	78	21	3.25	0.92	62	B
BG	57	45	2.58	2.5	70	C
LSC	46	79	4.46	1.3	58	A
BE	52	35	3.31	1.6	89	A
IA	38	102	1.58	1.0	77	B
CC	57	75	4.01	1.4	58	B
OU	57	69	3.52	1.6	54	A
AS	71	54	2.25	1.6	42	C
SM	51	32	4.01	0.9	86	A
GT	46	30	3.42	0.6	80	A
CI	59	26	3.03	1.5	65	B

were prepared.²² This method avoids the cumbersome preparation of the potentially explosive diazomethane, without any loss of sensitivity or precision. After evaporation of the solvents, the hexafluoroisopropyl derivatives were dissolved in ethylacetate and aliquots of 1–5 μ l injected in a Carlo Erba Fractovapp 4200, equipped with 180 cm spiral columns packed with SP-2401 on Supelcoport (100–120 mesh). The injection point temperature was 240°C, oven (column) 230°C, detector 250°C. The flow rate of the carrier gas (nitrogen for GLC) was 45–60 ml/minute.

STATISTICAL ANALYSIS

The results of the studies are expressed as mean values $\pm$ standard derivations of the means. The statistical significance of differences between means was assessed with paired Student's *t* tests.

Results

Changes of biliary bile acid composition induced by DCA feeding are shown in Figure 1. As expected, in patients with liver cirrhosis the sum of CDCA and cholic acid (CA) accounted for more than 90%, with CDCA being the most abundant in all the investigated patients. After DCA feeding the mean fraction of both primary bile acid was significantly

Fig. 1 Percentage of the three major bile acids in the duodenal aspirates of the 11 investigated patients before treatment, after DCA feeding, and one month from stopping treatment. Each column represents the mean value with the standard deviation.

reduced to 20.4 ± 6.9 and $32.6 \pm 10.5\%$ respectively ($p < 0.001$). All patients made a rapid return to the baseline values on stopping treatment. Before treatment, the mean fraction of DCA in the total pool was only $5.6 \pm 4.9\%$, as opposed to the 20–30% reported in normal subjects.^{12, 23}

After treatment, the fraction of DCA rose to $43.9 \pm 12.0\%$ ($p < 0.001$), returning to the basal values ($5.9 \pm 4.8\%$) upon cessation of treatment. Other secondary bile acids (UDCA and lithocholic acid) were present only in trace amounts (0.84 ± 1.36 and $1.80 \pm 2.25\%$ respectively) in the basal sample and did not show any significant change during the course of the study.

Deoxycholic acid was present virtually in trace amounts in about one third of the subjects, the highest baseline value (15% of the total) being below the mean value of those without cirrhosis or hepatobiliary diseases. After treatment, changes were variable but in all patients, however, DCA reached at least 30% of the total and in six of 11 it became the bile acid prevalent in the pool, ranging from 35–60%. After stopping treatment all patients immediately returned to the low basal values.

The effect of DCA feeding on bile cholesterol saturation and biliary lipid composition is shown in Figure 2 and in Table 2. The basal saturation index ranged between 0.7 and 1.5 with a mean of 0.92 ± 0.26 .

After treatment, bile was supersaturated with cholesterol in all but one patient, the mean value (1.34 ± 0.34) being significantly different from the pretreatment value ($p < 0.005$). As expected, after stopping treatment bile saturation decreased in all patients, the mean value (0.91 ± 0.44) being not significantly different from the pretreatment value.

No direct relationship was found between the fractional increase of DCA in the total bile acid pool and the rise of bile saturation ($r = 0.57$, ns).

Treatment with low doses of DCA was well tolerated and none of the investigated patients complained of diarrhoea or other side effects frequently observed with CDCA treatment or with higher doses of DCA.^{10, 12} Similarly, none of the liver function tests showed any consistent change throughout the study.

Table 2 Bile lipid composition (mmol/l) before, during and after DCA feeding (mean $\pm$ SD)

	Before	During	After
Bile acid	21 $\pm$ 18.7	22.2 $\pm$ 17.6	19.6 $\pm$ 10.2
Phospholipid	7.1 $\pm$ 4.8	6.6 $\pm$ 4.9	6.9 $\pm$ 4.1
Cholesterol	2.1 $\pm$ 1.7	2.4 $\pm$ 2.0	1.9 $\pm$ 1.1

Fig. 2 Changes of bile cholesterol saturation in each patient during the three phases of the study. The values observed after DCA administration were significantly ($p < 0.005$) different both from the basal values and from those seen after stopping treatment.

Discussion

This study has shown that when DCA is given in 'physiological' doses to subjects with a low fraction of this bile acid in their bile, this treatment is followed by a sharp (but reversible) increase of DCA in the total bile acid mixture and by a parallel and significant increase of bile saturation. These findings provide further support to the hypothesis that the detergent capacity of individual bile acids is one of the major determinants of bile cholesterol saturation.⁸

The effect of DCA feeding on bile cholesterol saturation has been frequently investigated (and debated) in the last few years. Low-Beer and Pomare first showed that feeding 100–150 mg DCA for two weeks to healthy volunteers led to a consistent increase of bile cholesterol saturation, suggesting that colonic bacterial metabolites predispose to cholesterol gall stones.¹² Subsequently, LaRusso *et al* and Ahlberg and colleagues were unable to find any significant change of bile saturation after feeding larger amounts (750 mg/day) of DCA to normal or hyperlipidemic subjects.^{9, 10} The reasons for these discrepancies are unclear but may

depend on the length of treatment, the type of patients investigated and particularly the dose of DCA given. It is noteworthy that in the two studies in which small doses of DCA were administered – to normal volunteers¹² or cirrhotic patients (present study) – a significant increase of bile cholesterol saturation was seen. When larger doses of DCA are given other 'pharmacological' effects of this bile acid presumably become more evident, with the net result of little change of bile saturation. Indeed, DCA feeding at the dose of 15 mg/kg/day reduces cholesterol absorption,¹¹ lowers hepatic cholesterol synthesis,²⁴ and may induce hepatocellular damage.^{8, 10, 11} All these effects may counterbalance or mask the supersaturating effect of DCA due to its intrinsic physical chemical properties.

Further confirmation that DCA increases the lithogenicity of bile comes from the results of many other studies. Thus, Low-Beer and Nutter showed that feeding metronidazole – an inhibitor of anaerobic bacteria which produces DCA from cholic acid – was followed by a significant decrease both of the fraction of DCA in the total pool and of bile saturation.²⁵ Similar effects on biliary bile acid composition and bile cholesterol saturation were observed after feeding large amounts of dietary fibre^{26, 27} or lactulose.²⁸ In a recent study from our Unit we investigated the effect of cholic acid + ampicillin on bile lipid composition. In those individuals in whom the simultaneous administration of cholic acid + broad spectrum antibiotics was followed by a selective expansion of cholic acid pool size, bile saturation tended to decline. In those subjects in whom both cholic acid and DCA pool size increased, however, presumably owing to a relative resistance of the colonic bacteria to the antibiotic, bile cholesterol saturation was actually increased.²⁹ Finally, it is worth mentioning that in patients with cholelithiasis the fraction of DCA is frequently higher than in normal controls,³⁰ and that in patients with type IV hyperlipidemia, whose bile is frequently supersaturated with cholesterol and who are at risk for gall stones, the proportion of DCA in the total bile acid pool is nearly double that of normal subjects.³¹

The results of the present studies, as well as the above mentioned observations from other laboratories, confirm the view that one of the major determinants of bile cholesterol secretion and saturation are the physical and chemical properties of bile acids. We have recently observed that when bile acid pool is acutely replaced by infusion of individual bile acids the observed ordering of biliary cholesterol secretion seems to be related to the hydrophilic-hydrophobic balance of the different bile acids. Thus, the secretion of less hydrophilic

(and more detergent) bile acids (like DCA) is followed by a biliary cholesterol output much higher than that observed with more hydrophilic (and less detergent) bile acids, such as cholic acid or UDCA.⁸

As all the other physiological bile acids are less detergent than DCA, the major practical implication of the present study is that whenever bile enriches with DCA this is associated with an increased risk of developing cholesterol gall stones. This contention, of course, does not exclude that other factors, such as the metabolic changes induced by chronic bile acid feeding or the presence of nucleating (or antinucleating) factors in hepatic or gall bladder bile, might also be relevant in determining the risk of gall stones.³²

The recent observation that the increase in cholesterol content of bile, in prairie dogs, stimulates gall bladder mucus secretion and this, in turn, is a nucleating agent for biliary cholesterol, might be taken as an even closer relationship between bile supersaturation and formation of nucleating agents.³³ If confirmed, this would mean that the prevention of cholesterol gall stones should continue to be done initially at the level of bile saturation before trying to interfere with the process of nucleation (for instance, by the administration of aspirin or other anti-inflammatory drugs).

Part of this work has been presented at the annual meeting of the British Society of Gastroenterology (Liverpool, September 12–14, 1984) and at the annual meeting of the Italian Gastroenterological Association (Catania, 8–10 October, 1984).

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