BILE LIPIDS IN EXPERIMENTAL EXTRAHEPATIC CHOLESTASIS

Yu. A. Bogdarin

UDC 616.36-008.811.6-092.9-07:616.36-008.839.15-074

KEY WORDS: lipid complex of the bile; extrahepatic cholestasis; cholelithiasis

Studies of lipid metabolism in extrahepatic cholestasis, leading under certain conditions to lithogenesis [10, 12], point to the necessity for both quantitative and qualitative evaluation of the composition of lipid complexes circulating in the enterohepatic system [3, 5]. However, much of this evidence requires clarification because of the nonhomogeneity of the results and the contradictory nature of opinions which have been expressed on the diagnostic value of levels of certain lipid fractions and fatty acids [4, 9].

The aim of this investigation was to study the lipid complex of the bile in experiments with extrahepatic cholestasis and to discover criteria for the early diagnosis of cholelithiasis.

EXPERIMENTAL METHOD

Experiments were carried out on 19 rabbits and 6 Java macaques from the Sukhumi Primatologic Nursery, weighing 1.2-2.0 kg and 2.8-6.0 kg respectively. All the animals were females. Graded occlusion of the bile duct by 40-60% in the region of the duodenum was carried out on 10 rabbits and 3 monkeys. The animals received a diet deprived of the lipid component. The rabbits were killed 4 weeks and the monkeys 12 weeks after the operation by intravenous injection of a 2% solution of hexobarbital. Five rabbits developed calculous cholecystitis, and in the other 5 rabbits and 3 monkeys a noncalculous form of cholecystitis (NCC) was found. The fractional composition of bile acids [2], neutral lipids [6], and phospholipids, and also the fatty acid composition of the principal lipid fractions of the bile in the gall bladder were analyzed. Fractions of bile acids obtained after fractionation on Silufol UV-254 plates (Czechoslovakia) in a toluene:glacial acetic acid:water (8:12:1 by volume) system were analyzed with the aid of a "Bian-170" model 821 densitometer. Fractions of neutral lipids and phospholipids were separated on glass plates measuring 18 × 30 cm on LSL_{254} silica-gel (Czechoslovakia) in corresponding solvent systems. Fractions of neutral lipids were obtained after chromatography in two systems of solvents: 1) diethylether:benzene:96% ethyl alcohol:glacial acetic acid (40:50:2:0.2 by volume), 2) hexane:diethyl ether (94:6 by volume). Phospholipids fractions were obtained after chromatography in a system of chloroform:methyl alcohol:water, twice in one direction. This was followed by densitometry on the IFO-451 microphotometer. The stain of the lipids was developed after staining with $K_2Cr_2O_7$ solution in an 80% solution of H_2SO_4. The quantities of the separate fractions were calculated with the aid of calibration curves plotted on the basis of determination of corresponding standard preparations: cholesterol, phosphatidylcholine (PC), lysophosphatidylcholine (LPC), and free fatty acids (FFA), including palmitic (16:0), oleic (18:1), and arachidonic (20:4). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Specific features of the composition of bile acids were found in in the bile of the experimental animals: in rabbits glycoconjugates were predominant, but tauroconjugates in monkeys. The total bile acid content in the bile of the rabbits was 115.2 ± 15.8 mmoles/liter, in the monkeys 197.6 ± 30.7 mmoles/liter.

Gor'kii Research Institute of Pediatrics, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR M. L. Studenikin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 144-146, August, 1991. Original article submitted October 1, 1990.

0007-4888/91/0008-1097$12.50

TABLE 1. Content of Principal Lipid Fractions and Fatty Acids in Bile of Animals with Extrahepatic Cholestasis (M ± m)

Lipid fraction	Rabbits			Monkeys	
	control (n=9)	NCC (n = 5)	CC (n = 5)	control (n = 9)	NCC (n = 3)
GDC + GCDC	94,9±14,4	76,1±16,7	8,8±0,9	29,3±6,1	37,5±7,1
DC	6,6±0,9	5,2±0,7	0,3±0,9*	8,3±1,6	6,5±1,2
CDC	—	6,0±0,7	5,2±2,4	23,1±4,5	19,3±1,5
TC	3,9±0,9	5,0±1,4	4,1±1,0	38,8±7,7	74,0±8,4*
TG	2,9±0,4	3,8±0,5*	6,4±0,6***	0,7±0,1	9,6±0,1
Cholesterol	5,0±0,7	4,6±0,7	12,4±2,8***	12,0±0,9	15,2±1,9
CE	0,5±0,2	13,6±1,7*	5,9±1,1**	0,5±0,1	0,9±0,2*
LPC	0,1±0,03	3,0±0,6*	0,5±0,1*	0,04±0,01	0,06±0,02
PC	0,4±0,1	0,5±0,1*	0,8±0,2*	0,7±0,1	1,3±0,3*
K_2	12,1±0,5	4,3±0,4*	3,4±0,5*	15,0±0,6	10,0±0,9*
16:0 acid	30,3±4,6	8,6±0,7*	4,3±0,6***	23,0±1,4	7,7±0,9*
TG					
PC	51,0±2,5	41,5±5,0*	34,8±2,1*	37,2±2,4	32,7±1,9*
18:1 acid	43,0±2,0	45,5±5,6	27,9±2,7***	33,8±1,4	50,6±4,9*
18:1 acid					
TG					
PC	11,9±2,1	7,4±1,8	19,9±3,4***	19,0±0,7	28,1±1,0*
20:4 acid	15,5±4,0	17,3±1,8	26,0±7,3*	23,8±1,2	25,7±2,3
TG					
PC	9,1±0,9	15,8±3,7	20,9±1,1*	6,0±0,5	2,9±1,0*
	4,8±0,6	1,9±0,5*	1,6±0,4*	4,6±0,4	2,0±0,3*
K_3					
TG	4,3±0,7	5,6±1,2	1,7±0,5***	2,0±0,2	1,2±0,2*
PC	2,8±0,6	2,6±0,8	1,1±0,3***	1,4±0,2	2,0±0,4

Legend. K_1) ratio between CE and cholesterol levels, K_2) ratio between PC and LPC levels, K_3) ratio between levels of 16:0 and 18:1 acids. * indicates significant difference compared with control, ** difference between results in groups of animals with NCC and CC.

Total quantity of bile acids in the bile of the animals with NCC remained unchanged, but chenodeoxycholic acid (CDC) appeared in their composition in rabbits, whereas in monkeys the quantity of taurocholic acid (TC) was increased. Meanwhile in the rabbits the content of neutral lipids was increased: from 7.8 ± 1.0 mmole/liter in the control to 22.6 ± 1.4 mmole/liter in NCC ($p < 0.001$), including an increase in the triacylglyceride (TG) content, whereas in monkeys the increase in the neutral lipid content was smaller. Among the phospholipids in the bile of these animals a decrease in the PC content was observed, and in monkeys, in addition, there was an increase in the LPC content. In the fatty acid spectrum of the TG and PC, which were dominant among the lipid fractions, there was an increase in the proportion of the 16:0 acid in TG and a decrease in the fraction of the 20:4 acid in PC; in monkeys there was a decrease in the content of the 16:0 acid and an increase in that of stearic (18:0) and the 18:1 acids in TG, accompanied by a decrease in the content of the 20:4 acid in TG and PC.

In the calculous form of cholecystitis (CC) in rabbits the bile acid pool in the liver was reduced to 38.0 ± 5.6 mM ($p < 0.001$) on account of a marked decrease in the total content of glycodeoxycholic (GDC) and glycochenodeoxycholic (GCDC) acids accompanied by an increase in the content of deoxycholic acid (DC) (Table 1). The content of neutral lipids was increased to 27.8 ± 1.9 mM ($p < 0.001$), and the concentration of cholesterol esters (CE) in their composition was increased by 11.7 times, that of cholesterol by 2.4 times, and of TG by 2.2 times. The content of phospholipids, including PC, was reduced by 3.6 times, and this was accompanied by doubling of the LPC concentration. In the composition of TG and PC the content of the 16:0 acid was reduced whereas that of the 18:1 acid was increased. Among the fatty acids in the TG fraction the level of the 20:4 acid was increased, whereas in PC it was reduced. Analysis of the chemical composition of the lipid component of cholesterol biliary calculi in rabbits showed that neutral lipids predominated in them: cholesterol 59.5 ± 3.1% TG 17.6 ± 2.2%, and CE 13.5 ± 1.7%.

Assessment of the total content of bile acids, cholesterol and phospholipids enables the lithogenic index (LI) to be calculated: this is a generally accepted index which characterizes the ability of the bile to solubilize cholesterol [3]. In the control rabbits it was 0.75 ± 0.05, in NCC it was 1.76 ± 0.29 ($p < 0.05$), and in CC it was 4.62 ± 0.68 ($p < 0.001$). In the control monkeys LI was 0.14 ± 0.01, in NCC it was 0.50 ± 0.04 ($p < 0.001$). Parallel with determination of LI in the present investigation we also calculated three coefficients: the CE/cholesterol ratio (K_1), the PC/LPC ratio (K_2), and the

ratio of 16:0/18:1 acids (K_3). The K_1 index in the experiments changed variously: in rabbits with NCC it was increased by 30 times, in those with CC by a lesser degree (by 5 times), and in monkeys with NCC it was unchanged. The value of K_2 was reduced in animals of both species with NCC, but in rabbits with CC it was smaller than both the control value and the ratio in CC. The values of K_3 for TG and PC in rabbits with CC did not differ from the controls; in rabbits with CC they were 2.5 times less for TG and 2.6 times less for PC than the control values; if the results of animals with CC and NCC are compared with one another, K_3 was 3.3 times less for TG and 2.4 times less for PC, and finally, in monkeys with NCC it was 1.7 times less for TG compared with the control. Since with low values of K_2 and K_3 (compared with the control) a difference existed in the rabbits with NCC and CC, these coefficients can be used for the differential diagnosis of different stages of cholelithiasis.

The lower initial level of LI in Javanese macaques than in rabbits is typical of several families of the lower monkeys that are used as models of cholelithiasis [13]. The differences in the degree of increase of the LI index in rabbits with NCC and CC do not permit it to be used as a differential, and it has therefore been suggested that instead of a triangular system, a quadrangular system of coordinates be used, with the introduction of FFA as parameter for the calculation of LI characteristic of both CC and NCC [9]. Elevation of the CE level in the bile is evidence of a change in the activity of the corresponding enzymes: ACAT, LCAT, and CE hydrolase. Reduction of ACAT (EC 2.3.1.26) activity is known to lead to cholesterol accumulation in the endoplasmic reticulum of the hepatocytes, to the formation of lithogenic bile, and to the appearance of cholesterol calculi [12]. A parallel considerable rise of the LPC level in the composition of the lipid complexes of the bile in rabbits and monkeys in extrahepatic cholestasis is characteristic of animals with a compressed bile duct [14] and, as a result, it leads to damage of the epithelium of the mucous membrane of the gall bladder [11]. The increase in the contribution of the 18:1 acid to the spectrum of fatty acids of the biliary lipids of the experimental animals is not accidental, for preferential incorporation of this acid into lipids is known to take place in the malabsorption syndrome, accompanying cholelithiasis, and also during the development of alimentary hypercholesterolemia in rabbits, accompanied by accumulation of cholesterol in the liver and by progressive saturation of the bile followed by the formation of cholesterol calculi. Yet another factor stimulating the formation of cholesterol concretions in the gall bladder may be a high level of the 20:4 acid, in particular in TG, in rabbits with CC, for it has been suggested that it can produce prostaglandins and the mucus of the mucous membrane of the gall bladder.

Thus under conditions of extrahepatic cholestasis in experimental animals, because of specific changes in the composition of the biliary lipids, objective pauses of the development of cholelithiasis are present.

LITERATURE CITED

1. Yu. A. Bogdarin, G. A. Boyarinov, and L. V. Gorbunova, Lab. Delo, No. 9, 17 (1982).
2. A. I. Ivanov, Lab. Delo, No. 8, 504 (1973).
3. Xh. Z. Mansurov, Problems in Gastroenterology, ed. by V. Kh. Vasilenko [in Russian], No. 7, Dushanbe (1991), pp. 3-20.
4. V. A. Smogol', L. A. Maksimova, et al., Advances in Hepatology: Methods, Facts, Concepts, ed. by A. F. Blyuger [in Russian], Riga (1988), pp. 126-131.
5. D. L. Alvaro, A. Bantafora, A. F. Attill, et al., Comp. Biochem. Physiol., 83, No. 3, 551 (1986).
6. C. P. Freeman and D. West, J. Lipid Res., **7**, 324 (1966).
7. C. P. Henegouwen, A. T. van Berge, et al., Clin. Chim. Acta, **165**, No. 1, 27 (1987).
8. S. P. Lee, C. Tasman-Jones, and V. Carlisle, Am. J. Pathol., **124**, No. 1, 18 (1986).
9. C. Mingrone, A. V. Grego, and A. Bertuzzi, Comput. Biomed. Res., **19**, No. 4, 311 (1986).
10. J. J. Müller, "Experimentelle Untersuchungen bei extrahetatischer Cholestase: Energieshaushalt und Raster-elektronen-mikroskopische Untersuchungen," Doctoral Dissertation, Munich (1988).
11. D. H. Neiderhiser, Am. J. Pathol., **124**, No. 3, 559 (1986).
12. J. L. Smith, J. Jorsey, et al., Clin. Chim. Acta, **158**, No. 3, 271 (1986).
13. A. Schwaier, H. I. Wies, and J. van der Linden, Res. Exp. Med., **176**, No. 2, 157 (1979).
14. R. Weiner, Ernahrungs-forsch., **28**, No. 3, 72 (1983).