Hepatoprotective Properties of Phospholiv, a Preparation Containing Phosphatidylcholine from Sunflower Seeds and Glycyrrhizic Acid, in Modeled Cirrhosis of Rat Liver

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Hepatoprotective effect of Phospholiv, a phospholipid preparation containing phosphatidylcholine from sunflower seeds and glycyrrhizic acid trisodium salt, is studied using a model of CCl_4-induced cirrhosis of the liver. Phospholiv protects hepatic tissues from necrotic and dystrophic changes and prevents the development of cirrhosis. Phospholiv restores impaired RNA and protein synthes is under conditions of chronic intoxication.

Key Words: *liver; experimental cirrhosis; RNA and protein synthesis; phospholipid preparation*

Structural and functional disturbances of cell membranes play an important role in the development of liver diseases [1]. Phospholipids are the major component of the plasma membrane. They determine its viscosity and permeability, modulate activity of membrane-bound enzymes, and participate in signal transduction [6,11]. Exogenous phospholipids, in particular phosphatidylcholine (PC) containing unsaturated fatty acids, are rapidly transported to the liver, incorporated into hepatocyte membrane, and repair its structure and function in various pathologies [3,4].

We have previously showed that Phospholiv, a phospholipid preparation containing PC from sunflower seed and glycyrrhizic acid (GA) trisodium salt, prevents dystrophic and necrotic changes in hepatocytes, activates macrophagal reaction, and enhances repair processes by restoring the system of protein synthesis, production of albumin and mitochondrial DNA in rat liver after acute poisoning with CCl_4 [9].

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In the present study we explored the hepatoprotective effect of Phospholiv in experimental hepatocirrhosis induced by long-term treatment with CCl_4. We studied the effect of the preparation on the development of fibrotic and cirrhotic changes in the liver and on DNA, RNA, and protein synthesis in hepatocytes. The pharmacopeial preparation Essentiale (Rhone-Poulenc Rorer) was used as the reference drug.

MATERIALS AND METHODS

Experiments were performed on Wistar male rats weighing 160-180 g kept under natural illumination and fed standard diet *ad libitum*.

CCl_4 (10 mmol/kg) was injected intraperitoneally in 50% olive oil twice per week. Control animals received olive oil.

For preparation of Phospholiv, 50 mg/ml PC and 10 mg/ml GA trisodium salt (Institute of Organic Chemistry, Ural Division of the Russian Academy of Sciences) were suspended in 100 mM Tris-HCl buffer, pH 7.4. The suspension was sonicated (30 sec, 10 times, at a 60-sec interval) in an MSE

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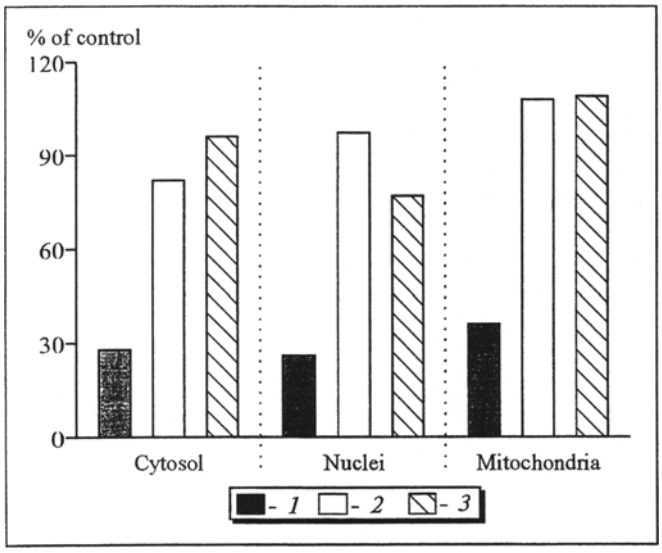

Fig. 1. Effect of preparations on ^{14}C-leucine incorporation into proteins of subcellular fraction from the liver of rats with CCl_4-induced cirrhosis. Control, cpm/mg DNA: 72320±1750 (cytosol), 12170±550 (nuclei), and 2830±260 (mitochondria). Here and in Fig. 2: means of 5 experiments are presented. *1*) CCl_4; *2*) CCl_4+Essentiale; *3*) CCl_4+Phospholiv.

Soniprer-150 ultrasound desintegrator at a medium power and constant cooling.

Phospholiv, PC and GA were tested for cytotoxicity on cultured L-929 cells before *in vivo* experiments. The tests showed that cultivation of L-929 cells for 4 days in the presence of Phospholiv or its components in the incubation medium in concentrations of 50 μg/ml-1 mg/ml has no effect on the rate growth.

Phospholiv and Essentiale (200 mg/kg) were administered intragastrally through a tube 3 times per week.

For determination of the rate of RNA and protein synthesis ^{14}C-orotic acid and ^{14}C-leucine (specific activity 40 and 80 mCi/mmol, respectively) were injected intraperitoneally in a dose of 50 μCi/100 g 1 h (^{14}C-orotic acid) and 10 min (^{14}C-leucine) prior to decapitation. The liver was rapidly frozen in liquid nitrogen and stored at -80°C.

Procedure of homogenization, isolation of nuclei and mitochondria, and radiometry of an acid-soluble fraction and of RNA and protein, as well as quantitative assay of DNA and RNA were described previously [13].

Histological and histochemical studies were performed as follows: the samples were fixed in 10% neutral formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin, with Van Gieson picrofuchsin, by the methods of Brachet (RNA-specific) and Feulgen (DNA-specific), with Sudan IV for neutral lipids, and Sudan Black for alcohol-resistance membrane-bound phospholipids. Morphological parameters of the liver tissue was assessed and expressed using a 5-point scale: no changes (0), minor (1), moderate (2), and pronounced (3) and very pronounced (4) changes.

The number of binucleated cells and mitoses per 1000 hepatocytes and their percentage were determined. The data were processed statistically using the Student test.

RESULTS

Histological and histochemical tests showed that treatment with CCl_4 for 1.5 months induced fibrotic process in the liver, which is a result of replacement of resorpted necrotic foci with the connective tissue and maturation of the granulation tissue (Table 1). This leads to the formation of false lobules due to extensive growth of fibrous septa and to the development of hepatocirrhosis. In animals receiving Essentiale for 1.5 months starting from day 2 of CCl_4 poisoning, the degree of dystrophic changes in the liver was similar to that in CCl_4-treated controls. Only slight inhibition of false lobule formation was noted.

In rats receiving Phospholiv, active regeneration of the liver tissue occurred as soon as 2 weeks after the start of treatment. This manifested itself in an increased number of mitoses and binucleated and polyploid hepatocytes (data not shown).

Phospholiv has no effect on the mitotic index in the liver of CCl_4-poisoned rats. Dystrophic changes in hepatocytes are markedly reduced; the development of connective tissue and cirrhotic rearrange-

TABLE 1. Effect of Phospholiv and Essentiale on Mitotic Index and Liver Morphology in CCl_4-Induced Cirrhosis

Experimental conditions	Dystrophic changes	Intensity of Necrosis	Fibrosis	False lobules	Fibroblastic reaction	Growth of granulation tissue	Inflammation	Mitoses (*M±m*)
Control animals	0.33	0	0	0	0	0	0	0.13±0.04
CCl_4 poisoning	2.3	0.33	2.3	3.0	2.3	0	2.3	0.3±0.07
Essentiale	2.3	0	2.3	2.3	1.7	0	2.0	0.47±0.07
Phospholiv	1.3	0	1.3	1.0	1.0	0	1.0	0.33±0.03

Note. Means of 4 experiments are presented.

ment of the liver are less pronounced in comparison with untreated CCl_4-poisoned rats. These data suggest that Phospholiv exhibits hepatoprotective properties; it protects liver cells against necrotic and dystrophic changes and prevents the development of hepatocirrhosis.

Data on protein and RNA synthesis in hepatocytes of rats receiving Essentiale and Phospholiv are summarized in Figs. 1 and 2. The content of newly synthesized proteins in various subcellular fractions from the liver of CCl_4-poisoned rats decreased 3-fold in comparison with the control. In Phospholiv-treated rats, protein synthesis in the cytoplasm returned to the control level (Fig. 1), while in the nuclei it increased but remained below the control level. In animals treated with Essentiale, the level of protein synthesis in the cytosol is below the control, while no differences in the content of labeled proteins in the nuclei were found.

Both preparations completely restored protein synthesis in mitochondria.

Although long-term treatment with CCl_4 had no effect on the synthesis of nuclear RNA precursors, the content of radiolabeled RNA in the cytoplasm (primarily mRNA) 1.5 months after the start of poisoning was reduced 2-fold in comparison with the control (Fig. 2). The decreased content of newly synthesized mRNA in the cytoplasm can presumably be attributed to disturbed transport of mRNA from the nucleus to the cytoplasm or its accelerated degradation under conditions of CCl_4 poisoning the synthesis of mitochondrial RNA either remained unaffected or returned to the control value by the end of the experiment. Phospholiv completely normalizes the content of newly synthesized mRNA in the cytoplasm. In rats receiving Essentiale, the content of newly synthesized mRNA was elevated in comparison with both the control rats and animals treated with CCl_4 but receiving no phospholipid preparations.

Thus, Phospholiv more effectively restores RNA and protein syntheses in liver cells under conditions of CCl_4-induced hepatocirrhosis. This is also confirmed by histological and histochemical analysis.

Preparation containing PC as the main biologically active component prevents alteration of cell membrane in toxic liver damage [2,5]. Intravenous injections of PC to patients with hepatocirrhosis normalizes lipid composition of the erythrocyte membrane [10]. PC-containing liposomes reduce the intensity of free radical processes in the liver and stabilize the phospholipid bilayer of hepatocyte plasma membranes [7]. The hepatoprotective properties of Phospholiv are determined not only by PC but also by high biological activity of GA, a structural analog of steroid hormones (mineral- and glucocorticoids).

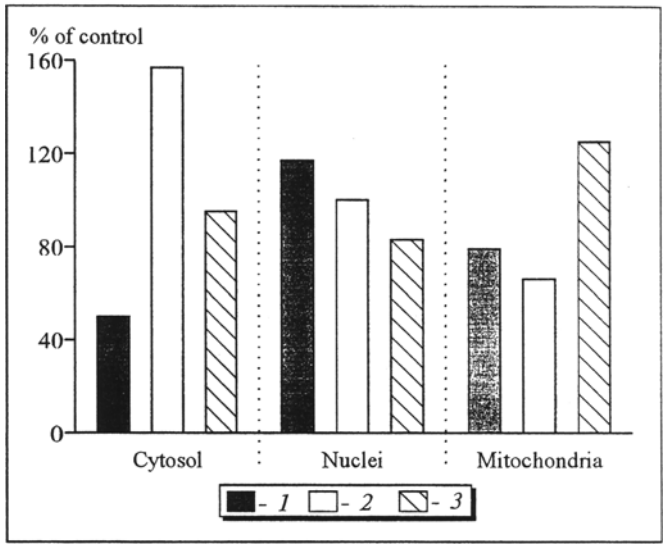

Fig. 2. Effect of preparations on ^{14}C-orotate incorporation into RNA of subcellular fraction from the liver of rats with CCl_4-induced cirrhosis. Control, cpm/mg DNA: 63650±3060 (cytosol), 40180±4700 (nuclei), and 48300±510 (mitochondria).

This substance is a component of various drugs for the treatment of liver and biliary duct diseases [12]. Histological and biochemical studies have shown that GA-containing preparations restore functional activity of liver cells and normalize serum enzymes in patients with acute and chronic hepatitis [12]. It has been recently demonstrated that polyunsaturated PC in the form of GA-stabilized mycelia reduced serum atherogenity *in vitro* [8]. Since GA salts act as a detergent, the GA trisodium salt was used as a safe solubilizer. Thus, our data confirm that Phospholiv can be successfully used as a hepatoprotective agent in the treatment of chronic liver diseases.

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