7. M. Belew, B. Gerdin, G. Lindeberg, et al., Biochim. Biophys. Acta, 621, 169 (1980).
8. V. A. Belitser, T. V. Varetska, V. P. Manyakov, et al., Thrombos. Res., 3, 265 (1983).
9. V. A. Belitser, E. V. Lugovskoy, A. A. Musjalkovskaya, and G. K. Gogolinskaja, Thrombos. Res., 27, 261 (1982).
10. K. H. Hsieh, M. S. Mudd, and G. D. Wilner, J. Med. Chem., 24, 322 (1981).
11. A. P. Laudano and R. F. Doolittle, Proc. Natl. Acad. Sci. USA, 75, 3085 (1978).
12. A. P. Laudano and R. F. Doolittle, Biochemistry (Washington), 19, 1013 (1980).
13. H. A. Scheraga and M. Laskowski, Adv. Protein Chem., 12, 1 (1957).
14. R. Scott, R. Bernstein, and F. C. Westall, Proc. Natl. Acad. Sci. USA, 81, 4339 (1984).

HEPATOTROPIC ACTION OF BENZOBAMIL IN CCl_4 POISONING

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Most of the known antiepileptic agents (phenobarbital, phenytoin, primidone, carbamazepine, sodium valproate) possess hepatotoxicity and are contraindicated by liver diseases [8]. The exception is benzobamil, a derivative of benzoylbarbituric acid, which not only does not disturb liver function, but can also stimulate regeneration of the liver as a mild inducer of the cytochrome P-450-dependent microsomal multipurpose oxidase system [6].

This paper gives data on the effect of benzobamil on metabolic parameters and liver function of rats with severe CCl_4 hepatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 180 male albino rats weighing 200-220 g. For 4 days the animals received by intragastric injection a 50% solution of CCl_4 in olive oil in a dose of 2.5 ml/kg body weight, accompanied by an aqueous suspension of benzobamil in a dose of 75 mg/kg, equivalent to 0.05 LD_{50}. Control animals received CCl_4 and the same volume of distilled water. Activity of various enzymes, and concentrations of RNA, glycogen, protein, and lipids were determined in frozen sections of the liver by histochemical and cytophotometric methods [5]. In survey films stained with hematoxylin and eosin the number of necrotic hepatocytes was counted among 2000 cells in 40 fields of vision. The state of the liver function was judged from the retention of bromsulphthalein (BSP) 45 min after intravenous injection of the dye, and the duration of sleep after intraperitoneal injection of hexobarbital (80 mg/kg body weight). The total content and content of individual fractions of lipids and phospholipids in lipid extracts of the liver [11] were studied by one-layer chromatography on Silufol UF-245 plates (Czechoslovakia) [1], diene conjugates (DC) were determined as in [3], Schiff bases as in [4], and antiradical activity of the lipids as in [12]. The content of reduced glutathione [13] and the kinetics of malonic dialdehyde (MDA) formation for a period of 60 min during stimulation of lipid peroxidation *in vitro* by Fe^{++} and ascorbic acid or by an enzymic method [3] were determined in homogenates of the liver, perfused with KCl and Tris-buffer (pH 7.4). Activity of urocaninase [2], alkaline phosphates (AlP) [10], phospholipase A [7], lactate dehydrogenase (LDH) isozymes [9], aspartate-aminotransferase (AST), and alanine aminotransferase (ALT), and concentrations of total lipids, total and bound bilirubin, and low- and very low-density lipoproteins [4] were measured in the blood serum.

EXPERIMENTAL RESULTS

CCl_4 causes severe liver damage, affecting the bioenergetics and carbohydrate, lipids, and pigment metabolism. On the 4th day of poisoning activity of the cytoplasmic enzymes LDH

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and glucose-6-phosphate dehydrogenase was reduced by half, that of mitochondrial succinate, isocitrate, malate, and α-hydroxybutyrate dehydrogenases by 2.3-2.8 times, and that of glucose-6-phosphatase, a marker of the endoplasmic reticulum, by 3.3 times. Conversely, activity of acid phosphatase, located in the lysosomes, was increased 3.4 times. Concentrations of RNA, protein, and glycogen were recorded by 1.8-2.6 times. Abundant accumulation of neutral fat took place, mainly in the central zones of the lobule. These metabolic disturbances were accompanied by severe damage to the liver structure in the form of loosening of the trabeculae, perisinusoidal edema, and an increase in the number of necrotic hepatocytes (karyopycnosis, lysis, or rhexis) to 5.9% (from 0.89% in intact rats). AlP activity in the endothelium of the sinusoids was increased.

Under the influence of benzobamil activity of enzymes of the tricarboxylic acid cycle, LDH, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, and acid phosphatase of the rats poisoned with CCl_4 was largely restored to normal, although deviation from values observed in the intact control amounted to 30-35%. The RNA and protein concentrations were 47 and 21% higher respectively than normal, the regular radial arrangement of the trabeculae was preserved, and the number of dying hepatocytes was 2.5%. Benzobamil did not prevent inhibition of β-hydroxybutyrate dehydrogenase, the development of fatty degeneration, and glycogen deficiency in the liver cells, dilatation of the perisinusoidal spaces, and increased AlP activity in the sinusoids.

CCl_4 depressed the parameters of liver function. In the group of poisoned animals hexobarbital sleep was lengthened to 43.8 ± 3.14 min compared with the normal value of 19.6 ± 0.89 min (by 2.3 times), a fourfold increase of BSP retention and hyperbilirubinemia developed, whereas the conjugation of bilirubin with glucuronic acid was reduced by half (Table 1).

When rats poisoned with CCl_4 were treated with benzobamil partial normalization of the liver function tests took place. Hexobarbital sleep lasted 26.5 ± 1.06 min. Compared with its value in hepatitis, BSP retention was reduced by 2.4 times, the total bilirubin content by 1.4 times, and the binding of this pigment with glucoronic acid was increased almost to normal (Table 1).

The CCl_4 induced syndrome of hepatocytolysis was accompanied by a significant increase in permeability of the cytolemma for the release of hepatic enzymes into the blood stream. Serum urocaninase activity increased by 25 times, ALT by 8.6 times, ACT by 2.8 times, AlP by 2.5 times, and hepatic LDH isozymes by twice. The De-Ritis ratio (AST/ALT) decreased to 0.9, reflecting the acute course of the hepatitis. Benzobamil inhibited most strongly the increase in LDH activity; activity of the remaining enzymes also was significantly lower than in CCl_4 hepatitis, but ALT, AST, and AlP activity was 2.6, 2, and 1.6 times higher than normal respectively, and urocaninase activity was increased by 8 times (in intact animals traces of this enzyme were present in the blood). The De-Ritis ratio was 2 (Table 1).

The ability of CCl_4 to damage the phospholipid matrix of the membranes and its pro-oxidative properties made it necessary to investigate the antioxidative and membranotropic action of benzobamil. CCl_4 increased the total lipid content in the liver homogenates by 2.4 times, mainly due to an increase of 5.4 times in the triglyceride content, whereas the total phospholipid content showed a moderate decrease, with a change in their fractional composition: the lysophosphatidylcholine content was increased by 1.8 times, cardiolipin by 2.1 times, and the phosphatidylcholine and phosphatidylethanolamine content was reduced by 1.6 and 1.3 times respectively; there was no significant change in the content of phosphoinositiol, sphingomyelin, phosphatidylserine, or phosphatidic acid. The ratio of the total neutral phospholipids to the total acid phospholipids fell to 1.4 (normally 2). The content of total lipids in the serum of rats with CCl_4 hepatitis was increased by 1.6 times and activity of phospholipase A was doubled, whereas the content of low- and very low-density lipoproteins was reduced by 1.7 times (Table 1).

Despite its inducting effect on the microsomal biotransformation system for xenobiotics, including CCl_4, benzobamil thus does not impair the function, metabolism, or structure of the liver in CCl_4 poisoning. Moreover, benzobamil exhibits hepatoprotective antinecrotic properties: it improves oxidative processes in the mitochondria, endoplasmic reticulum, and cytoplasm of the hepatocytes, activates the antitoxic and excretory function of the liver, and reduces raised blood enzyme levels. As a mild inducer, benzobamil evidently stimulates the formation of hepatotoxic metabolites of CCl_4 by only a slight degree with the participation of cytochrome P-450. This unfavorable effect of the drug is completely balanced by its ability to stabilize lysosomal membranes and to inhibit the release of necrosis-

TABLE 1. Effect of Benzobamil on Metabolic Parameters of the Liver and Blood Serum of Rats with CCl_4 Hepatitis (M ± m, mean results of 8-10 determinations)

Parameter	Intact animals	CCl_4 hepatitis	Benzobamil + CCl_4
		Liver	
Total lipids, mg/g liver	48,6±2,12	114,7±4,72*	90,9±11,31
Phospholipids, μg lipid phosphorus/g liver:			
total phospholipids	1103,4±30,51	980,7±37,88*	1028,6±67,17
lysophosphatidylcholine	62,3±6,49	112,4±8,12*	72,5±1,57*
phosphatidylcholine	408,3±16,22	249,1±8,01*	325,7±15,20*
phosphatidylethanolamine	218,3±17,67	166,2±11,04*	172,7±17,15
Cardiolipin	80,4±12,39	173,3±12,12*	117,2±10,25*
DC, optical density units/mg lipids	0,24±0,02	0,61±0,03	0,39±0,04
Schiff bases, relative units/mg lipids	2,5±0,20	8,8±0,44*	7,5±0,59
NADPH-dependent MDA, μmoles/g protein	0,35±0,03	1,3±0,04*	1,0±0,08*
Antiradical activity of lipids, μmoles/g lipid	31,1±1,34	17,2±0,60*	27,8±2,44*
Reduced glutathione, μmoles/g liver	5,5±0,31	2,8±0,26*	3,9±0,36*
		Serum	
Retention of BSP, %	2,2±0,37	11,1±1,03*	4,6±0,71*
Total bilirubin, μmoles/liter	10,2±1,01	38,6±3,89*	27,4±1,18*
Bilirubin glucuronide, μmoles/liter	8,2±1,09	15,9±2,82*	20,4±1,52
Urocaninase, μmoles/liters/h	4,6±1,06	117,4±20,97*	38,8±18,21*
AST, moles/liter/h	0,72±0,071	6,2±0,06*	1,9±0,40*
ALT, moles/liter/h	1,8±0,19	5,6±0,24*	3,7±0,42*
AlP, μmoles/liter/h	8,3±0,36	21,0±1,30*	15,3±1,61*
Liver enzymes: LDH, conventional units/liter	198,2±9,91	392,6±71,33*	189,9±21,25*
Phospholipase A, conventional units/liter	723±64,2	1583±169,1*	665±47,2*
Total lipids, g/liter	2,0±0,10	3,2±0,30*	2,8±0,19
Low- and very low-density lipoproteins, conventional units	15,5±1,14	9,3±1,31*	10,2±0,76

Legend. *$p < 0.05$: for CCl_4, compared with intact animals; for benzobamil, compared with CCl_4.

inducing hydrolases, including phospholipase A, followed by reduction of the formation of the detergent lysophosphatidylcholine. The antioxidative action of benzobamil is of definite importance, for it prevents the stabilizing effect of CCl_4 on DC production, protects natural water- and lipid-soluble antioxidants from destruction, although it does not inhibit the genesis of secondary lipid peroxidation products. The positive side of the pharmacodynamics of benzobamil is its ability to abolish the inhibition of protein synthesis and to intensify regeneration of the parenchyma of the liver.

LITERATURE CITED

1. E. K. Alimova and A. T. Astvatsatur'yan, "Investigation of fatty acids and lipids by chromatography," Advances in Laboratory and Clinic [in Russian], Moscow (1967).
2. V. A. Burobin, N. V. Likhacheva, and G. E. Abgaforova, Lab. Delo, No. 11, 650 (1978).
3. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
4. V. G. Kolb and V. S. Kamyshnikov, A Guide to Clinical Chemistry [in Russian], Minsk (1982).
5. A. G. E. Pearse, Histochemistry, Theoretical and Applied [Russian translation], Moscow (1962).
6. A. S. Saratikov. T. P. Novozheeva, and A. I. Vengerovskii, Vorp. Med. Khimii, No. 2, 23 (1983).
7. S. A. Tuzhilin and A. I. Saluén'ya, Lab. Delo, No. 6, 334 (1975).
8. I. S. Chekman, Complications of Pharmacotherapy [in Russian], Kiev (1980).
9. H. Bergmeyer and E. Bernt, Methods of Enzymatic Analysis, New York (1974), pp. 223-226.
10. O. Bessey, O. Lowry, and M. Brock, J. Biol. Chem., 164, 321 (1948).
11. J. Folch, M. Lees,and G. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
12. J. Glevind, Acta Chem. Scand., 17, 1635 (1963).
13. M. Moron, J. Depierre, and B. Mannervik, Biochem. Biophys. Acta, 582, 67 (1979).
14. A. Tappel, Pathology of Cell Membranes, Vol. 1, New York (1975), pp. 145-150.