Ursocholic acid: bile acid and bile lipid dose response and clinical studies in patients with gall stones

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SUMMARY The biliary bile acid and bile lipid responses to six weeks treatment with approximately 5, 10, and 15 mg/kg/day of ursocholic acid (UCA) were studied in 11 gall stone patients. Maximum enrichment of bile with UCA (24 (SE) 4.9%) occurred with 15 mg UCA/kg/day. The maximum reduction in biliary cholesterol saturation was seen with the 10 mg/kg/day dose when the moles % cholesterol fell from 14 (2.4)% before treatment to 5.6 (0.83)% ($p < 0.02$) and the saturation index fell from 1.4 (0.23) to 0.77 (0.13) ($p < 0.05$). Clinical studies of the safety and efficacy of UCA in dissolving gall stones were carried out in 13 patients treated for up to two years with a dose of approximately 10 mg/kg/day. Diarrhoea caused withdrawal of treatment in three patients. There were no significant changes in liver function or haematology tests but fasting serum cholesterol tended to rise during treatment. Of nine patients treated for > 6 months, only one showed complete gall stone dissolution. As UCA may cause diarrhoea and hypercholesterolaemia, has only a modest effect on biliary cholesterol saturation and low gall stone dissolution efficacy, it is unlikely to replace existing forms of gall stone dissolution therapy.

Both chenodeoxycholic (CDCA) and ursodeoxycholic acid (UDCA) are established gall stone dissolving agents.^{1,2} Chenodeoxycholic acid, however, causes dose related diarrhoea^{3,4} and hypertransaminasaemia.^{4,6} Although UDCA does not have these disadvantages, we have found that it is less effective than CDCA in dissolving gall stones completely,⁷ partly because of acquired gall stone calcification.⁷⁻⁹ The efficacy of a combination of CDCA and UDCA is variable¹⁰⁻¹³ and the ideal bile acid therapy for gall stones has still to be found.

Ursocholic acid (UCA) is the 7 beta epimer of the naturally occurring primary bile acid, cholic acid (Fig. 1). Cholic acid (CA) administration does not reduce biliary cholesterol saturation, unless its 7 alpha dehydroxylation to deoxycholic acid (DCA) is prevented by concomitant antibiotic treatment.¹⁴ Because 7 beta dehydroxylation is less efficient than 7

alpha dehydroxylation,¹⁵ we reasoned that UCA might not be degraded to DCA, might not cause diarrhoea and might, therefore, be an effective gall stone dissolving agent. Information about UCA treatment in man is limited. Loria *et al*¹⁶ studied the effects of acute (five hour) intraduodenal infusions of 1 g UCA/h on T-tube bile in three postcholecystectomy patients. This enriched the bile with UCA conjugates to a plateau of 90%. They also examined the effects of two weeks oral treatment with either 10 or 15 mg UCA/kg/day, on the bile acid and bile lipid composition of fasting, bile rich duodenal fluid. Arguably, however, two weeks is too short a time to establish a new steady state in bile composition.¹⁷ Zuin and Podda¹⁸ treated six patients with UCA, all of whom had stones which were ideally suited for dissolution therapy and found complete gall stone dissolution in three.

The aims of the present study were two-fold: first, to establish the dose response of biliary bile acid and bile lipid composition to treatment with UCA and second, to examine the gall stone dissolution efficacy, side effects and safety of longterm UCA treatment.

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Fig. 1 Structure of ursocholic acid (UCA) contrasted with its parent bile acid, cholic acid (CA). As indicated by the ringed groups, the only difference is in the configuration of the 7 hydroxyl group – beta for UCA and alpha for CA.

Methods

PATIENTS

Eleven patients took part in the dose response study (patients 1–11, Table 1). One patient (number 2) had small, asymptomatic recurrent gall stones, after dissolution with UDCA. Four patients presented with non-specific dyspepsia. The remaining six had a clinical history and/or biochemical evidence of biliary colic.

Before the study, all patients underwent ultrasonography and cholecystography, all had radiolucent gall stones and all but two had stones measuring <20 mm (maximum diameter) in 'functioning' gall bladders (patent cystic ducts).

Table 1 Clinical details of the 13 patients who took part in the dose response and efficacy studies with ursocholic acid

Patient no	Sex	Age	Weight (kg)	% IBW	Stones (n)	Stones max diameter (mm)
1	M	46	84	99	3	13
2	M	67	83	118	3	5
3	M	66	92	114	>10	5
4	M	45	81	100	2	<5
5	F	52	87	141	1	28
6	M	59	83	101	6	10
7	F	57	74	117	2	20
8	F	75	61	104	2	11
9	F	56	78	123	2	17
10	F	27	75	131	>15	18
11	M	55	71	87	1	14
12	F	57	65	98	3	5
13	F	61	73	116	>20	<5

In addition to the patients entered into the dose response study, two further patients were treated with UCA in a dose of approximately 10 mg/kg/day for up to 24 months to assess its gall stone dissolution efficacy.

BILIARY BILE ACIDS AND BILE LIPIDS

For the dose response studies, fasting bile rich fluid was aspirated from the duodenum after an intravenous injection of 100 Ivy Dog Units of cholecystokinin (Pancreozymin, Boots PLC, Nottingham, UK). In the first seven patients, the bile rich fluid was obtained before treatment and again after six week courses each of 5, 10, and 15 mg UCA/kg/day. In the remaining four, duodenal bile was studied before and after six weeks treatment with 15 mg/kg/day of UCA. The bile samples were stored at -20°C until analysed for their bile acid, phospholipid and cholesterol content, as previously described.¹⁹ Individual bile acids were analysed by gas liquid chromatography.

The biliary cholesterol saturation indices (SIs) were calculated using the Thomas and Hofmann polynomial equation²⁰ and the solubility limits of Hegardt and Dam²¹ and Holzbach *et al.*²² No correction was made for changes in biliary bile acid composition.²³

SERUM LIPIDS AND LIVER FUNCTION TESTS

Blood samples were taken for full blood counts, serum bilirubin, alanine transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, total protein and albumin concentrations and fasting lipids before treatment, at the time of each intubation, and every six months thereafter. Lipoprotein electro-

Fig. 2 Moles % cholesterol (upper panel) and the cholesterol saturation index, as defined by Hegardt and Dam²¹ and Holzbach et al²² (lower panel), in fasting bile rich duodenal fluid obtained before treatment (0 mg UCA/kg/day) and after six weeks treatment with approximately 5, 10, and 15 mg UCA/kg/day. The saturation index values are uncorrected for non-micellar solubilisation of cholesterol (individual data points with means (SE)).

phoresis was done only if abnormalities of serum total lipid concentrations were found.

STATISTICAL ANALYSIS

Results were expressed as means (SE). Statistical analysis was by Student's paired or unpaired *t* tests or analysis of variance, as appropriate.

Results

BILE ACIDS AND BILE LIPIDS: DOSE RESPONSE STUDIES

The effect of UCA treatment on the moles % cholesterol in bile and on the associated SIs, is

Table 2 Biliary bile acid composition (duodenal bile) in gall stone patients studied before (baseline) and during six weeks treatment with approximately 5, 10, and 15 mg UCA/kg/day

	LCA %	DCA %	CDCA %	UDCA %	CA %	7KLCA %
Baseline						
Number	11	11	11	11	11	11
Mean	4.6	20.4	28.0	2.0	43.4	0.72
SE	1.3	2.4	3.4	1.8	3.4	0.5
5 mg/kg/day						
Number	7	7	7	7	7	7
Mean	4.2	28.8	21.6	1.4	29.9	0.00
SE	0.7	4.8	4.9	1.4	2.3	0.00
10 mg/kg/day						
Number	7	7	7	7	7	7
Mean	5.5	27.2	19.9	1.1	23.0	0.00
SE	1.3	5.4	4.6	1.1	5.4	0.00
15 mg/kg/day						
Number	10	10	10	10	10	10
Mean	4.0	29.0	17.4	3.1	19.9	1.6
SE	1.3	3.6	2.0	2.1	4.9	1.2

illustrated in Figure 2. The corresponding changes in biliary bile acid composition are given in Table 2. The data for the per cent UCA conjugates in bile with the different UCA doses, are shown in Figure 3.

BILE LIPIDS

Before treatment, the moles % bile acids, phospholipids and cholesterol were 64.4 (SE 1.95), 23.3 (1.7)%, and 11.4 (1.27)% respectively, giving a mean calculated SI of 1.44 (0.22).

After six weeks treatment with approximately 5 mg and 10 mg UCA/kg/day, the moles % cholesterol fell significantly to 8.5 (0.94)% ($p < 0.05$, compared with baseline), and to 5.6 (0.83)% ($p < 0.02$) respectively, then rose slightly to 7.7 (0.76) on the 15 mg/kg dose. The corresponding SIs were 1.25 (0.14), 0.77 (0.13) ($p < 0.05$), and 1.26 (0.16), respectively.

Therefore, SIs were significantly lower than baseline values only with 10 mg/kg dose and even then, two patients (numbers 2 and 6) had supersaturated bile (SIs of 1.3 and 1.2 respectively).

BILIARY BILE ACIDS

The molar percentage of UCA conjugates in bile rose from undetectable levels before treatment to 14 (3.6)% with 5 mg ($p < 0.001$), 23 (3.6)% with the 10 mg ($p < 0.001$) and 24 (5.1)% with the 15 mg UCA/kg/day ($p < 0.001$) doses (Fig. 3).

The enrichment of bile with UCA was 'accommodated' by reciprocal falls in the per cent of both the primary bile acids CA and CDCA. The reduction in the proportion of CA, however, was greater than that of CDCA with the result that the molar ratio of

CA:CDCA which was 1:50:1 before treatment, fell to 1:38:1 with the 5 mg, 1:15:1 with the 10 mg and 1:14:1 with the 15 mg UCA/kg/day doses, respectively.

Whilst the molar percentage of CA (normally the main precursor of DCA) fell, there was a 41% increase in the mean per cent DCA conjugates between the pretreatment and the 5 mg/kg UCA treatment periods, but with little overall change thereafter (Table 2). This implies that during UCA therapy, either there was a major increase in bacterial 7 alpha dehydroxylation of the substrate CA or, more likely, in 7 beta dehydroxylation of the administered UCA and its conjugates. This suggestion is supported indirectly by the molar ratios of product: potential substrate. The DCA:CA ratio increased markedly from 0.47:1 before treatment to 0.96:1 with the 5 mg, 1.17:1 with the 10 mg and 1.45:1 with the 15 mg UCA doses. If both CA and UCA had acted as substrates for DCA, however, one would have expected a more fixed DCA:(CA+UCA) ratio. This, essentially is what was found, the mean DCA:(CA+UCA) ratios being 0.46:1, 0.66:1, 0.56:1, and 0.66:1.

The fact that the molar percentage of the potentially hepatotoxic bile acid, lithocholic acid (LCA), was maintained during UCA therapy, despite falling proportions of its precursor CDCA, suggests that the dehydroxylation fraction must have increased – although only to a modest extent (Table 2).

RELATIONSHIP BETWEEN MOLES % CHOLESTEROL AND THE % UCA CONJUGATES IN BILE

The relationship between the moles % cholesterol and the % UCA conjugates in bile, is shown in Figure

Fig. 4 Relationship between the moles % cholesterol and the per cent UCA conjugates in fasting bile-rich duodenal fluid. From these data, a linear regression line ($n=35$; $y=25.67-1.22x$; $r=0.468$; $p<0.005$) may be derived such that, on average, for every 10% increase in the moles % UCA conjugates, there is a 16% drop in the moles % cholesterol in bile (95% confidence limits 9–22%).

4. There was a trend towards lower moles % cholesterol values as the bile became enriched with UCA conjugates.

GALL STONE DISSOLUTION EFFICACY

Of the 13 patients who started UCA treatment, four stopped in less than six months before any on-treatment ultrasound or x-ray studies had been carried out. In three, this was because of intolerable diarrhoea whilst the fourth, who had been symptom free, simply defaulted from follow up.

Nine patients remained on treatment at six months, seven at 12 months, three at 18 months and one at 24 months. Of these nine patients, only one (patient 2) showed complete gall stone dissolution (confirmed by two consecutive cholecystograms and two ultrasonograms three months apart during continued treatment), after 18 months UCA therapy in a dose of 10.8 mg/kg/day. In the dose response study, his bile had been supersaturated with cholesterol (SI of 1.3) during treatment with 10 mg/kg/day. Four patients underwent elective cholecystectomy, of whom three had experienced episodes of mild biliary colic during treatment.

ADVERSE SIDE EFFECTS

Caused by gall stones

No patient experienced cholecystitis, cholangitis, obstructive jaundice, pancreatitis, or severe biliary colic whilst on treatment.

Caused by UCA therapy

The only adverse symptom during UCA therapy was

Table 3 Results of liver function tests and fasting serum lipids before and during the UCA dose response study (see text) and in patients on longterm UCA therapy

		Pretreatment baseline (10 mg/kg/day)	Dose-response studies			Longterm therapy treatment with approxi- mately 10 mg UCA/kg/day (months)		
			5 mg/kg/day	10 mg/kg/day	15 mg/kg/day	6	12	18
Liver function tests								
ALT (n<43 IU/l)	Number	11	7	7	10	8	6	4
	Mean	31.9	32.9	36.4	32.6	27.7	26.9	33.2
	SE	3.2	3.7	4.6	2.8	2.7	2.3	4.4
ALP (n=20–90 IU/l)	Number	11	7	7	10	9	6	4
	Mean	54.8	54.9	53.4	55.9	55.2	55.7	56.5
	SE	4.6	6.6	5.3	4.3	4.8	5.5	10.9
Bilirubin (n<17 µmol/l)	Number	11	7	7	10	9	6	4
	Mean	10.5	13.9	12.4	11.1	7.9	6.0	6
	SE	1.8	2.7	2.1	3.0	1.4	1.1	1.8
GGT (n<51 U/l M) (n<35 U/l F)	Number	11	6	7	9	8	4	3
	Mean	29.5	41.1	41.9	36.4	34.4	31.5	38.0
	SE	8.5	9.9	12.2	10.0	12.0	16.5	21.9
Fasting serum lipids								
Cholesterol (N<30 yr=3.1 –6.2; n>50 yr=4.1–8.5 mmol/l)	Number	11	6	7	9	8	5	4
	Mean	6.3	6.5	6.6	7.6	7.3	7.2	7.8
	SE	0.4	0.5	0.6	0.4	0.6	0.6	0.7
Triglyceride (n<2.2 mmol/l)	Number	11	6	7	9	8	5	4
	Mean	2.0	1.7	1.7	2.5	2.2	2.6	2.1

diarrhoea, which was experienced by, and necessitated treatment withdrawal in three patients.

HAEMATOLOGY AND CLINICAL CHEMISTRY

With one exception, there were no haematological abnormalities in any patient before or during UCA treatment. The exception was in patient 2, who had a raised MCV of 103fl (N86–96) and a high serum gamma glutamyl transpeptidase (GGT) before and during treatment. These abnormalities were attributed to excessive alcohol intake.

The clinical chemistry results (liver function tests and fasting serum lipids), are summarised in Table 3.

Liver function tests

Although there was a tendency to hypertransaminasaemia during the dose response study, the mean serum alanine transaminase levels remained within the normal range (0–43 U/l) and, in fact, fell after 6, 12, and 18 months treatment with UCA.

Mean serum bilirubin concentrations increased slightly during the dose response studies but again, remained within the normal range. Serum alkaline

phosphatase values remained normal in all patients. There were slight, non-sustained increases in serum gamma glutamyl transpeptidase in patients 4 and 5, which fell to within normal limits during continued treatment.

Fasting serum lipids

Mean serum cholesterol concentrations were higher at every six week period during the dose response study, compared with baseline, although these differences were not statistically significant. Two of the nine patients treated for six months or more, had raised concentrations of fasting serum cholesterol – patient 6 after six months therapy (8.3 mmol/l) and patient 2 at 18 months (9.3 mmol/l). Both these patients had increased beta and prebeta lipoproteins.

The mean concentrations of fasting serum triglycerides increased in the patients given 15 mg/kg/day during the dose response study when there was a 25% increase above the pretreatment baseline value (NS). Again, patients 2 and 6 had raised serum triglycerides during continued treatment.

Discussion

The results of this study fall into two parts: the bile acid and bile lipid dose response studies and the subsequent clinical studies of gall stone dissolution.

DOSE RESPONSE STUDIES

The decision to treat the patients for six weeks with each UCA dose was based on the results of our previous speed-of-change study with CDCA in which we showed that it took up to four weeks before the bile lipid changes reached a new steady state.¹⁷ The progressive increase in dose was designed to reduce any carryover effect and to restrict the number of duodenal intubations to a maximum of four.

The dose response study suggested an optimal dose of 10 mg UCA/kg/day for gall stone dissolution. Why there should be an apparently less favourable bile lipid response with the 15 mg/kg dose is not known, although a similar 'plateau' effect has been reported for UDCA.²⁴

The enrichment of bile with the administered bile acid during UCA therapy is much less (approximately 25% of the bile acid total) than that seen during treatment with either CDCA (75–90%) or UDCA (40–55%). This implies poorer bioavailability of the ingested UCA, greater bacterial degradation, hepatic biotransformation of UCA, or a less potent effect of UCA on primary bile acid synthesis, than occurs with CDCA or UDCA.

Considering each of these points in turn, we have no direct information about UCAs bioavailability. Hepatic biotransformation of ingested UCA seems unlikely. The human liver is not known to isomerise bile acids by converting 7 beta hydroxyl groups to the 7 alpha configuration. Had it done so, the proportion of CA in bile might have risen, rather than fallen. Furthermore, the gas liquid chromatographic results showed no accumulation of 7 keto bile acids in bile (intermediates in the conversion of 7 beta to 7 alpha bile acids). For the reasons already discussed in the Results, UCA may have selectively depressed CA synthesis. We are left, therefore, with the possibility that intestinal bacteria 7 beta dehydroxylate the ingested UCA, to form DCA. This is supported by the 41% increase in DCA conjugates in bile during UCA therapy – particularly since the proportion of CA, which is the usual precursor of DCA, was decreased.

CLINICAL TRIAL OF DISSOLUTION EFFICACY AND SAFETY

In contrast to the results of Zuin and Podda,¹⁸ who reported complete gall stone dissolution in three of six patients with small (<5 mm diameter), floating

stones, we found that only one patient achieved complete gall stone dissolution.

The present results have shown no evidence that UCA is hepatotoxic. Indeed, the mean ALT concentrations fell during UCA therapy. The significance of this observation is unknown but an apparent improvement in liver function has been reported during CDCA and UDCA treatment in gall stone patients²⁵ and in patients with primary biliary cirrhosis during UDCA therapy.²⁶

The fasting serum cholesterol results seen in the present dose response studies, are a cause for concern, in spite of the small number of patients studied.

Unfortunately, UCA therapy produced troublesome diarrhoea in three of the 13 patients. This was probably caused by 'spillover' of cathartic bile acids, particularly DCA, into the colon.

In conclusion, UCA has limited efficacy in dissolving gall stones and as it caused diarrhoea in three of 13 patients and increased mean fasting serum cholesterol concentrations, it confers no advantages over existing forms of bile acid therapy. Nevertheless, the present study makes important contributions to our knowledge about the biochemical and potential therapeutic effects of short term and long term treatment with different bile acids in man, knowledge which can only be gained through clinical experience.

This study was presented in part at a meeting of the Medical Research Society (Howard *et al* [Abstract]. *Clin Sci* 1984; 67: 100–1).

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