II Effect of chenodeoxycholic acid treatment in gallstone subjects

T. C. NORTHFIELD, N. F. LARUSSO, A. F. HOFMANN, AND J. L. THISTLE

From the Gastroenterology Unit, Mayo Clinic, Rochester, Minnesota, USA

SUMMARY Oral treatment with chenodeoxycholic acid causes dissolution of cholesterol gallstones in man. In order to determine the mechanism of this effect, we have measured 24-hour biliary lipid output, lipid composition of fasting gallbladder bile, and bile acid pool sizes before and during such treatment in six patients with radiolucent gallstones in functioning gallbladders. In all six patients, the degree of cholesterol saturation of fasting-state gallbladder bile was decreased during treatment to a level below the thermodynamic solubility line. This effect was due to a decrease in biliary cholesterol output, associated with conversion of more than 90% of the total bile acid pool to chenodeoxycholic acid. It could not be attributed to an increase in total bile acid pool size nor to an increase in biliary bile acid or phospholipid output.

We have previously demonstrated that oral administration of chenodeoxycholic acid causes dissolution of cholesterol gallstones in man (Danzinger, Hofmann, Schoenfield, and Thistle, 1972; Thistle and Hofmann, 1973). The initial observation (Danzinger et al, 1972), made in a small uncontrolled series, has now been confirmed in a separate uncontrolled trial (Bell, Whitney, and Dowling, 1972) and in a larger controlled clinical trial (Thistle and Hofmann, 1973). Cholic acid, the other primary bile acid synthesized by the human liver, did not cause gallstone dissolution in this controlled trial (Thistle and Hofmann, 1973).

The exact mechanism for this therapeutic effect is not known, although it has been shown that chenodeoxycholic acid treatment makes fasting gallbladder bile less saturated in cholesterol (Thistle and Schoenfield, 1971; Danzinger, Hofmann, Thistle, and Schoenfield, 1973), and that cholic acid does not have this effect (Thistle and Schoenfield, 1971). It is generally assumed that the mechanism of action involves an increase in biliary bile acid secretion rate, because total bile acid pool size is usually increased by bile acid feeding (Danzinger et al, 1973); but this would only decrease cholesterol saturation of bile if unaccompanied by an equivalent increase in biliary cholesterol secretion. Alternative explanations are a specific effect of chenodeoxycholic acid in enhancing biliary phospholipid secretion or a specific effect in decreasing biliary cholesterol secretion or both.

In order to differentiate among these possibilities, we measured the 24-hour biliary output of bile acids, phospholipid, and cholesterol in six patients with radiolucent gallstones in functioning gallbladders, using a duodenal perfusion technique, and repeated these measurements during oral treatment with chenodeoxycholic acid. Lipid composition of fasting gallbladder bile, bile acid pool sizes, and gallstone size were also measured before and during treatment.

Some of our findings were reported at the annual meeting of the British Society of Gastroenterology, 1973 (Northfield, Larusso, Thistle, and Hofmann, 1973).

Materials and Methods

All six patients had radiolucent gallstones in a functioning gallbladder, as demonstrated by a cholecystogram immediately before the first study. The group included three men and three postmenopausal women, ages 42 to 69 years (mean, 55 years); their weights ranged from 59·1 to 88·6 kg (mean 72·0 kg). Informed consent was obtained before the study.

Measurements of bile acid pool size, lipid composition of fasting gallbladder bile, and 24-hour biliary lipid output were made in all six patients before chenodeoxycholic acid treatment, and these measurements have already been reported in detail (Northfield and Hofmann, 1975). The patients restudied during treatment were subjects 1 to 6 inclusive in that report, and they have been assigned the same numbers in the present communication.

After completion of these baseline studies, the patients were discharged with instructions for an oral chenodeoxycholic acid regimen, the dose being adjusted to the maximum that did not cause diarrhoea. The dose taken ranged from 1·0 to 2·25 g/day (mean, 1·6 g/day). No instructions were given about diet. Dietary histories provided an estimate of cholesterol intake of 316 ± 70 mg/day (mean ± SD) before the first study and 378 ± 139 mg/day before the second study. Another cholecystogram was taken after six months and gallstone size was
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compared without knowledge of treatment status.

The patients were readmitted to a metabolic unit for repeat studies after five to six months of treatment (patients 1, 2, 3, 5, and 6) or after two months (patient 4). Measurements of bile acid pool size and biliary lipid output were carried out in exactly the same order as in the pretreatment observations. The patients continued to take chenodeoxycholic acid during the studies according to the regimen that they had followed at home in the preceding months. Measurements of biliary lipid output were further modified to distinguish ingested chenodeoxycholic acid from secreted biliary bile acid. Ingested cheno-
doxycholic acid, which was unconjugated, was extracted directly from duodenal samples and determined by gas-liquid chromatography as described previously (Northfield and Hofmann, 1975), except that before the extraction procedure, a known amount of nordeoxycholic acid was added as an internal standard, and the saponification procedure was omitted. The value obtained for the concentration of free chenodeoxycholic acid was subtracted from the total bile acid concentration, which was determined enzymatically, in order to obtain the biliary output of conjugated bile acids. Apart from this, the methods used for all measurements and calculations were exactly the same as for the baseline studies (Northfield and Hofmann, 1975).

Results

LIPID COMPOSITION OF FASTING GALLBLADDER

BILE

In all six patients, fasting gallbladder bile became less saturated with cholesterol (fig 1; table I). Before treatment, samples from patients 1 and 4 were supersaturated with cholesterol according to the criterion of Admirand and Small (1968), and those from all six patients were supersaturated according to the criteria of Dam and Hegardt (1971) and of Holzbach, March, Olszewski, and Holan (1973). During treatment, samples from all six patients became unsaturated in cholesterol according to both sets of criteria.

|                                      | Before Treatment | During Treatment | p 1
|--------------------------------------|-----------------|-----------------|---
| **Cholesterol saturation of fasting gallbladder bile (%) according to:** |                 |                 |   |
| Admirand and Small (1968)            | 103 ± 55        | 45 ± 13         | <0·01 |
| Dam and Hegardt (1971) and Holzbach et al (1973) | 190 ± 97        | 73 ± 19         | <0·025 |
| **Bile acid pool sizes (umol/kg)**   |                 |                 |   |
| Chenodeoxycholic acid                 | 20·4 ± 7·2      | 103·1 ± 38·3    | <0·00005 |
| Cholic acid                           | 15·0 ± 4·7      | 0·3 ± 0·5       | <0·00005 |
| Deoxycholic acid                      | 23·5 ± 14·1     | 1·0 ± 0·9       | <0·01 |
| Total                                | 65·3 ± 11·5     | 110·5 ± 44·7    | <0·05 |
| Percentage of total as chenodeoxycholic acid | 31·5 ± 10·8    | 94·5 ± 5·2      | <0·00005 |
| Recycling frequency of bile acid pool (cycles/day) | 7·2 ± 2·5   | 4·6 ± 1·5       | NS  |
| **Total daily biliary outputs (umol/kg)** |                 |                 |   |
| Bile acids                            | 451·6 ± 116·0   | 456·2 ± 93·5    | NS  |
| Phospholipid                          | 215·0 ± 136·3   | 193·8 ± 45·4    | NS  |
| Cholesterol                           | 56·0 ± 12·5     | 35·3 ± 4·5      | <0·025 |
| **Duration of lithogenic bile (hours/day) according to** |     |                 |   |
| Admirand and Small (1968)             | 7·8 ± 7·6       | 1·8 ± 1·6       | <0·05 |
| Dam and Hegardt (1971) and Holzbach et al (1973) | 16·0 ± 7·0    | 3·3 ± 2·7       | <0·005 |

Table I Mean values (± SD) before and during oral treatment with chenodeoxycholic acid

1 p values are based on a paired t test.
Bile acid pool size
In all six patients, there was an increase in the pool size of chenodeoxycholic acid, accompanied by a reciprocal decrease in the pool sizes of cholic acid and deoxycholic acid, so that during treatment 94.5 ± 5.2% of the total bile acid pool was composed of chenodeoxycholic acid (fig 2; table I). Total bile acid pool size increased in four of the six patients. The patient without any change in bile acid pool size (patient 3) was one of those in whom gallstone size had decreased by the end of six months. The daily recycling frequency of the bile acid pool tended to decrease, but this change was not significant.

Total daily biliary lipid output
There was no significant change in mean total daily outputs of bile acids and phospholipid, although the change in phospholipid output paralleled the change in bile acid output in individual patients (fig 3; table I). In two patients, bile acid and phospholipid outputs increased; in two they were unchanged; and in the two patients who failed to show an increase in total bile acid pool size, there was a decrease in both bile acid and phospholipid outputs. There was a significant decrease in mean biliary cholesterol output, that was also present in five patients individually. In the sixth patient (patient 1) there was a slight absolute increase in cholesterol output. This, however, was accompanied by a marked increase in the bile acid and phospholipid outputs, so that cholesterol output decreased relative to bile acid and phospholipid outputs in this patient also.

Hourly output
During treatment there was a significant decrease in the number of hours per day during which bile samples were saturated or supersaturated with cholesterol, according to both sets of criteria (table I).

There was a curvilinear relationship between the hourly rate of bile acid output and the percentage saturation with cholesterol (fig 4), but during treatment this curve was shifted downward and to the left so that samples were rarely supersaturated with cholesterol, even at low bile acid outputs, and were more unsaturated at high bile acid outputs. The shift in this curve could not be accounted for by any alteration in bile acid-phospholipid coupling, but it could be accounted for by a change in bile acid-cholesterol coupling that occurred in all six patients.
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Fig 4 Relationship between rate of bile acid (BA) output and percentage cholesterol (XOL) saturation, according to criterion of Admirand and Small (1968), before (---) and during (--·) treatment. Cholesterol saturation was calculated as described by Thomas and Hofmann (1973). Each line is a moving average of data points, each point depicting secretion during a one-hour collection period.

There was a linear relationship between the hourly rates of bile acid and cholesterol output before and during treatment (fig 5). The two regression lines, before and during treatment, had the same intercept, which was not significantly different from zero, but the slope was twice as steep before treatment as during treatment (p < 0.0005). Thus, for an increase in bile acid output of 20 μmol/kg per hour, there was an increase in cholesterol output of 2 μmol/kg per hour before treatment but of only 1 μmol/kg per hour during treatment.

Gallstone size

In patients 1 and 3, gallstone size was diminished (as judged by oral cholecystography) at the end of six months of treatment; in patients 4, 5, and 6 there was no change; and in patient 2, it was not possible to reassess gallstone size at six months because of poor concentration of the dye.

Discussion

This study has shown that oral chenodeoxycholic acid results in a decrease in biliary cholesterol output. Particularly strong evidence for this is provided by the change in the dose-response curve for bile acid-cholesterol coupling shown in figure 4. This could not be attributed to a decrease in dietary cholesterol intake before the second study. One possible explanation for this effect is that chenodeoxycholic acid decreases hepatic cholesterol concentration in man (Salen, Nicolau, and Shefer, 1973).

In patients with gallstones, chenodeoxycholic acid is considered to suppress hydroxy-methyl glutaryl coenzyme A reductase, the rate-limiting enzyme for cholesterol synthesis (Salen et al, 1973; Coyne, Bonorris, Goldstein, and Schoenfield, 1974). Based on isotope dilution studies, cholesterol synthesis is probably also suppressed (Pedersen, Arnfred, and Hess Thaysen, 1974), but total body cholesterol pools do not appear to change (Hoffman, Hofmann, and Thistle, 1974). Chenodeoxycholic acid administration has been shown to suppress cholic acid synthesis (Danzinger et al, 1973), but as yet it is not known whether chenodeoxycholic acid suppresses its own synthesis in man. No information is available on the effect of chenodeoxycholic acid on the absorption of dietary or biliary cholesterol, so as yet our findings that chenodeoxycholic acid diminishes cholesterol secretion in bile cannot be incorporated into an overall description of cholesterol metabolism in patients receiving chenodeoxycholic acid.
Although gallstone dissolution by chenodeoxycholic acid appears to be entirely explicable by decreased cholesterol secretion in bile, it cannot be assumed that gallstone formation is caused solely by increased cholesterol secretion. We have previously shown, in a small series, that Caucasian with gallstones had the same total daily biliary cholesterol output as controls carefully matched for weight, age, and sex (Northfield and Hofmann, 1973). Furthermore, control subjects had the same regression line for bile acid-cholesterol coupling as did untreated gallstone patients (Northfield and Hofmann, 1975).

Since this decrease in biliary cholesterol output during chenodeoxycholic acid treatment was not accompanied by any consistent change in bile acid or phospholipid output, the degree of cholesterol saturation of bile was altered. We have previously shown, in both gallstone and control subjects, a curvilinear relationship between bile acid output and the degree of cholesterol saturation of bile, such that hepatic bile is supersaturated with cholesterol at low bile acid outputs and unsaturated at high bile acid outputs (Northfield and Hofmann, 1975). During treatment with oral chenodeoxycholic acid, this curve was shifted downwards and to the left, so that samples were rarely supersaturated with cholesterol at low bile acid outputs and were more unsaturated at high bile acid outputs. For expression of these results (fig 4), the line representing 100% saturation is based on the limit for cholesterol saturation defined in an in vitro system of bile acids, phospholipid, and cholesterol containing 80-95% water by Admirand and Small (1968). This figure is intended only to illustrate changes in the relative degree of cholesterol saturation at different bile acid outputs, and not the absolute position of the line for 100% saturation, since hepatic bile samples may contain more than 95% water, and since more recent in vitro experiments (Dam and Hegardt, 1971; Mufson, Triyanond, Zarembo, and Ravin, 1972; Holzbach et al, 1973; Tamesue, Inoue, and Juniper, 1973) suggest that the true solubility line at thermodynamic equilibrium requires a slightly lower relative proportion of cholesterol than described by Admirand and Small (1968). The effect of chenodeoxycholic acid in reducing the degree of cholesterol saturation of hepatic bile was reflected also in the results obtained from fasting gallbladder bile. In showing these results (fig 1) we have drawn both solubility lines. The absolute position of these in-vitro lines can be applied directly to fasting gallbladder bile, which almost invariably contains less than 95% water. Theoretically, for gallstone dissolution to occur, the lipid composition of bile entering the gallbladder would have to fall below the true solubility line at thermodynamic equilibrium. Our results are consistent with this prediction: before treatment, samples from all six patients lay above the line of Dam and Hegardt (1971) and of Holzbach et al (1973), whereas during treatment samples from all six patients fell below this line.

Published clinical trials have used the maximal tolerated dose of chenodeoxycholic acid in order to have the best chance of a therapeutic effect. Because gallstone patients have a decreased bile acid pool size (Vlahcevic, Bell, Buhac, Farrar, and Swell, 1970), it has been thought necessary to give a dose sufficient to increase bile acid pool size and secretion rate. In the present study, the dose of chenodeoxycholic acid was adjusted according to bowel function in exactly the same way as in the previous clinical trials (Danzinger et al, 1972; Thistle and Hofmann, 1973) reported from this institution, and treatment was continued during the repeat studies so as to reproduce the therapeutic situation as closely as possible. The results obtained confirm our previous study (Danzinger et al, 1973) in showing that an increase in bile acid pool size is not essential to make fasting gallbladder bile unsaturated in cholesterol or to dissolve gallstones. In addition, this study shows that bile acid secretion may actually decrease in patients whose gallstones are dissolving. What appears to be required is the conversion of a sufficient proportion of the total bile acid pool to chenodeoxycholic acid for a critical effect on biliary cholesterol output. The dose required for this has not been defined but may be considerably less than that currently used in clinical trials (about 20 mg/kg). This is important because of concern over a possible hepatotoxic effect of high doses of chenodeoxycholic acid. In the rhesus monkey, chenodeoxycholic acid at 20 mg/kg causes an increase in serum glutamic-pyruvic transaminase levels, associated in some instances with histological changes of bile duct proliferation (Webster, Lancaster, Wease, Hofmann, and Baggenstoss, 1973). In man, chenodeoxycholic acid at doses of 1 to 2 g/day causes a one- to two-fold increase in serum glutamic-oxaloacetic transaminase levels in about one-fourth of patients (Bell et al, 1972; Thistle and Hofmann, 1973). However, the levels subsequently decrease to normal, and these increases are not associated with an alteration in any other liver function tests or with significant histological abnormality.

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