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EFFECT OF HEPATOPROTECTORS ON LIPID METABOLISM IN HEPATITIS INDUCED BY CARBON TETRACHLORIDE

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An important role in the pathogenesis of toxic hepatitis is played by the detergent action of lysophospholipids and activation of phospholipases and of lipid peroxidation (LPO), leading to the development of cytolysis of the hepatic parenchyma [2, 3]. The mechanism of the hepatoprotective effect of phospholipid preparations and antioxidants of phenolic nature is considered to be through their inhibitory effect on individual components of the "lipid triad" [5, 11, 13].

The aim of this investigation was to study the effect of the widely used hepatoprotective agents — the flavonoid silybinin and the phosphatidylcholine-containing substance essentiale — on the combination of disturbances of lipid metabolism present in severe toxic hepatitis induced by carbon tetrachloride (CCl_4).

EXPERIMENTAL METHOD

Experiments were carried out on 180 male albino rats weighing 200-220 g. For 4 days the animals were given 2.5 ml/kg of a 50% solution of CCl_4 in olive oil by gastric tube daily, accompanied by an aqueous suspension of silybinin (200 mg/kg; Karsil, from Bulgaria) or a solution of essentiale (80 mg/kg; from Yugoslavia) in ampuls. The doses of the hepatoprotective agents were chosen beforehand in a screening experiment. Control animals received CCl_4 and, instead of the hepatoprotective agents, the same volume of distilled water. The concentrations of total lipid and phospholipid fractions in the lipid extracts of the liver [7] were determined by one-way thin-layer chromatography on Silufol UF-254 plates (Czechoslovakia) [1], and the concentrations of diene conjugates (DC) [3] and Schiff bases [14], and the antiradical activity of lipids [10] also were determined. The reduced glutathione concentration [12] and the kinetics of malonic dialdehyde (MDA) formation were studied in liver homogenates perfused with KCl solution and Tris-buffer (pH 7.4), during stimulation of LPO in vitro by Fe^{++} and ascorbic acid, or by an enzymic method [3]. Activity of acid phosphatase and β-hydroxybutyrate dehydrogenase (HBDH) was determined histochemically in frozen sections of the liver, followed by cytophotometry. The number of necrotic hepatocytes was counted in survey sections through the liver stained with hematoxylin and eosin. Phospholipase A activity [6] and concentrations of total lipids, phospholipids, and low-density and very low-density lipoproteins [4] were measured in the blood serum.

EXPERIMENTAL RESULTS

CCl_4 caused a profound disturbance of lipid metabolism. On the 4th day of poisoning the lipid concentration in liver homogenates was increased by 2.4 times, mainly due to an increase (by 5.4 times) in the amount of triglycerides (Table 1). The concentration of free cholesterol and of its esters also was increased. The relative percentage of triglycerides was increased by 2.3 times, the level of free cholesterol was not significantly changed, and concentrations of monodiacylglycerides, free fatty acids, and cholesterol esters were reduced by 1.7-1.9 times.

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Silybinin and essentiale prevented accumulation of total lipids and triglycerides in the liver by about the same degree, their concentration falling by 1.4-2.1 times compared with those found in CCl_4 hepatitis, but they were 1.6-2.5 times higher than their levels in intact animals. The decrease in the free cholesterol concentration was very small, and the concentration of its esters remained the same as in hepatitis (Table 1).

Under the influence of CCl_4 moderate destruction of phospholipids took place, the concentration of lysophosphatidylcholine increased by 1.8 times and that of cardiolipin by 2.1 times, whereas the concentrations of phosphatidylethanolamine and phosphatidylcholine fell by 1.3-1.6 times. There was no change in the content of fractions of phosphoinositols, sphingomyelin, phosphatidylserine, and phosphatidic acids. Essentiale prevented the decrease in concentration of liver phospholipids, whereas silybinin weakened the damaging action of CCl_4 only a little. Both preparations delayed the formation of lysophosphatidylcholine and cardiolipin and phosphatidylcholine catabolism (Table 1). Silybinin reduced the sphingomyelin concentration by 31% compared with its level in intact animals and restored the phosphatidylethanolamine concentration to normal. Essentiale increased the phosphatidylserine concentration by 28% above normal.

The damaging action of CCl_4 is based on its powerful pro-oxidant effect. The DC content in liver homogenates from poisoned rats was increased by 2.5 times, and the content of Schiff bases by 3.5 times. The rate of MDA formation by nonenzymic and enzymic pathways was increased by 3.4 times. Parallel with this the antiradical activity of the lipids was reduced, with degradation of reduced glutathione. Silybinin and essentiale are characterized by marked antioxidant properties. They prevented the formation of DC, Schiff bases, and MDA, and enhanced the function of tissue lipid-soluble antioxidants and increased the concentration of reduced glutathione (Table 1).

Phospholipase A activity in the blood serum was doubled in CCl_4 hepatitis, the total lipid concentration was increased by 1.6 times, the phospholipid concentration did not change significantly, and the total content of low-density and very low-density lipoproteins was reduced by 1.7 times. The raised blood enzyme and lipid levels were reduced in animals treated with the hepatoprotective agents and the lipoprotein concentration remained low (Table 1).

In the liver parenchyma activity of mitochondrial HBDH was inhibited by the hepatic poison by 2.4 times, lysosomal acid phosphatase activity was increased by 3.4 times, and the number of necrotic hepatocytes was increased to 5.9% (from 0.9% in intact rats). Silybinin and essentiale normalized enzyme activity and reduced the number of dying cells to 2.2 and 3%, respectively.

TABLE 1. Effect of Silybinin and Essentiale on Parameters of Lipid Metabolism in the Liver and Blood Serum in CCl_4 Hepatitis (M ± m)

Parameters studied	Intact animals	CCl_4 hepatitis	Silybinin + CCl_4	Essentiale + CCl_4
	Liver			
Total lipids, mg/g liver	48,6±2,1	114,7±3,8*	75,1±4,8	79,5±8,5*
Total lipid fraction, mg/g liver				
phospholipids	8,5±0,8	6,6±0,2*	6,7±0,5	8,1±0,5*
free cholesterol	6,3±0,5	11,9±0,7*	8,4±1,7	9,8±0,9
triglycerides	12,6±0,6	67,8±4,1*	31,7±3,9*	31,9±4,6*
cholesterol esters	13,1±0,9	18,4±1,1*	17,4±1,1	18,4±1,2
Phospholipid fraction, μg lipid phosphorus/g liver				
lysophosphatidylcholine	63,3±6,5	112,4±8,1*	62,5±11,6*	86,0±4,9*
phosphatidylcholine	408,3±16,2	249,1±8,0*	357,8±23,6*	310,8±13,3*
phosphatidylethanolamine	218,3±17,7	166,2±11,0*	199,5±8,9*	136,8±10,8
cardiolipid	80,4±12,4	173,3±12,1*	127,5±14,7*	107,7±15,0*
Diene conjugates, optical density units/mg lipids	0,24±0,02	0,61±0,03*	0,39±0,04*	0,41±0,02*
Schiff bases, relative units/mg lipids	2,5±0,2	8,8±0,4*	5,6±0,3*	4,6±0,3*
NADPH-dependent MDA, μmoles/g protein	0,35±0,03	1,32±0,04*	0,64±0,04*	0,42±0,02*
	Serum			
Phospholipase A, conventional units/liter	723±64,1	1583±169,1*	954±42,0*	704±30,4*
Total lipids, g/liter	2,0±0,1	3,2±0,3*	1,9±0,3*	2,1±0,3*
Low-density and very low-density lipoproteins, conventional units	15,5±1,1	9,3±1,3*	8,6±0,5	8,0±0,4

Legend. *$p < 0.05$; for CCl_4 – compared with intact animals, for silybinin and essentiale – relative to CCl_4. Mean results of 8-10 determinations shown.

The hepatoprotective effect of silybinin and essentiale is thus due to their antioxidant action and to normalization of function of the liver phospholipids. CCl_4 and its metabolic activation products are known to be membranotropic poisons: they intensify LPO and destroy phospholipids of the mitochondria, microsomes, and other structures of the hepatocytes. In the outer and inner membranes of the mitochondria and microsomal fraction CCl_4 depresses phospholipid biosynthesis, inhibits the conversion of phosphatidylethanolamine into phosphatidylcholine, and causes destruction of arachidonic and decosahexaenoic acids [8, 9]. The mechanism of destruction of the phospholipid matrix of the membranes is connected with accumulation of primary and secondary LPO products coupled with inhibition of the antioxidative defense of the cells, their labilizing action on lysosomes, and consequent release of phospholipase A. The most toxic breakdown product of phospholipids is lysophosphatidylcholine, which leads to destruction of membranes and ultimately to necrosis of the liver parenchyma. The CCl_4-induced steatosis is due to disturbance of mitochondrial function, inhibition of β-oxidation of fatty acids, and delay of lipoprotein transport from the liver into the blood. The hepatoprotective agents silybinin and essentiale, by neutralizing free radicals in LPO reactions, prevent destruction of phospholipids by phospholipase A, reduce the production of lysophosphatidylcholine, steatosis, and necrosis of the liver parenchyma, and activate β-oxidation of fatty acids. The therapeutic action of essentiale in CCl_4-induced hepatitis is evidently due to the antioxidant properties of the phospholipids and not to their replacement effect, for the flavonoid silybinin increases the concentration of endogenous phosphatidylcholine by a greater degree than essentiale.

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