Efficiency of Enzyme-Inducing Agents in Rats with Intrahepatic Cholestasis

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Inductors of the monooxygenase system benzonal, halonal, and halodif prevented the development of intrahepatic cholestasis induced by α-naphthylisothiocyanate and stimulated detoxifying function of the liver in rats. These agents increased the content of microsomal protein and cytochrome P-450 and accelerated metabolism of types I and II substrates. This was accompanied by a decrease of serum concentrations of total and free bilirubin and activity of liver-specific enzymes. Phenobarbital did not prevent the development of hepatocyte cytolysis.

Key Words: *intrahepatic cholestasis; enzyme-inducing agents*

Intrahepatic cholestasis is caused by impaired transport of bile acids and sodium in biliary tubules and damage to membranes and cytoskeleton of their cells [2,7]. Cholestasis is associated with inhibition of the monooxygenase system (MOS). This substantiates the use of enzyme-inducing agents stimulating oxidative elimination of bilirubin and bile acids synthesis and conjugation in this condition [1]. Phenobarbital was successfully used as inductor of metabolism for prevention of cholelithiasis in children and adults with intrahepatic cholestasis [9].

Liver disease in rats induced with α-naphthylisothiocyanate (ANIT cholestasis) is an adequate model of intrahepatic cholestasis [6,11].

We investigated the therapeutic effects of cyclic and linear urea derivatives benzonal, halonal (*o*-fluorobenzonal), and halodif (*m*-chlorobenzohydrylurea), the most active and low-toxic inductors of hepatic MOS [10], and compared these agents with reference enzyme inducers phenobarbital and zixorin (flumecinol) in ANIT cholestasis.

MATERIALS AND METHODS

Experiments were carried out in winter on 180 random-bred male and female albino rats (190-230 g) kept under natural day/night regimen on a standard diet with free access to water and food. Cholestasis was induced by single intragastric administration of 100 mg/kg ANIT in oil. After 24 h the animals received phenobarbital (50 mg/kg), benzonal and halonal (70 mg/kg), zixorin and halodif (100 mg/kg) suspensions in starch gel. The drugs were given intragastrically once a day for 4 days.

Controls received equivalent volumes of the solvent. The animals were sacrificed by decapitation under ether narcosis on the next day after the last drug dose. The duration of hexobarbital-induced sleep (80 mg/kg intraperitoneally) was evaluated as described previously [3,5], the content of protein, cytochrome P-450 and b_5, levels of high-spin (P-450-H) and low-spin (P-450-L) cytochrome P-450 forms, rates of aminopyrine N-demethylation and aniline *n*-hydroxylation, and activity of NADPH-cytochrome *c*-reductase in the liver microsomal fraction were measured. Activities of glutathione-S-transferase and glutathione reductase were measured in liver cytosol. Serum activities of alanine (AlAT) and aspartate aminotransferases (AsAT), alkaline phosphatase (AP), and the levels of total, free, and conjugated bilirubin were measured.

The results were processed by methods of variation statistics; the arithmetic mean (*M*) and standard error (*m*) were calculated and significance of differences was analyzed using Student's *t* test.

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RESULTS

On day 5 of the experiment, blood concentrations of total and free bilirubin in rats poisoned with ANIT increased 7- and 16.6-fold, respectively, compared to intact animals; activity of microsomal oxidation was markedly changed. The duration of hexobarbital-induced sleep increased 2.3 times, the content of cytochrome P-450 in hepatocytes decreased by 2.5 times (Table 1). Redistribution of high- and low-spin cytochrome P-450 forms was observed: the content of active high-spin forms decreased 9.3-fold and the content of low-spin forms of hemoprotein increased 1.6-fold. The development of ANIT cholestasis was associated with inhibition of catalytic activity of cytochrome P-450-dependent animopyrine-N-demethylase and aniline-*n*-hydroxylase (by 2 and 1.3 times, respectively, Table 1).

Cholestasis activated membrane lipid peroxidation [4,8] promoting the development of the cytolysis syndrome (activities of AsAT and AlAT in the blood increased 1.3-1.5-fold compared to intact controls). Activities of glutathione-S-transferase and glutathione reductase in the hepatocyte cytosol of experimental rats decreased 2.3 and 1.6-fold, respectively (Table 1).

Enzyme inductors prevented the development of after ANIT poisoning. Blood concentration of total and free bilirubin decreased 2.5-3.8- and 3.1-6-fold, respectively. All test agents improved functioning of the liver microsomal oxidation system. They 1.7-2.5-fold reduced the duration of hexobarbital-induced sleep compared to the control. In rats with ANIT cholestasis receiving benzonal, halonal, and phenobarbital the content of microsomal protein increased by 1.5-1.6 times. All test drugs 1.6-3.8-fold increased the content of cytochrome P-450 without changing the content of cytochrome b_5 (Table 1). Analysis of the ratio of high- to low-spin forms of hemoproteins also confirmed the positive effect of the test compounds on liver MOS.

TABLE 1. Effects of Enzyme Inductors on Liver and Serum Metabolic Parameters in Rats with ANIT Cholestasis ($M\pm m$, n=6-8)

Parameter	Intact animals	Control (cholestasis)	Benzonal	Halonal	Phenobarbital	Halodif	Zixorin
Hexobarbital-induced sleep, min	27.1±2.2	62.0±4.4*	30.5±2.5+	25.0±2.3+	34.6±3.4+	28.5±2.6+	36.0±3.9+
Liver microsomes							
Protein, mg/organ	56.5±4.9	44.8±4.1	71.5±7.2+	68.0±6.5+	73.1±7.2+	62.0±6.0	52.7±5.4
Cytochromes, nmol/mg protein:							
P-450	1.22±0.07	0.48±0.06*	0.77±0.07+	1.83±0.08+	0.80±0.06+	1.18±0.06+	0.94±0.11+
b_5	0.48±0.07	0.40±0.08	0.36±0.03	0.46±0.05	0.40±0.02	0.48±0.06	0.33±0.04
Enzyme activities, nmol/mg protein/min:							
aminopyrine-N-demethylase	0.60±0.03	0.30±0.03*	1.20±0.10+	1.82±0.21+	1.21±0.12+	0.88±0.12+	0.94±0.11+
aniline hydroxylase	0.31±0.01	0.23±0.02*	0.50±0.05+	0.62±0.06	0.45±0.04+	0.63±0.07+	0.53±0.03+
cytochrome *c*-reductase	80.5±6.0	85.0±5.3	164.0±12.4+	188.0±16.8+	176.0±18.1+	128.0±12.6+	165.0±13.0+
glutathione-S-transferase	283.0±19.2	127.0±12.4*	362.0±26.1+	320.0±32.5+	380.0±28.3+	350.0±27.4+	283.0±23.3+
glutathione reductase	45.5±2.5	30.5±2.7*	64.5±5.6+	75.0±6.2+	60.5±5.2+	68.5±6.2+	58.5±5.7+
Serum							
Bilirubin, μmol/liter:							
total	1.50±0.24	10.6±1.0*	3.84±0.26+	2.82±0.11+	3.57±0.30+	2.85±0.14+	4.27±0.36+
free	0.31±0.05	5.03±0.55*	1.08±0.21+	0.86±0.08+	1.64±0.31+	1.17±0.12+	1.64±0.06+
conjugated	1.24±0.14	5.66±0.58*	2.82±0.08+	2.01±0.22+	2.13±0.06+	1.76±0.42+	2.65±0.53+
Enzymes, μmol/liter/h:							
AlAT	1.16±0.04	1.40±0.07*	1.01±0.05+	1.16±0.07+	1.47±0.05	0.81±0.08+	1.26±0.12
AsAT	1.23±0.04	1.52±0.06*	1.33±0.12+	1.23±0.10+	1.44±0.08	1.10±0.09+	1.21±0.06+
AP	1.12±0.05	1.64±0.18*	1.21±0.06+	1.21±0.06+	1.42±0.12	1.24±0.12+	1.34±0.11

Note. p<0.05: *compared to intact animals, +compared to cholestasis.

All test drugs accelerated aminopyrine N-demethylation (type I substrate) and aniline (type II substrate) *n*-hydroxylation (2.9-6- and 2-2.7-fold, respectively) and 1.5-2.2 times activated electron transport to cytochrome *c*-reductase in animals with ANIT cholestasis. This can be explained by increased content of this flavoprotein in microsomal membranes in the presence of inductors.

Judging from decreased serum levels of liver-specific enzymes (AlAT, AsAT, and AP), benzonal, halonal, and halodif prevented hepatocyte cytolysis (by 1.3-1.7 times). In contrast to benzoyl derivatives, phenobarbital did not promote normalization of enzyme activities.

In rats with ANIT cholestasis enzyme inductors increased activities of glutathione-S-transferase (by 2.3-3.0 times) and glutathione reductase (by 2.0-2.5 times) catalyzing phase II of xenobiotic detoxication and involved in the hepatocyte antiperoxide defense.

Hence, benzonal, halonal, and halodif prevented the development of intrahepatic ANIT cholestasis in rats and stimulated detoxifying function of the liver. They increased the content of microsomal protein and cytochrome P-450, accelerated types I and II substrate metabolism and, in parallel, reduced the levels of total and free bilirubin and serum activities of liver-specific enzymes. Phenobarbital did not prevent the development of ANIT-induced hepatocyte cytolysis. Benzonal, halonal, and halodif can be recommended for the treatment of intrahepatic cholestasis as stimulators of antitoxic function of the liver.

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