Effects of Phospholipid-Containing Hepatoprotectors on Cytochrome P-450-Dependent Antitoxic Function of the Liver during Experimental Toxic Hepatitis

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Phospholipid-containing hepatoprotectors essentiale and eplir inhibited conversion of cytochrome P-450 into cytochrome P-420 and restored aminopyrine N-demethylase and aniline *n*-hydroxylase activities of cytochrome P-450 in rats during acute hepatitis induced by CCl_4 and allyl alcohol. The polyphenol phytopreparation legalon did not prevent degradation of cytochrome P-450. Differences in the effects of hepatoprotectors on the impaired antitoxic function of the liver are probably associated with the abilities of essentiale and eplir to provide phospholipids for regeneration of endoplasmic reticulum membranes of hepatocytes.

Key Words: *hepatoprotectors; phospholipids; cytochrome P-450; experimental toxic hepatitis*

The main component of the hepatic microsomal monooxygenase system cytochrome P-450 is a hydrophobic protein with the transmembrane localization in the endoplasmic reticulum (ER) of hepatocytes. Hydrophobic surface formed by the α-helix of the cytochrome P-450 peptide chain stabilizes its structure in the catalytically active state due to multipoint contacts with phospholipids of ER membranes [4,7]. The attack of free-radical oxygen intermediates and oxidized hepatotoxin and activation of lipid peroxidation (LPO) inactivate cytochrome P-450 during toxic hepatitis. Free radicals of CCl_4 formed during its homolytic oxidation by cytochrome P-450 directly affect the hem of this enzyme and violate its phospholipid microenvironment by activating LPO [1]. The allyl alcohol metabolite acrolein that exhausts resources of reduced glutathione also contributes to degradation of cytochrome P-450 during LPO activation [6].

Here we presented the data on the effects of various hepatoprotective agents on the function of hepatic microsomal monooxygenase systems inhibited during acute hepatitis induced by CCl_4 or allyl alcohol. Phospholipid-containing hepatoprotectors essentiale and eplir were used. Polyphenol phytopreparation legalon was used for a comparative analysis. Essentiale contains phosphatidylcholine (PC) linoleate and vitamins, and legalon contains plant polyphenols [5]. An original agent eplir consists of PC, phosphatidylethanolamine (PEA), sulfolipids, and tetraterpene pigments [3]. All these preparations display considerable antioxidant activities, improve the bioenergetics, stabilize lysosoms, and prevent hepatocyte dystrophy and necrosis [2].

MATERIALS AND METHODS

Experiments were performed on 72 outbred albino male rats weighing 180-200 g that were kept under standard vivarium conditions. Animals were daily intragastrically administered with 1.25 ml/kg CCl_4 in 50% oil solution (for 4 days) or 100 mg/kg allyl alcohol in 1% water solution (for 2 days). These rats received simultaneously 80 mg/kg essentiale (in ampoules, Bosnalijek), 30 mg/kg eplir, or 200 mg/kg legalon (Silimarin, Madaus) (suspensions in 1% starch gel). Control animals received CCl_4, allyl alcohol, and equivalent volumes of distilled water or starch gel. These

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doses of hepatoprotectors were the optimum therapeutic doses. The rats were decapitated under Nembutal anesthesia (40 mg/kg intraperitoneally). The content of phospholipid fractions in liver microsomes was measured by thin-layer chromatography on Silufol UV-254 plates [3]. The concentrations of protein and cytochromes P-450, P-420, and b_5 and activities of aminopyrine N-demethylase and aniline *n*-hydroxylase were determined. Results were analyzed by Student's *t* test.

RESULTS

The composition of microsomal phospholipids was altered, and the catalytic activity of microsomal monooxygenase systems was inhibited during acute hepatitis. The total phospholipid content in ER fragments of poisoned animals decreased by 25-28% in comparison with intact rats. The content of lysophosphatidylcholine (LPC) increased, and the concentrations of PC and PEA decreased. The contents of other phospholipid fractions changed insignificantly. Conversion of cytochrome P-450 into functionally inactive cytochrome P-420 resulted in a 2.1-2.3-fold decrease in its concentration. The ratio between cytochromes P-450 and P-420 was 42-46% to 54-58%, respectively, *vs.* 80% to 20% in the control. Activity of aminopyrine N-demethylase oxidizing type I substrate decreased by 3-3.4 times. Aniline *n*-hydroxylase activity (monooxygenase of type II substrate) decreased by 3.6-5.7 times. The content of cytochrome b_5 decreased by 1.8-1.9 times (Table 1).

Hepatoprotectors differently prevented hepatic microsomes from damaging effects of CCl_4 and allyl alcohol. The therapy of toxic hepatitis with essentiale, eplir, and legalon normalized total level of microsomal phospholipids. The content of LPC slightly increased. Phospholipid preparations prevented degradation of PC more efficiently than legalon. Eplir was the only preparation that protected microsomes from PEA depletion (Table 1).

Phospholipid preparations contributed to the maintenance of functionally active cytochrome P-450 in microsomes of poisoned animals. The ratio between cytochromes P-450 and P-420 was 75-80% to 20-25%. Microsomal aminopyrine N-demethylase and aniline *n*-hydroxylase activities reached 67-78% of the control levels. By contrast, legalon did not prevent conversion of cytochrome P-450 into cytochrome P-420 (their ratio was 53% and 47%) and did not restore activity of biotransformation enzymes. The content of cytochrome b_5 returned to normal in all groups of hepatoprotector-treated animals (Table 1).

Phospholipid and polyphenol hepatoprotectors have similar antioxidant activities [2]. Therefore, the ability

TABLE 1. Effects of Hepatoprotectors on the Contents of Phospholipids and Cytochromes and Enzyme Activities in Microsomes of Rat Liver during Toxic Hepatitis (*M*±*m*, *n*=8)

Index	Intact animals	CCl_4	Hepatoprotectors+CCl_4			Allyl alcohol	Hepatoprotectors+allyl alcohol		
			essentiale	eplir	legalon		essentiale	eplir	legalon
Phospholipids, μg lipid phosphorus/g liver									
Total content	1037±37	782±24*	1106±26+	1091±34+	884±27+	746±25*	1203±41°	1148±21°	891±24°
LPC	52.2±8.3	89.3±5.4*	62.6±5.2+	63.4±7.6+	76.4±4.8+	95.1±6.0*	71.2±3.9°	67.3±4.8°	76.2±4.2°
PC	534±25	269±18*	568±22+	504±22+	430±19+	291±10*	289±12°	520±18°	439±14°
PEA	208±12	159±8*	178±15	236±6+	153±16	167±16*	181±16	224±8°	59±12
Cytochromes, nmol/mg protein									
P-450	0.65±0.05	0.31±0.03*	0.58±0.08+	0.59±0.05+	0.33±0.03	0.28±0.02*	0.56±0.06°	0.57±0.07°	0.34±0.05
P-420	0.16±0.01	0.36±0.04*	0.19±0.02+	0.16±0.02+	0.29±0.04	0.38±0.03*	0.14±0.02°	0.16±0.02°	0.31±0.02
b_5	0.30±0.03	0.17±0.02*	0.27±0.03+	0.33±0.04+	0.28±0.02+	0.16±0.02*	0.28±0.0°	0.24±0.02°	0.28±0.03°
Enzyme activities, nmol/mg protein/min									
Aminopyrine N-demethylase	2.50±0.12	0.73±0.10*	1.96±0.18+	1.68±0.21+	1.01±0.09	0.83±0.09*	1.76±0.23°	1.92±0.26°	0.98±0.08
Aniline *n*-hydroxylase	0.68±0.10	0.12±0.02*	0.48±0.06+	0.51±0.05+	0.16±0.03	0.19±0.02*	0.46±0.04°	0.47±0.06°	0.24±0.05

Note. $p<0.05$: *compared with intact animals, +compared with CCl_4, °compared with allyl alcohol.

of preparations to supply ER membranes with phospholipids plays the major role in preventing cytochrome P-450 from inactivation during intoxication with prooxidants producing electrophilic metabolites. The role of phospholipids in the restoration of normal functioning of cytochrome b_5 the amphipathic component of monooxygenase system, is lower.

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