Dysfunction and Correction of Microsomal Enzyme Oxidation, Glucuronidation and Glutathione Conjugation of Xenobiotics in the Liver of Deoxycholate-Intoxicated Rats

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Intoxication with deoxycholic acid is accompanied by destruction of hepatocyte plasma membrane (increased serum alanine aminotransferase activity) and inhibition of monooxygenase, glucuronidase and glutathione systems of rat liver. Heptral and cordiamine (mitethamide) had no effect on membrane integrity, while α-tocopherol and ursofalk (ursodeoxycholic acid) protected membranes. Ursofalk and cordiamine are superior a α-tocopherol and heptral by their ability to activity of monooxygenase, glucuronidase, and glutathione conjugating systems in the liver.

Key Words: *liver damage by deoxycholic acid; biotransformation of xenobiotics; heptral; cordiamine; ursofalk; α-tocopherol*

Blood from animals and humans with bile flow disturbances is toxic due to the presence of cholates, bile components, and intermediate products of impaired metabolism associated with cholestatic damages to the liver. Cholestatic endotoxemia results in liver dysfunction, in particular, in impaired biotransformation of xenobiotics and other drugs, which promotes their accumulation in the organism and aggravates intoxication. Experiments on animals showed that hydrophobic bile acids play the key role in cholestatic hepatotoxicity [12].

We examined impaired metabolism of xenobiotics in the liver under conditions of intoxication with deoxycholic acid (DOC). Heptral, α-tocopherol, ursofalk, and cordiamine were used as possible correctors.

MATERIALS AND METHODS

Experiments were performed on 62 male rats weighing 200-240 g. DOC was administered intragastrialy (i/g) via a metal probe (250 mg/kg/day with starch mucus) during 8 days. Heptral (100 mg/kg), cordiamine, and vitamin E (50 mg/kg each) were injected intraperitoneally (i/p), ursofalk (100 mg/kg) was administed i/g with starch mucus (8 days). Control rats received the same amounts of DOC or starch mucus (i/g) and 0.85% NaCl (i/p). Twenty four hours after the last injection the animals were decapitated and microsomal liver fraction was isolated. The following parameters were determined by the methods described elsewhere [3]: total protein and cytochrome b_5+P450 content, the rate of NADPH and NADH oxidation, aminopyrine demethylation, and aniline hydroxylation, activity of NADPH-cytochrome P450 and NADH-cytochrome b_5 oxidoreductases, uridindiphosphate (UDP), glucuronide and glutathione transferases, and UDP-glucose dehydrogenase. Urinary excretion of glucuronic acid was determined by the method proposed by H. Yuki and W. Fishman [14] and serum alanine aminotransferase (ALT) activity by the method described elsewhere [5].

RESULTS

Eight days after DOC injection, ALT activity in rat serum increased indicating impairment of hepatocyte

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plasma membrane permeability. Activity of microsomal monooxygenase system was inhibited by 23-66%, which manifested in a decreased cytochrome P450 and b_5 content reduced NADPH cytochrome P450 and NADH cytochrome b_5 reductase activities, and inhibition of NADPH oxidation, aminopyrine demethylation, and aniline hydroxylation. Microsomal glutathione-S-transferase activity decreased by 31%, while the function of cytoplasmic 1-chloro-2,4-dinitrobenzene and sulfobromophthalein glutathione-S-transferases did not significantly change (Table 1).

DOC also inhibited glucuronidation of xenobiotics. This was confirmed by inhibition of UDP-glucuronyltransferase and UDP-glucose dehydrogenase, decreased urinary excretion of free glucuronic acid, and reduced conjugated/total glucuronic acid ratio (Table 1).

Heptral did not affect permeability of hepatocyte plasma membrane in DOC-intoxicated rats. It slightly activated the monooxygenase and glucuronidase systems in the liver: normalized cytochrome b_5 content, NADPH oxidation rate, UDP-glucuronyltransferase activity, glucuronic acid excretion and congugated/total glucuronic acid ratio (Table 1).

α-Tocopherol normalized the content of cytochrome b_5 and activities of NADPH cytochrome P450 and NADH cytochrome b_5 reductases, microsomal glutathione-S and glucuronyltransferases, and UDP-glucose dehydrogenase. It also reduced urinary excretion of free glucuronic acid and conjugated/total glucuronic acid ratio. Vitamin E decreased serum ALT activity by 33% compared to untreated DOC-intoxicated animals. Activity of NADH-cytochrome b_5 reductase and the rate of aminopyrine N-demethylation increased by 20 and 117%, respectively (Table 1).

Ursofalk normalized ALT activity and activities of monooxygenase (content of cytochrome P450 and b_5, cytochrome reductase activities, and the rate of NADPH oxidation and aniline p-hydroxylation), glutathione conjugating (microsomal glutathione-S-transferase activity), and glucuronidase systems (UDP-glucuronyltransferase and UDP-glucose dehydrogenase activities, excretion of free glucuronic acid, and conjugated/total glucuronic acid ratio). Ursofalk decreased ALT activity by 46% and increased cytochrome P450 and b_5 content by 100 and 66%, respectively, and NADH-cytochrome b_5 reductase activity by 54%. It also stimulated NADPH oxidation rate by 64%, aminopyrine N-demethylation and aniline p-hydroxylation by 146 and 104%, respectively, compared to untreated DOC-intoxicated animals. Ursofalk reduced urinary excretion of free glucuronic acid and increased conjugated/total glucuronic acid ratio (Table 1).

Injection of cordiamine to DOC-intoxicated rats normalized NADPH oxidation rate, cytochrome P450 and b_5 content, cytochrome reductase activity, and microsomal glutathione-S-transferase and UDP-glucose dehydrogenase activities, as well as the excretion of unconjugated glucuronic acid. Cordiamine-induced aminopyrine N-demethylation rate, UDP-glucuronyltransferase activity, excretion of free glucuronic acid and conjugated/total glucuronic acid ratio surpassed those of intact animals by 13-90% (Table 1).

Cordiamine increased cytochrome P450 and b_5 content (by 103 and 83%), cytochrome reductase activity (by 20 and 55%), NADPH oxidation rate (by 69%), aminopyrine demethylation and aniline hydroxylation (by 268 and 75%, respectively). It stimulated activity of microsomal and cytoplasmic1-chloro-2, 4-dinitrobenzene and sulfobromophthalein gluthatione-S-transferases (by 64, 72, and 38%, respectively), UDP-glucuronyltransferase (by 99%), and UDP-glucose dehydrogenase (by 40%) compared to DOC-intoxicated untreated rats. Urinary excretion of free glucuronic acid decreased by 47%, while excretion of conjugated glucuronic acid and conjugated/total glucuronic acid ratio surpassed those in untreated rats by 64 and 24%, respectively (Table 1).

Increased serum ALT activity in DOC-intoxicated rats and inhibition of monooxygenase glucuronidase and gluthatione transferase systems in the liver confirm hepatotoxicity of the test compound, which agree with published data. Thus, addition of DOC to food (0.5% of weight, 2 weeks) [12] induced inflammation of the portal triad, proliferation of bile ducts, necrosis and nuclear polymorphism of hepatocytes. It was shown that hydrophilic cholic acid produces no hepatotoxic effect. Moreover, direct *in vitro* inhibition of liver monooxygenase system by DOC was demonstrated [7].

Activation of lipid peroxidation (LPO) probably plays a role in the increase of plasma membrane permeability and inhibition of the enzyme systems involved in biotransformation of xenobiotics in the liver of DOC-intoxicated rats. This assumption agrees with the data on accumulation of malonic dialdehyde in the liver of rats receiving DOC with food [12]. Earlier, LPO activation in liver microsomes *in vitro* exposed to DOC addition was shown [8].

Cell membranes with incorporated enzymes are the main targets for reactive peroxides. This is confirmed by preferential inhibition by DOC of membrane-bound enzymes, but not glutathione-S-transferase (Table 1). Enhanced hepatocyte membrane permeability and the release of ALT from cells into circulation can be interpreted similarly.

Apart from LPO activation, enzyme inhibition by DOC can be realized via other mechanisms. Binding of DOC to cytochrome P450 (type I bond) and oxidation by this enzyme determine its competition with other substrates for binding sites on this hemoprotein,

TABLE 1. Effect of Intoxication by Deoxycholic Acid and Its Correction on Alanine Aminotransferase Activity and Functions of Monooxygenase, Glutathione Conjugation and Glucuronidation Systems (*M±m*)

Parameters	NaCl	Deoxycholic acid				
		+NaCl	+heptral	+vitamin E	+ursofalk	+cordiamin
ALT, mmol/liter	2.06±0.14	4.69±0.56*	4.02±0.55*	3.16±0.37*+	2.51±0.33+	3.97±0.48*
Monooxygenase system						
cytochrome P450, nmol/mg	0.82±0.05	0.38±0.09*	0.38±0.12*	0.53±0.10*	0.76±0.04+	0.77±0.15+
cytochrome b_5, nmol/mg	0.54±0.07	0.35±0.05*	0.37±0.06	0.49±0.06	0.58±0.04+	0.64±0.06+
NADPH-cytochrome P450 reductase, μmol/min/mg	0.26±0.02	0.20±0.01*	0.20±0.01*	0.23±0.02	0.22±0.01	0.24±0.01+
NADPH-cytochrome b_5 reductase, μmol/min/mg	4.40±0.23	2.51±0.30*	2.82±0.39*	3.59±0.39+	3.87±0.18+	3.88±0.30+
NADPH oxidation, nmol/min/mg	2.71±0.21	1.73±0.30*	2.14±0.40	1.97±0.21*	2.83±0.17+	2.92±0.46+
aminopyrine N-demethylation, nmol/min/mg	11.33±0.09	3.83±1.04*	5.45±1.44*	8.30±0.37*+	9.41±0.39*+	14.79±0.71*+
aniline p-hydroxylation, nmol/min/mg	0.67±0.09	0.24±0.04*	0.30±0.05*	0.37±0.07*	0.49±0.08+	0.42±0.03*+
Glutathione conjugation system						
microsomal glutathione-S-transferase, nmol CDNB/min/mg	78.11±6.98	53.82±9.22*	45.43±10.58*	63.05±11.89	0.31±6.17	88.1±10.85+
cytosolic gluthatione-S-transferase,						
μmol CDNB/min/mg	0.60±0.06	0.46±0.10	0.44±0.13	0.54±0.11	0.48±0.08	0.78±0.12+
nmol SBP/min/mg	7.99±0.37	6.61±0.75	7.76±1.08	7.64±0.74	7.42±0.81	9.14±0.92+
Glucuronidation, system						
UDP-glucurotransferase, nmol/min/mg	3.46±0.22	2.59±0.29*	2.97±0.37	2.65±0.42	3.57±0.40	5.17±0.69*+
UDP-glucose dehydrogenase, nmol/min/mg	14.33±1.17	10.42±0.69*	10.37±1.43*	11.65±1.32	12.82±1.03	14.62±0.65+
glucuronic acid, mg/15 h						
total	3.48±0.23	3.87±0.57	4.44±0.51	3.32±0.60	3.87±0.44	6.53±1.07*+
conjugated	2.60±0.24	3.00±0.44	3.21±0.45	2.72±0.42	2.91±0.30	4.93±0.58*+
free	0.77±0.12	1.24±0.16*	1.05±0.29	0.80±0.21	0.80±0.06+	0.67±0.11+
conjugated/total	0.77±0.03	0.70±0.02*	0.74±0.03	0.77±0.03	0.77±0.02+	0.87±0.01*+

Note. CDNB: 1-chloro-2,4-dinitrobenzene, SBP: sulfobromophthalein; $p<0.05$ compared to rats receiving NaCl (*) or deoxycholic acid+NaCl (+).

decreased metabolism of many xenobiotics [2]. Moreover, DOC metabolites produced by cytochrome P450 act as detergents and destruct substrate-binding site in the enzyme, which considerably impairs its interaction with substrates and reduces its catalytic activity. Impaired hydroxylation of cholesterol backbone results in accumulation of cytotoxic mono- and dihydroxy bile acid derivatives in hepatocytes [2,4,10]. Their high concentrations or prolonged exposure produces a detergent effect on hepatocyte endoplasmic reticulum membranes resulting in uncoupling of membrane enzyme complexes, conversion of cytochrome P450 into inactive cytochrome P420 and destruction of substrate-binding centers in the enzyme molecule.

Similar to phospholipidase, DOC attacks membrane phospholipids, thus enhancing permeability of hepatocyte plasma membrane. Moreover, it impairs normal biogenesis of endoplasmic reticulum membranes and cytochrome P450 not incorporated into membrane is washed away or inhibited [2].

In DOC-intoxicated rats, heptral produced no significant effect of enzyme activity.

α-Tocopherol produced more potent protective effect on hepatocyte plasma membrane (judging from ALT activity) than on endoplasmic reticulum membranes. These data were confirmed by E. Serbinova *et al.* [11], who showed inefficacy of α-tocopherol (in contrast to another tocol 2,2,5,7,8-pentamethylchroman) against LPO-induced damage of cytochrome P450. The mechanism of this selective antioxidant action of α-tocopherol on different cell membranes remains unclear.

In contrast to α-tocopherol, ursofalk produced stabilizing action on hepatocyte plasma membrane (decreased ALT release into circulation) and endoplasmic reticulum membrane (reactivation of microsomal oxidation enzymes, glucuronidation and glutathione conjugation of xenobiotics). It rats, dietary treatment with nontoxic hydrophilic ursodeoxycholic acid (ursofalk, 1% of the food weight) prevented DOC-induced cholestasis and hepatotoxicity (increased serum content of bile acids, inflammation of the portal triada, proliferation of bile ducts, necrosis and nuclear polymorphism of hepatocytes) [12].

Enzyme-stabilizing effect of ursofalk can be mediated by several mechanisms: 1) decrease in concentration of DOC and other cytotoxic cholate due to inhibition of their absorbtion in the small intestine; 2) stimulation of bile acid transport; 3) formation of nontoxic mixed micelle of ursofalk and hydrophobic cholates [13], and 4) ursofalk incorporation into biomembranes and their subsequent stabilization.

The ability of cordiamine to reactivate DOC-impaired enzyme systems of microsomal oxidation systems and stimulate glucuronidation and glutathione conjugation of xenobiotic probably depends on its enzyme-activating (substrate induction of cytochrome P450, elevation of NADPH concentration) and antioxidant properties [1,6,9].

The obtained results show that DOC inhibits membrane bound enzymes of microsomal oxidation, glucuronidation and glutathione conjugation of xenobiotics and impairs the integrity of hepatocyte plasma membrane. Heptral produced no protective effect, α-tocopherol markedly prevented damage to hepatocyte membrane and slightly improved function of enzyme systems. Ursofalk completely normalized permeability of plasma membrane and activity of the enzyme systems of oxidation and conjugation of xenobiotics with glucuronic acid and glutathione. Cordiamine produced a pronounced stimulatory effect on drug metabolism in the liver.

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