

Effect of Rowachol on biliary lipid secretion and serum lipids in normal volunteers

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SUMMARY The effect of Rowachol (200 mg tid), an essential oil preparation, on biliary lipid secretion and serum lipids was measured in six healthy male volunteers before and after four weeks of treatment. Biliary cholesterol and phospholipid secretion increased significantly from 113 ± 36 (SD) $\mu\text{mol/h}$ to 155 ± 52 $\mu\text{mol/h}$ ($p < 0.05$) and from 409 ± 145 $\mu\text{mol/h}$ to 587 ± 185 $\mu\text{mol/h}$ ($p < 0.05$), respectively. Bile acid secretion increased from 1519 ± 662 $\mu\text{mol/h}$ to 2287 ± 1175 $\mu\text{mol/h}$ ($p > 0.05$ and > 0.10). This marked increase in biliary lipid secretion was not followed by a change in molar composition of biliary lipids and lithogenicity of bile. Serum cholesterol and triglycerides declined from 4.9 mmol/l to 4.1 mmol/l ($p < 0.05$) and from 1.2 mmol/l to 0.9 mmol/l ($p < 0.05$) respectively. The ratio of high-density-lipoprotein cholesterol to total cholesterol increased from 0.22 to 0.31 ($p < 0.05$). Although it has been shown previously that Rowachol could dissolve cholesterol gall stones the present results indicate that Rowachol alone has only weak litholytic properties, at least in normal volunteers, but might have several advantages when combined with chenodeoxycholic or ursodeoxycholic acid.

Chronic administration of either chenodeoxycholic acid or ursodeoxycholic acid has been shown to reduce lithogenicity of bile thereby promoting dissolution of cholesterol gall stones.¹⁻³ Recently, another agent, Rowachol, has been shown to be effective in dissolving gall stones^{4,5} and biliary tract stones.⁶ This essential oil preparation containing the mono- and bicyclic monoterpenes menthol (32% w/v), pinene (17% w/v), menthone (6% w/v), borneol (5% w/v), camphene (5% w/v) and cineole (2% w/v) in olive oil (ROWA-Wagner KG, 5060 Bergisch-Gladbach 1, FRG) has been used as a choleric and therapeutic agent for biliary tract diseases in Europe for more than 25 years.^{7,8} Whereas chenodeoxycholic acid and ursodeoxycholic acid reduce the lithogenicity of bile by decreasing biliary lipid output of cholesterol⁹⁻¹¹ the mechanism by which Rowachol effect biliary lipid metabolism has not been elucidated. The purpose of the present study was to examine the effects of Rowachol on biliary lipid secretion and serum lipid concentration in normal volunteers.

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Methods

EXPERIMENTAL DESIGN

Six healthy young male volunteers were studied as outpatients at the Metabolic Unit, Department of Medicine, University of Bonn. The study was conducted in accordance with the principles of the Helsinki declaration and informed consent was obtained from each subject. None of the subjects had evidence of gastrointestinal disease and routine liver function tests were normal. Age, body weight, and height are given in Table 1. During the study period each subject was on his usual diet and no changes in body weight occurred.

Biliary lipid secretion rates were measured before

Table 1 Age, body weight and height of the volunteers

Subject	Age (yr)	Body weight (kg)	Height (cm)
1	23	84	184
2	22	76	192
3	25	77	190
4	26	74	187
5	25	70	176
6	26	69	180

and after four weeks of administration of Rowachol (200 mg tid). Blood was taken after an overnight fast before and after two and four weeks of administration of Rowachol. Serum was separated and stored at -20°C until serum lipid analysis.

EXPERIMENTAL PROCEDURE

Biliary lipid secretion was measured by the intestinal perfusion technique described by Grundy and Metzger.¹² The evening before the study the subjects were admitted to the Metabolic Ward where they swallowed a triple lumen tube. After an overnight fast the tube was positioned with two proximal outlets adjacent to the ampulla of Vater and a third outlet 10 cm distally just beyond the ligamentum of Treitz. A liquid formula diet (142 cal/kg/h) containing 36% of calories as fat (70% in the form of lard), 16% as protein and 48% as carbohydrates (Nutrodrip, Wander GmbH, 6522 Osthofen, FRG) together with beta-sitosterol (22.5 mg/h) as a non-absorbable marker were infused constantly through the most proximal outlet. After allowing four hours for gall bladder contraction and stabilisation of hepatic bile secretion hourly samples were obtained for the following six hours from the second proximal and the distal outlet by continuous aspiration. During the regimen of Rowachol administration 400 mg of this terpene mixture was given in the morning of the study before liquid formula infusion was started.

Storage of the proximal samples and separation of bile acids from cholesterol and phospholipids were performed according to the method used in the National Cooperative Gallstone Study.¹³ Ten millilitres of the distal samples were added to ethanol. In every proximal and distal sample, cholesterol and beta-sitosterol were measured by gas liquid chromatography as trimethylsilylethers¹⁴ using 5- α -cholestane as internal standard. In every proximal sample bile acids were determined enzymatically^{15,16} and phospholipids were measured by the method of Bartlett.¹⁷ Hourly outputs of cholesterol, bile acids and phospholipids were then calculated according to equations given by Grundy and Metzger¹² along with correction factors for cholesterol content of the perfused formula. Biliary lipid composition was expressed as molar per cent for each lipid component according to Admirand and Small¹⁸ and per cent saturation was calculated by the criteria of Carey and Small¹⁹ assuming a total lipid content of hepatic bile of 5 g/dl.

Individual bile acids were determined in every proximal sample by gas liquid chromatography. The conjugated bile acids were deconjugated enzymatically (cholyglycin hydrolase; Sigma Chemicals Co, St. Louis, Mo. (20)) and solvolised.²¹

After methylation²² and derivatisation to their trimethylsilylethers¹⁴ the individual bile acids were separated on a fused silica capillary column (SP 2100, 25 m, ID 0.20–0.21 mm, Hewlett Packard, Palo Alto, CA) using an automatic injection system (7671 A Automatic Sampler, Hewlett Packard).

SERUM LIPIDS

Total lipids, cholesterol, phospholipids and triglycerides were determined by the micromethod of Egge *et al.*²³ High-density-lipoprotein cholesterol was determined in the supernatant after precipitation of apolipoprotein-B containing lipoproteins with dextran sulphate/magnesium chloride²⁴ using a micromethod described recently.²⁵ Previous studies by one of us²⁵ have shown that high-density-lipoprotein-cholesterol determined by this micromethod was in good agreement with high-density-lipoprotein-cholesterol determined by ultracentrifugation according to Havel *et al.*²⁶

STATISTICAL ANALYSIS

The results are expressed as mean \pm SD. Results between control period and Rowachol administration were compared with the Wilcoxon's signed rank test.

Results

EFFECT OF ROWACHOL ON BILIARY LIPID SECRETION

Biliary lipid outputs during control period and after administration of Rowachol are given in Table 2. Biliary cholesterol secretion averaged 113 $\mu\text{mol/h}$ during control period and increased after administration of Rowachol by 37% to 155 $\mu\text{mol/h}$ ($p < 0.05$). Biliary secretion of phospholipids averaged 409 $\mu\text{mol/h}$ during control period and increased significantly by 36% to 587 $\mu\text{mol/h}$ during Rowachol treatment ($p < 0.05$). The average increase in bile acid secretion from 1519 $\mu\text{mol/h}$ to 2287 $\mu\text{mol/h}$ (51%) was not significant ($p > 0.05$ and < 0.10). Administration of Rowachol had no effect on molar composition of cholesterol, bile acids, phospholipids or the lithogenic index (Table 3). Molar ratios of cholesterol to bile acids, cholesterol to phospholipids and phospholipids to bile acids during the control period averaged 0.09 ± 0.03 , 0.29 ± 0.09 and 0.28 ± 0.07 , respectively. Administration of Rowachol did not alter these ratios (0.08 ± 0.03 , 0.27 ± 0.05 and 0.29 ± 0.09 , respectively). No change in the molar composition of individual bile acids was observed during administration of Rowachol, except for lithocholic acid, the smallest fraction, which increased (Table 4).

Table 2 Biliary lipid secretion rates in normal volunteers during control period and after four weeks of treatment with Rowachol (200 mg tid)

Subject	Cholesterol ($\mu\text{mol/h} \pm \text{SD}$)		Phospholipids ($\mu\text{mol/h} \pm \text{SD}$)		Bile acids ($\mu\text{mol/h} \pm \text{SD}$)	
	CO	ROW	CO	ROW	CO	ROW
1	151±16	133±43	579±92	582±269	1484±252	1845±894
2	90±14	141±22	253±65	673±173	913±177	2752±548
3	122±17	155±18	590±146	568±117	2689±755	2570±720
4	57±8	119±43	334±149	473±166	1415±240	1090±354
5	151±5	255±41	405±34	884±191	1725±179	4245±596
6	109±23	125±22	295±59	339±65	888±214	1219±265
$\bar{x} \pm \text{SD}$	113±36	155±51†	409±145	587±185†	1519±662	2287±1175‡

Abbreviations: CO = Control period; ROW = Rowachol period.

* Values for each subject represent the average for 6 determinations during the steady state period of formula infusion.

† Significantly different from CO ($p < 0.05$).

‡ p between 0.05 and 0.10.

EFFECT OF ROWACHOL ON SERUM LIPIDS

Mean total lipids, cholesterol, triglycerides and phospholipids before, after two and four weeks of treatment with Rowachol are presented in Table 5. A significant reduction of triglyceride concentration in serum was observed after two and four weeks. Phospholipids and total lipids showed a significant decrease after four weeks. High-density-lipoprotein-cholesterol increased slightly (14%) after four weeks, and with the parallel small but not significant decrease in total cholesterol the ratio of high-density-lipoprotein-cholesterol to total cholesterol increased significantly ($p < 0.05$).

Discussion

EFFECTS OF ROWACHOL ON BILIARY LIPID SECRETION

The results of this study in normal volunteers show that administration of Rowachol (200 mg tid) for

four weeks increases biliary outputs of bile acids, phospholipids, and cholesterol to almost the same rate extending previous studies in animals²⁷ and preliminary results in patients with indwelling T-tube after cholecystectomy.²⁸ The obtained secretion rates of biliary lipids are in the same range as in other groups of normal volunteers published previously²⁹ as well as studied recently in our department. (In 20 healthy volunteers the hourly secretion rates of cholesterol, phospholipids, bile acids and the LI of hepatic bile averaged 120 ± 19 , 420 ± 140 , 1776 ± 277 $\mu\text{mol/h}$ and 0.96 ± 0.26 , respectively). Whether the present results obtained in normal volunteers might be extrapolated to patients with cholesterol gall stones has to be elucidated. How Rowachol promote this parallel increase in output of all biliary lipids remains unclear. Other drugs for treatment of cholesterol gall stone disease or hyperlipidemia do not induce such remarkable effects on biliary lipid secretion.

Table 3 Biliary lipid composition and lithogenic index of stimulated hepatic bile in normal volunteers during control period and after 4 weeks of treatment with Rowachol (200 mg tid)

Subject	Cholesterol (molar %) (mean±SD*)		Phospholipids (molar %) (mean±SD*)		Bile acids (molar %) (mean±SD*)		Lithogenic Index (CS) (mean±SD*)	
	CO	ROW	CO	ROW	CO	ROW	CO	ROW
1	6.9±0.7	5.6±1.3	26.2±1.5	24.0±6.5	66.9±2.0	70.4±6.8	0.93±0.11	0.84±0.19
2	7.2±0.7	4.0±0.3	20.3±5.2	18.7±2.1	72.5±5.6	77.3±2.1	1.18±0.08	0.71±0.05
3	3.7±0.6	4.9±0.8	17.5±1.7	17.4±1.1	78.8±2.0	77.7±1.9	0.70±0.11	0.91±0.14
4	3.2±0.2	7.1±1.3	18.1±5.1	28.2±4.7	78.7±5.2	64.7±5.9	0.59±0.12	0.93±0.20
5	6.6±0.4	4.7±0.5	17.8±0.5	16.3±1.8	75.6±0.8	79.0±1.9	1.20±0.07	0.92±0.07
6	8.5±0.1	7.5±0.7	23.0±2.3	20.3±1.4	68.5±2.8	72.2±2.0	1.29±0.09	1.23±0.10
$\bar{x} \pm \text{SD}$	6.0±2.1	5.6±1.4	20.5±3.5	20.8±4.5	73.5±5.1	73.6±5.5	0.98±0.29	0.92±0.17

Abbreviations: CO = Control period; ROW = Rowachol period; CS = Lithogenic Index calculated according to Carey and Small¹⁹ assuming a total lipid content of hepatic bile of 5 g/dl.

* Values for each subject represent the average for 6 determinations during the steady state period of formula infusion.

Table 4 Mean molar per cent of individual bile acids in bile in normal volunteers during control period and after four weeks of treatment with Rowachol (200 mg tid)

Subject	LICA (molar %*)		DCA (molar %*)		CDCA (molar %*)		CA (molar %*)		UDCA (molar %*)	
	CO	ROW	CO	ROW	CO	ROW	CO	ROW	CO	ROW
1	0.29	1.09	19.95	31.58	39.24	38.82	37.64	29.48	2.87	1.04
2	0.52	0.72	24.03	24.16	41.15	47.33	30.03	24.07	4.26	3.72
3	0.12	0.98	20.80	19.45	43.37	43.48	30.64	32.03	5.08	4.06
4	0.98	1.03	25.79	23.01	53.12	46.58	15.32	26.14	4.80	3.24
5	0.85	1.86	15.20	19.42	43.92	44.55	39.07	33.15	0.96	1.02
6	1.26	1.17	17.32	12.29	49.62	49.33	30.11	36.10	1.69	1.11
Mean	0.67	1.14†	20.51	21.65	45.07	45.02	30.16	30.47	3.28	2.37
±SD	0.43	0.38	3.98	6.39	5.27	3.67	4.50	8.43	1.71	1.46

Abbreviations: LICA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; CA = cholic acid; UDCA = ursodeoxycholic acid; CO = Control period; ROW = Rowachol period.

* Values for each subject represent the average for 6 determinations during the steady state period of formula infusion.

† Significantly different from CO ($p < 0.05$).

The observed increase of biliary lipid output had no consistent effect on lithogenicity of hepatic bile. Molar ratios of cholesterol to bile acids, cholesterol to phospholipids and phospholipids to bile acids were not changed after Rowachol administration indicating that this terpene mixture did not alter the coupling of cholesterol or phospholipids to bile acids. As shown in Table 3, however, in four of the volunteers with a lithogenic index greater than 0.9 treatment with Rowachol slightly decreased the lithogenic index. These results are comparable with those of acute studies by Doran *et al.*³⁰ With the same dose regimen these investigators found a significant decrease in cholesterol saturation of gall bladder bile obtained by needle aspiration in patients at the time of abdominal surgery.

EFFECTS OF ROWACHOL ADMINISTRATION ON SERUM LIPIDS

The beneficial effects of Rowachol on serum lipids

Table 5 Mean serum lipids during control period and after 2 weeks and 4 weeks of administration of Rowachol (200 mg tid)

	CO (mean±SD)	2 weeks (mean±SD)	4 weeks (mean±SD)
Total lipids (g/l)	6.53±0.91	6.40±0.69	5.22±1.14*
Triglycerides (mmol/l)	1.22±0.29	0.95±0.22*	0.87±0.29*
Phospholipids (mmol/l)	2.89±0.92	3.09±0.68	2.09±0.62*
Total cholesterol (mmol/l)	4.91±0.38	4.89±0.63	4.07±0.64
HDL-cholesterol (mmol/l)	1.09±0.14	1.38±0.32	1.25±0.23
HDL-cholesterol Total cholesterol	0.22±0.04	0.28±0.06*	0.31±0.05*

Abbreviations: CO = Control period; ROW = Rowachol period.

* Significantly different from CO ($p < 0.05$).

support previous studies in patients with cholestasis²⁸ and hyperlipoproteinaemia.³¹⁻³³ The significant increase in the absolute values of high-density-lipoprotein-cholesterol during treatment with this terpene preparation observed by Bell *et al.*³⁴ and Hordinsky and Hordinsky³³ could not be confirmed in the present study, probably because normolipidemic volunteers were studied during shorter treatment periods. When expressed as relative values, however, (ratio of high-density-lipoprotein-cholesterol to total cholesterol) the change from 0.22 to 0.31 was statistically significant ($p < 0.05$). The beneficial effect of this terpene preparation on serum lipids might be of advantage for treatment of hyperlipidemic patients with increased risk factors for gall stone formation as common lipid lowering drugs like clofibrate,³⁵ bezafibrate,³⁷ and probably nicotinic acid³⁶ increase lithogenicity of bile.

MECHANISM OF CHOLESTEROL GALL STONE DISSOLUTION BY ROWACHOL

The mechanism whereby Rowachol induces cholesterol gall stone dissolution is not clear. In the present study the lithogenic index in normal volunteers was not influenced consistently by Rowachol, in contrast with known effects of chenodeoxycholic acid and ursodeoxycholic acid. Thus, other effects after Rowachol administration seems also to be important for gall stone dissolution. In addition to the relative concentration of bile acids, phospholipids and cholesterol total lipid content of bile is another important determinant of cholesterol solubility.¹⁹ In the present study Rowachol increased total lipid output and total lipid concentration of duodenal content (0.67 g/l versus 0.87 g/l; $p < 0.05$). This observation is in line with results of

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