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ROLE OF ESTROGENS IN REGULATION OF THE BILE ACID COMPOSITION OF THE ENTEROHEPATIC SYSTEM OF RABBITS AND MONKEYS WITH EXTRAHEPATIC CHOLESTASIS

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UDC 616.36-008.811.6-092.9-07:
616.36-008.829.3-02:615.256.51

KEY WORDS: extrahepatic cholestasis; bile acids; enterohepatic system; estrogens.

The study of the action of estrogens on lipid metabolism in cholestasis is extremely important because this disease is sex-dependent [8, 10]. The link between induction of cholecystasis by retention of bile and its formation after the use of contraceptives is known [7]. During the formation of cholesterol biliary calculi (CBC) a leading role is ascribed to changes in the metabolism of bile acids on whose biosynthesis and secretion into the bile complex formation between biliary lipids in a solution of bile depends, in the hepatocytes [5]. Since the liver is an extragenital organ for endogenous and exogenous sex steroids, and is influenced by these substances [1], analysis of the bile acid composition of the enterohepatic system (EHS), which depends to a certain degree on the estrogen level [9], is important in the study of the mechanism of CBC formation. This paper gives the results of a comparative study of bile acid levels in EHS of rabbits and monkeys with extrahepatic cholestasis associated with estrogen deficiency and excess.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 6, pp. 599-601, June, 1991. Original article submitted December 17, 1990.

EXPERIMENTAL METHOD

Experiments were carried out on 24 female rabbits weighing 1.2-2.0 kg and nine male Java macaques weighing 2.8-6.0 kg from the Sukhumi Primatologic Nursery. Nine rabbits and three monkeys served as the control. Measured constriction of the bile duct by 40-60% of its lumen was carried out in the region of the duodenum of 15 rabbits and six monkeys. On 10 rabbits and three monkeys unilateral ovariectomy (ULA) was carried out, and another five rabbits additionally received injections of estradiol dipropionate (EDP) intramuscularly, in an oily solution, in a sessional daily dose of 100 μg/kg for 4 weeks, with intervals of 3 days. The animals received a fat-free diet. The rabbits were killed 4 weeks and the monkeys 12 weeks after the operations by intravenous injection of 2% hexobarbital solution. The contents were taken from the gall bladder, blood flowing from the intestine was taken from the portal vein, and the liver was perfused and treated to separate the cells [4]. The fraction or composition of the bile acids in the hepatocytes and of the bile was determined by thin-layer chromatography [1], and in the blood by gas chromatography [2]. Acids were analyzed by means of a Bian-170 densitometer, model 821. Quantities of individual fractions were calculated from calibration curves plotted on the basis of determination of corresponding standard preparations (Biolar, Olaine, Latvia). Thee serum estradiol was determined by radioimmunoassay, using highly specific antiserum. The statistical analysis was carried out by Student's t test.

EXPERIMENTAL RESULTS

As a result of measured constriction of the bile duct in the experimental animals a noncalculous form of cholecystitis (NCC) was formed, and was accompanied by specific changes in bile acid levels (Table 1). Concentrations of glycodeoxycholic (GDC) and glycochenodeoxycholic (GCDC) acids were changed in the liver cells of rabbits with NCC, and as a result, the ratio of conjugated derivatives of glycocholic and taurocholic acids (G/T) also was changed. The fraction of taurocholic acid derivatives was increased, reflecting a defensive-adaptive process in the hepatocytes essential for inhibiting cholelithiasis [3]. Meanwhile there was an increase in the concentration of free forms of cholic and chenodeoxycholic (CDC) acids: by 2.1 and 1.6 times respectively. In a similar experiment on monkeys with NCC the cholic acid concentration was increased by 1.8 times. After ULO in rabbits and monkeys with NCC, the blood estradiol concentration was down to the trace level, the total bile acid concentration in the hepatocytes was unchanged, but levels of free bile acids were down to the control values. Thus the rabbits had a tendency for the total acid level to fall on account of a decrease in cholic acid and CDC, accompanied by a simultaneous increase in levels of glycocholic acids (GDC and GCDC) and restoration of the normal G/T ratio. In monkeys a tendency was noted for levels of conjugated derivatives of glycocholic acid to increase. Consequently, ULO under conditions of NCC has a similar action in rabbits and monkeys on bile acid levels: it increases the formation of conjugated glycocholic acid derivatives and reduces concentrations of free forms of bile acids. This process during NCC is evidently not accidental, despite the fact that in rabbits concentrations of conjugated glycocholic acid derivatives predominate, but taurocholic acid derivatives predominate in monkeys, and changes in their levels are less marked in monkeys In a solution of bile from animals with NCC the G/T ratio changed: in rabbits toward an increase in conjugated taurocholic acid derivatives (15.8 ± 1.3 in the control and 8.9 ± 1.3 in NCC, $p < 0.05$), and in monkeys the changes were not significant (0.42 ± 0.09 in the control and 0.40 ± 0.10 in NCC, $p < 0.001$). After gonadectomy in rabbits and monkeys with NCC the level of conjugated taurocholic acid derivatives increased to 7.0 ± 1.4 ($p < 0.05$) in rabbits and to 0.18 ± 0.04 ($p < 0.001$) in monkeys. Levels of free forms of bile acids in the animals, both in the bile and in hepatocytes, were raised in NCC but lowered, basically to the control values, after ULO. Consequently, a lower level of bile acids is found in the bile of rabbits with NCC after ULO than in the bile of the corresponding group of monkeys. In both species of animals, in this case a similar reduction in the bile cholesterol level was found: in rabbits 14.7 ± 0.8 mmole/liter in the control and 3.6 ± 0.8 mmole/liter in NCC + ULO ($p < 0.001$); in monkeys 2.2 ± 0.2 mmole/liter in the control and 1.7 ± 0.2 mmole/liter in NCC + ULO ($p > 0.001$). The lithogenic index in rabbits was 0.75 ± 0.05 in the control and 0.40 ± 0.04 in NCC + ULO ($p < 0.001$), and in monkeys 0.14 ± 0.02 in the control and 0.09 ± 0.02 in NCC + ULO ($p < 0.001$). ULO in rabbits with NCC lowered the blood levels of three free forms of bile acids, increased the level of conjugated glycocholic acid derivatives, increased the G/T ratio, whereas in monkeys it reduced the concentrations of two free forms of bile acids, namely deoxycholic (DC) and CDC, without any change in the G/T ratio.

TABLE 1. Effect of ULO and Injection of EDP on Composition of Bile Acids of EHS of Rabbits and Monkeys with Extrahepatic Cholestasis (M ± m)

Biological medium	Group	GC	GDC + GCDC	DC	GDC + GCDC	DC	CDC	Cholic	Total acids	G/T
Hepatocytes	1-	49±11	57±15	40±12	55±16*	42±13	53±7*	30±4*	396±70	0,9±0,1
	2-	40±10	40±10	49±7	83±12**	36±6	22±4**	10±3*	280±75	1,7±0,3**
	3-	60±13	53±11	39±5	118±16	57±7***	26±5	57±6***	412±93	1,4±0,3
	4-	150±20	100±27	100±29	80±16	37±6	6±1	35±3*	628±88	0,5±0,1
	5-	160±43	200±29	140±41	127±25	7±1**	10±2	24±3	668±96	0,7±0,04**
Bile	1-	5,0±1,4	4,3±0,4*	6,8±0,9	76,1±16,7	5,2±0,7	6,9±0,7	—	104,3±18,0	8,9±1,3*
	2	6,2±0,7	6,1±0,6**	5,4±0,5	80,9±11,6	1,8±0,1**	11,3±1,9	—	110,7±21,1	7,0±1,4*
	3-	5,3±1,0	3,3±0,5***	11,3±1,4***	85,6±36,2	9,1±0,9***	2,3±0,6***	—	107,8±39,6	11,3±0,9**
	4-	74,0±8,4*	81,4±7,2	26,2±4,0	37,5±7,1	6,5±1,2	19,3±1,5	9,0±1,0	253,7±24,1	0,4±0,03
Blood draining from intestine	5-	60,7±9,3	131,1±4,6**	12,6±1,0**	21,6±0,8**	13,7±0,9**	5,3±0,3**	6,0±0,4***	235,0±36,5	0,2±0,04**
	1-	3,7±0,5*	2,6±0,6	3,2±1,3	3,9±1,3	6,0±0,6*	4,0±0,5	4.6±0,6*	28,0±6,0	1.1±0,2*
	2-	2,2±0,4**	2,0±0,4	5,9±1,1	5,9±1,4**	3,2±0,4**	2,2±0,6**	2,1±0,5**	26,2±5,4	3,5±0,5
	3-	2,6±0,7	2,9±0,5	2,4±0,6	5,4±0,9***	7,1±1,1***	3,0±0,6	2,2±0,5	25,5±6,1	1,4±0,3
	4-	40,3±7,6	31,0±5,5	8,2±1,1	6,4±0,7	9,1±1,5	8,0±1,0	100±16	113,0±23,3	0,2±0,04
	5-	38,1±9,0	24,3±6,9	10,5±1,9	5,2±0,9	4,3±0,6**	3,3±0,6	83±7	94,0±16,9	0,3±0,04

Legend. Groups of rabbits: 1) NCC, 2) NCC + ULO, 3) NCC + ULO + EDP; groups of monkeys; 4) NCC, 5) NCC + ULO. *) Difference between controls; **) Difference between data in groups 2 and 1, and 5 and 4; ***) Difference between data in groups 3 and 2. In hepatocytes acids were determined in mg/g, in bile and blood flowing from intestine, in millimoles/liter.

In the case of animals with cholecystitis, injection of EDP into the ovariectomized rabbits did not change the total content of bile acids or their conjugated forms, but raised the cholic acid and DC levels in the hepatocytes, so that the G/T ratio fell to values determined in rabbits with NCC. Meanwhile, levels of GC and DC in the bile rose, those of GDC, GCDC, and CDC fell, and the normal G/T ratio was restored Meanwhile, levels of free forms of bile acids rose in the blood, their concentrations of the three types of glycocholic acids fell to values corresponding to their blood levels in rabbits with NCC.

Estrogens and their synthetic analogs are known to modify bile acid synthesis in the liver [6]. In particular, during the use of contraceptives synthesis and the pool of cholic acid rise by 30.3 and 37.4%, whereas CDC concentrations fall by 6.4 and 11.8%. The total pool of bile acids, under these circumstances, correlates with cholesterol synthesis de novo [6]. Estrogens, by modifying bile acid synthesis in the liver, increase the risk of gall stones [6, 11]. In the period from 15 to 40 weeks of pregnancy cholesterol synthesis is increased in the liver and the flow of the main components of the bile — cholesterol, phosphatidylcholine, and to a lesser degree, bile acids, from the liver into the bile is increased, and this is accompanied by a simultaneous decrease in the aqueous fraction of the bile [13]. Parallel with this, the fraction of GC in the serum increases from 0.3 to 0.6 μmole/liter. This parameter during pregnancy, which is accompanied by itching of the skin in 48% of cases, is a more sensitive test for the determination of liver function than the traditional biochemical parameters [12]. However, in the case of experimental extrahepatic cholestasis, no change in the blood GC level was observed in our experiments.

Thus the results of this study of the composition of the bile acids in the main organs and tissues of the EHS in ovariectomized rabbits and monkeys with cholecystitis, and also in rabbits receiving injections of EDP, point to the specific action of sex steroid hormones on regulation of the conjugation of bile acids in the liver, as a result of which more marked changes take place in levels of free forms of bile acids.

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COMPARATIVE STUDY OF ACTIVATION OF LIPID PEROXIDATION AFTER ELECTRICAL DESTRUCTION OF THE MYOCARDIUM AND DURING EARLY DEVELOPMENT OF ACUTE MYOCARDIAL INFARCTION IN EXPERIMENTS ON DOGS

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UDC 616.127-005.8-036.4+616.127-005.4-085.842]-07:616.153.915-39-074

KEY WORDS: arrhythmia; ischemia; lipid peroxidation; electrical destruction

Important factors in the etiology and pathogenesis of many cardiovascular diseases and their associated arrhythmias are stress-induced and ischemic lesions of the myocardium, which are accompanied by activation of free-radical lipid oxidation with the release of toxic products of incomplete lipid peroxidation (LPO) into the blood stream [1, 5, 7, 9, 10, 12]. For that reason the level of LPO metabolites in the general blood stream may be a criterion reflecting the degree of injury of biomembranes. Electrical destruction of the conducting pathways of the heart is nowadays used for the treatment of arrhythmias [2], but no research into the effect of electrical stimulation on LPO processes in cardiomyocyte membranes could be found. Knowledge of the possible contribution of several damaging factors of electrical destruction (the shock wave, the polarizing action of the current, the thermal factor) to activation of LPO is extremely important, for in clinical practice electrical destruction has to be undertaken on patients with arrhythmias based on ischemic heart disease and atherosclerosis, an important component of whose pathogenesis is activation of LPO [1, 7, 9].

The aim of this investigation was a comparative experimental study of the dynamics of LPO metabolites, namely conjugated dienes (CD) and malonic dialdehyde (MDA), in the blood serum of dogs after electrical destruction of the myocardium and after acute myocardial infarction (AMI).

EXPERIMENTAL METHOD

Experiments were carried out on 22 mongrel dogs of both sexes weighing 15-25 kg, anesthetized intravenously with thiopental sodium (1% solution, 0.5-1.0 g per animal), undergoing operations with the assistance of artificial ventilation of the lungs by the RO-2 apparatus. Thoracotomy was performed in the fourth right intercostal space, and this was followed by longitudinal pericardiostomy. The control group 1 contained three animals undergoing mock operations. In the animals of group 2 a model of AMI was created by ligation of the posterior interventricular artery (the presence of AMI was confirmed electrocardiographically and morphologically). In 16 dogs of experimental group 3, complete transverse heart block was created through the aorto-atrial groove by electrical destruction by a discharge from the DI-03 defibrillator, with

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 0007-4888/91/0006-0786$12.50