Journal of Hepatology, 1989; 8: 1–6
Elsevier

JHEP 00491

Low-dose ursodeoxycholic acid prolongs cholesterol nucleation time in gallbladder bile of patients with cholesterol gallstones

Dieter Jüngst[1], Gerhard Brenner[1], Ekkehard Pratschke[2] and Gustav Paumgartner[1]

Departments of [1]Internal Medicine II and [2]Surgery, Klinikum Grosshadern, University of Munich, Munich (F.R.G.)

(Received 21 April 1988)
(Accepted 11 August 1988)

Summary

The high rate of stone recurrence represents a drawback of non-surgical therapy of cholesterol gallstone disease. Although most studies report that long-term bile acid treatment does not have protective effects, preliminary results suggest that low-dose ursodeoxycholic acid decreases the rate of gallstone recurrence in a subgroup of younger patients. To clarify the underlying mechanism we investigated whether low-dose ursodeoxycholic acid treatment influences biliary cholesterol saturation and/or nucleation time of cholesterol. Ten patients with cholesterol gallstones and functioning gallbladder received 250 mg ursodeoxycholic acid/day at bedtime 6–10 days prior to cholecystectomy. Eleven patients with cholesterol gallstones without treatment served as controls. Cholesterol crystals were present in the gallbladder bile of 7 out of the 10 patients receiving ursodeoxycholic acid and in all control biles. Ursodeoxycholic acid treatment significantly ($P < 0.02$) decreased the cholesterol saturation index (mean ± S.E.: 0.94 ± 0.05 vs. 1.43 ± 0.18) and led to an approximately 5-fold prolongation ($P < 0.005$) of the cholesterol nucleation time (mean ± S.E.: 12.0 ± 2.4 vs. 2.3 ± 0.7 days). We conclude that low-dose ursodeoxycholic acid might be effective in the prevention of post-dissolution gallstone recurrence by both decreasing cholesterol saturation and prolonging cholesterol nucleation time.

Introduction

Several studies have shown that supersaturation of bile with cholesterol and a rapid formation of cholesterol crystals play a key role in the pathogenesis of cholesterol gallstones [1–8].

Dissolution of the stones within the gallbladder can be achieved by oral administration of chenodeoxycholic acid (CDCA) [9–11], ursodeoxycholic acid (UDCA) or a combination of both [12–15]. More recent studies have shown that extracorporeal shockwave lithotripsy combined with bile acid therapy for fragment dissolution is a safe and effective treatment in selected patients with radiolucent gallstones

Part of this work was presented at the annual meeting of the American Gastroenterological Association in Chicago, Illinois on May 13, 1987 and appeared in abstract form in Gastroenterology 1987; 92: 1742.

Correspondence: Dieter Jüngst, M.D., Dept. of Internal Medicine II, Klinikum Grosshadern, Marchioninistr. 15, 8000 München 70, F.R.G. Tel. (49-89) 7095-2573.

[16,17]. However, supersaturation of bile and a reduced nucleation time of cholesterol persist in most patients after stone dissolution and contribute to stone recurrence which has been observed at a rate of about 10% per year during the first years [18–20]. Oral administration of low-dose CDCA has been shown not to be effective in the prevention of stone recurrence after successful litholytic therapy [21]. However, preliminary results suggest that low-dose UDCA decreases the rate of gallstone recurrence in a subgroup of younger patients [22].

To clarify the underlying mechanism we investigated whether low-dose UDCA treatment influences biliary cholesterol saturation and/or nucleation time in patients with cholesterol gallstones.

Materials and Methods

Patients and collection of bile

Twenty one patients who underwent elective cholecystectomy because of symptomatic gallstones were selected for the study. Out of the entire population undergoing cholecystectomy the patients of the study were selected according to the following criteria: functioning gallbladder documented by visualization of the gallbladder by oral cholecystography or scintigraphy within 6 weeks prior to surgery and cholesterol content of the removed stones above 60%. Ten patients, 9 females and 1 male, with a mean age of 50.8 years (range: 23–68 years) and a mean bodyweight of 66.6 kg (range: 47–90 kg) were treated 6–10 days prior to cholecystectomy with 250 mg UDCA per day given at bedtime. Eleven patients, 7 females and 4 males, with a mean age of 46.2 years (range: 19–79 years) and a mean bodyweight of 68.0 kg (range: 48–89 kg) served as controls. Gallbladder bile was aspirated during surgery by puncture of the gallbladder after ligation of the cystic duct using a sterile needle and syringe. Particular care was taken to collect gallbladder bile completely, in order to avoid effects of stratification [23]. The stones were removed with the gallbladder, washed, and analyzed for cholesterol content. The mean cholesterol content of the stones (71.3% vs. 76.8%) was not significantly different between the groups of patients.

Biliary microscopy

After collection, bile samples were mixed thoroughly and one drop was immediately examined by polarized light microscopy for cholesterol crystals. Because small numbers of crystals might be overlooked by the microscopic examination of unspun bile, the sediment of the bile samples was re-examined for cholesterol crystals after ultracentrifugation (100 000 × *g* for 2 h).

For the determination of cholesterol nucleation time 4 ml of gallbladder bile were centrifuged for 2 h at 100 000 × *g* in a Beckman L 50 ultracentrifuge (Beckman Instruments, Fullerton, CA, U.S.A.) to obtain crystal-free bile as described by Holan et al. [2]. The top 1 ml was discarded and the next 2 ml (interphase) were removed by aspiration. An aliquot of the interphase was immediately examined microscopically to confirm the absence of crystals; the rest was placed in sterile tubes, flushed with nitrogen, sealed and incubated at 37 °C. Twice daily for up to 3 weeks the interphase was investigated for the appearance of cholesterol crystals. The interval between time zero and the first detection of a cholesterol crystal in the sample was taken as the nucleation time.

Analysis of bile

For analysis of bile lipids, duplicate aliquots were stored at −40 °C prior to determination. Cholesterol was determined colorimetrically after extraction with petroleum ether [24]. Phospholipids were measured using the colorimetric assay of Fiske and SubbaRow [25] and total bile salts were determined by a modified 3α-hydroxysteroid dehydrogenase method [26]. The saturation index of each sample was calculated by dividing the cholesterol concentration by the maximum cholesterol solubility according to Carey [27].

Individual bile acids were determined by capillary gas liquid chromatography. A 20-m polyethylene glycol 20 000 column (i.d. 0.32 mm) and hydrogen as carrier gas were used [28]. The gas chromatograph was a Carlo Erba 4160 model with an on-column injection system and flame ionization detection (detec-

for temperature 250 °C). A temperature program (60–240 °C, 30 °C/min) allowed an optimal injection technique. Quantitation was performed using an internal standard (hyodeoxycholic acid) and a multipoint calibration obtained with a Spectra Physics SP-4100 integrator plotter. Sample preparation included enzymatic hydrolysis of glycine and taurine conjugates and extraction with diethyl ether after acidification to pH 1 with concentrated hydrochloric acid. The free acids were methylated using an acidified (pH 1) mixture of methanol and 2,2-dimethoxypropane (1:1 v/v) and trimethyl silylated with a mixture of hexamethyldisilasane and trimethylchlorosilane in pyridine (2:1:3 v/v) at room temperature for 30 min.

Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.). The Student *t*-test was applied to assess the statistical significance of differences between means. A P value of < 0.05 was considered statistically significant.

Results

Biliary microscopy and cholesterol nucleation time

Cholesterol crystals were present in the native bile in 7 out of 19 patients under UDCA therapy and in all biles of the 11 control patients. The cholesterol nucleation time was significantly ($P < 0.005$) longer (12.04 ± 2.40 vs. 2.35 ± 0.72 days) in patients receiving low-dose UDCA than in untreated controls. The single values are given in Tables 1 and 2.

Biliary lipid composition

The single values of both groups for total bile acids, phospholipids, cholesterol, total lipids and the cholesterol saturation index (CSI) in the gallbladder biles are displayed in Tables 1 and 2. Statistical analysis of the data revealed significantly ($P < 0.05$) higher values of total bile acids (94.88 ± 9.27 vs. 70.54 ± 4.78 mmol/l) and significantly ($P < 0.02$) lower CSI (0.94 ± 0.05 vs. 1.43 ± 0.18) in subjects treated with UDCA compared to controls. No significant difference between the groups could be obtained for biliary phospholipids, cholesterol or total lipids.

Individual bile acids

The molar percentages of individual bile acids in gallbladder bile of both groups of patients are compared in Fig. 1. The relative amount of CDCA was significantly ($P < 0.001$) lower in patients receiving UDCA while cholic, deoxycholic and lithocholic acid

TABLE 1

COMPOSITION AND CHOLESTEROL NUCLEATION TIME OF GALLBLADDER BILES OF 10 PATIENTS UNDER TREATMENT WITH LOW-DOSE UDCA (250 mg/day) 5–10 DAYS PRIOR TO CHOLECYSTECTOMY

Patient	Age (yrs)	Nucleation time (days)	Bile acids (mmol/l)	Phospholipids (mmol/l)	Cholesterol (mmol/l)	Total lipids (g/dl)	CSI
E.K.	60	4.3	43.0	16.3	2.9	3.5	0.69
A.S.*	57	21.0	52.2	13.0	4.8	3.8	1.27
K.A.	61	3.9	114.9	39.0	13.1	9.2	1.06
M.A.	55	3.9	181.7	68.5	14.9	14.8	0.68
B.B.	55	5.0	81.5	34.0	7.7	7.1	0.81
R.E.	35	16.6	88.4	38.0	11.3	7.7	1.07
K.S.*	65	20.0	105.8	40.3	9.7	8.7	0.82
L.G.	50	3.8	83.5	32.5	7.0	6.9	0.78
I.P.	68	21.0	95.4	44.5	14.1	8.5	1.14
U.S.*	36	21.0	148.0	69.0	19.2	13.4	0.95
Mean ± S.E.	50.8 ± 4.4	12.0 ± 2.4	94.9 ± 9.3	39.5 ± 5.8	10.4 ± 1.4	8.1 ± 0.9	0.98 ± 0.05

* No cholesterol crystals in bile.
CSI = cholesterol saturation index.

TABLE 2

COMPOSITION AND CHOLESTEROL NUCLEATION TIME OF GALLBLADDER BILES OF 11 PATIENTS WITH CHOLESTEROL GALLSTONES WITHOUT TREATMENT PRIOR TO CHOLECYSTECTOMY

Patient	Age (yrs)	Nucleation time (d vs)	Bile acids (mmol/l)	Phospholipids (mmol/l)	Cholesterol (mmol/l)	Total lipids (g/dl)	CSI
K.M.	36	1.8	68.2	27.5	10.0	5.9	1.31
U.G.	19	1.8	85.1	25.5	10.0	6.5	1.28
H.B.	64	0.9	46.5	28.0	7.0	4.7	1.17
I.F.	61	1.8	75.6	28.0	24.3	6.8	2.64
N.L.	58	3.9	56.6	37.5	13.7	6.2	1.57
F.N.	46	8.9	66.5	24.5	19.1	5.90	2.44
L.M.	53	2.7	74.9	20.0	5.0	5.5	0.85
A.A.	28	0.8	87.2	33.0	8.4	7.5	0.89
T.H.	79	0.6	52.2	28.0	7.5	5.0	1.15
R.R.	35	0.7	64.2	42.0	12.0	6.9	1.32
B.W.	29	1.9	99.2	67.0	17.4	10.7	1.14
Mean ± S.E.	46.2 ± 5.5	2.3 ± 0.7	70.5 ± 4.8	32.8 ± 3.8	12.2 ± 1.8	6.5 ± 0.5	1.43 ± 0.18

CSI = cholesterol saturation index.

were not significantly different. As expected, the percentage of UDCA in bile was much higher (18.5 ± 3.2 vs. 2.7 ± 0.9%) under the administration of UDCA.

Correlation of nucleation time to CSI, biliary lipids and age

No correlations were found between the cholesterol nucleation time and the CSI (Fig. 2), the total lipids, total bile acids, phospholipids, cholesterol and age in patients receiving UDCA and control subjects.

Discussion

In our study we administered a short-term treat-

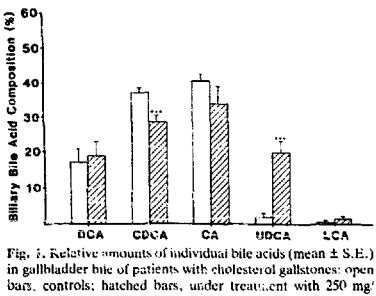

Fig. 1. Relative amounts of individual bile acids (mean ± S.E.) in gallbladder bile of patients with cholesterol gallstones: open bars, controls; hatched bars, under treatment with 250 mg/day UDCA 6–10 days prior to cholecystectomy. ***$P < 0.001$. DCA, deoxycholic acid; CA, cholic acid; LCA, lithocholic acid.

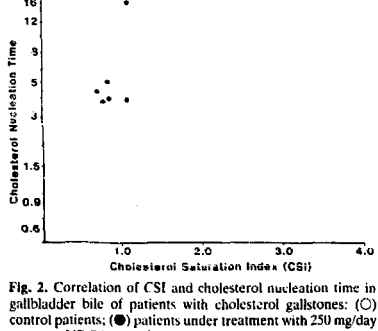

Fig. 2. Correlation of CSI and cholesterol nucleation time in gallbladder bile of patients with cholesterol gallstones: (○) control patients; (●) patients under treatment with 250 mg/day UDCA 6–10 days prior to cholecystectomy.

ment with a low UDCA dose (250 mg/day given at bedtime) in patients who were referred to the surgical department for elective cholecystectomy because of symptomatic gallstones. Drug compliance was documented by measurements of elevated UDCA concentrations in gallbladder bile in all treated patients. In patients under UDCA treatment the percentage of UDCA in bile was higher (mean: 18.5 vs. 2.7%) than in control biles.

These findings are similar to the results of Stiehl et al. [29], who reported a mean relative amount of 28% of UDCA in cholecystokinin-stimulated duodenal bile of patients receiving 250 mg UDCA/day. The higher biliary percentage of UDCA in their study might be caused by the much longer treatment period of 3 months as compared to 6–10 days in our investigation. The period of treatment with UDCA may have been too short to achieve a steady state at the time of bile sampling. The mean proportions of cholic (32 vs. 25%), chenodeoxycholic (28 vs. 31%), deoxycholic (19 vs. 16%) and lithocholic (1.5 vs. 1.8%) acid were similar in both studies.

Compared to controls, UDCA-treated patients revealed a significantly higher mean concentration of total bile acids (94.9 vs. 70.5 mmol/l) and a significantly lower mean CSI (0.94 vs. 1.43). In 6 patients receiving UDCA a CSI below 1 was calculated; the remaining 4 were slightly supersaturated. Cholesterol monohydrate crystals were detected in 7 of the 10 patients. There was no clear relation between the presence of crystals and the CSI. In one bile with the highest degree of saturation (1.27) no cholesterol crystals were found and two biles with the lowest CSI values (0.65 and 0.78) contained multiple crystals (Table 1).

Although the number of crystal-containing biles was similar in UDCA-treated patients (7/10) and in untreated controls (11/11) there was a striking difference in the cholesterol nucleation time between the groups. The mean cholesterol nucleation time in biles from UDCA-treated patients (12 days) was about 5-fold longer than in control biles (2.3 days). These findings indicate that even short-term treatment with low-dose UDCA has a beneficial effect on the rapid cholesterol crystallisation in gallbladder bile of patients with cholesterol gallstones. The results are in agreement with the long-term clinical study of Villanova et al. [22], who showed that low-dose UDCA (300 mg/day) might prevent gallstone recurrence after successful stone dissolution in a subgroup of younger patients.

Our data suggest that the administration of low-dose UDCA may have a protective effect against the recurrence of gallstones after successful dissolution therapy. This short-term study shows favourable alterations of both cholesterol saturation and cholesterol nucleation time of gallbladder bile and should stimulate further long-term clinical studies to substantiate these results.

Acknowledgement

The authors are grateful to Mrs. Benedikta Zündt and Mrs. Elke König for their skillful technical assistance.

References

1 Sedaghat A., Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. N Engl J Med 1980; 302: 1274–1277.
2 Holan KR, Holzbach RT, Hermann RE, et al. Nucleation time: key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 1979; 77: 711–717.
3 Gollish SH, Burnstein MJ, Ilson RG, et al. Nucleation of cholesterol monohydrate crystals from hepatic and gallbladder bile of patients with cholesterol gallstones. Gut 1983; 24: 836–844.
4 Burnstein MH, Ilson RG, Petrunka CN, et al. Evidence for a potent nucleating factor in the gallbladder bile of patients with cholesterol gallstones. Gastroenterology 1983; 85: 801–807.
5 Admirand WH, Small DM. The physicochemical basis of cholesterol gallstone formation in man. J Clin Invest 1968; 47: 1043–1052.
6 Carey MC, Small DM. The physical chemistry of cholesterol solubility in bile. J Clin Invest 1978; 61: 998–1026.
7 Cave PE, Smith BF, LaMont JT. Human gallbladder mucin accelerates nucleation of cholesterol in artificial bile. Gastroenterology 1984; 87: 270–275.
8 Green AK, Ottenhoff R, Noordam C, Zwiers hondt FMJ, Hock FJ, Tytgat GNJ. Cholesterol nucleation promoting activity shifts cholesterol (CH) and phospholipid (PL) from the micellar to the vesicular phase in model bile [Abstract].

Gastroenterology 1987; 92: 1737.
9 Dowling RH. Chenodeoxycholic acid therapy of gallstones. Clin Gastroenterol 1977; 6: 141–163.
10 Thistle JH, Hofmann AF, Ott BJ, Stephens DH. Chemotherapy for gallstone dissolution. I. Efficacy and safety. JAMA 1978; 239: 1941–1946.
11 Schoenfield LJ, Lachin JM. The Steering Committee, The National Cooperative Gallstone Study Group. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: The National Cooperative Gallstone Study. A controlled study of efficacy and safety. Ann Intern Med 1981; 95: 257–282.
12 Tokyo Cooperative Gallstone Study Group. Efficacy and indications of ursodeoxycholic acid treatment for dissolving gallstones. A multicenter double blind trial. Gastroenterology 1980; 78: 542–548.
13 Tint GS, Salen G, Colalillo A, et al. Ursodeoxycholic acid: a safe and effective agent for dissolving cholesterol gallstones. Ann Intern Med 1982; 97: 351–356.
14 Bachrach WH, Hofmann AF. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. Dig Dis Sci 1982; 27: 737–761, 833–856.
15 Roehrkasse R, Fromm H, Malavotti M., Tunuguntla AK, Ceryak S. Gallstone dissolution treatment with a combination of chenodeoxycholic and ursodeoxycholic acids. Studies of safety, efficacy and effects on bile lithogenicity, bile acid pool and serum lipids. Dig Dis Sci 1986; 31: 1032–1040.
16 Sauerbruch T, Delius M, Paumgartner, G, et al. Fragmentation of gallstones by extracorporeal shock waves. N Engl J Med 1986; 314: 818–822.
17 Sackmann M, Delius M, Sauerbruch T, et al. Shock-wave lithotripsy of gallbladder stones. N Engl J Med 1988; 318: 393–397.
18 Dowling RH, Gleeson D, Kuppan DC, Murphy GM, The British/Belgian Gallstone Study Group. Gallstone recurrence and post-dissolution management. In: Paumgartner G, Stiehl A, Gerok W, eds. Enterohepatic Circulation of Bile Acids and Sterol metabolism. Falk Symposium 42. Lancaster: MTP Press, 1985; 361–385.
19 Lanzini A, Jazrawi RP, Kupfer RM, et al. Gallstone recurrence after medical dissolution. An overestimated threat? J Hepatol 1986; 3: 241–246.
20 O'Donnel LDJ, Heaton KW. Recurrence and re recurrence of gall stones after medical dissolution: a longterm follow up. Gut 1988; 29: 655–658.
21 Marks JW, Lan SP, The Steering Committee. Low dose chenodiol to prevent gallstone recurrence after dissolution therapy. Ann Intern Med 1984; 100: 377–381.
22 Villanova N, Bazzoli F, Trabbani R, et al. Gallstone recurrence after successful oral bile acid treatment: a follow-up study and evaluation of long term post-dissolution treatment [Abstract]. Gastroenterology 1987; 92: 1789.
23 Tera H. Stratification of human gallbladder bile in vivo. Acta Chir Scand 1960; 256: (suppl) 4–85.
24 Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952; 195: 357–366.
25 Fiske CH, SubbaRow Y. The colorimetric determination of phosphorus. J Biol Chem 1925; 66: 375–400.
26 Talalay P. Enzymatic analysis of steroid hormones. Biochem Anal 1960; 8: 119–143.
27 Carey MC. Critical tables for calculating the cholesterol saturation of native bile. J Lipid Res 1978; 19: 945–955.
28 Karlaganis G, Paumgartner G. Determination of bile acids in serum by capillary gas-liquid chromatography. Clin Chim Acta 1979; 92: 19–26.
29 Stiehl A, Raedsch R, Rudolph E, Walker S. Effect of ursodeoxycholic acid on biliary bile acid and bile lipid composition in gallstone patients. Hepatology 1984; 107–111.