Correction of Dysfunction of Enzyme Systems of Microsomal Oxidation, Glucuronidation, and Glutathione Conjugation of Xenobiotics in the Liver of Rats with Deoxycholate Intoxication

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Intoxication with deoxycholic acid disturbed the integrity of hepatocyte plasma membranes (elevation of alanine transaminase activity in the plasma) and inhibited the monooxygenase, glucuronosyltransferase, and glutathione S-transferase systems in rat liver. Heptral and cordiaminum did not affect the integrity of plasma membranes, while α-tocopherol and, especially, ursofalk produced membrane-protective effects. Ursofalk and, especially, cordiaminum are far superior to α-tocopherol and heptral in their ability to normalize functions of the monooxygenase, glucuronosyltransferase, and glutathione S-transferase systems in rat liver.

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

The blood of animals and humans with cholestasis contains considerable amounts of cholates, other bile components, and intermediate products of impaired metabolism and, therefore, is toxic to the body. Endotoxemia in cholestasis leads to liver dysfunction and impairs biotransformation of drugs and other xenobiotics. This promotes their accumulation in the body and aggravates intoxication. Experiments on animals showed that hydrophobic fatty acids play the major role in the pathogenesis of hepatotoxicity during cholestasis [12].

Here we studied disturbances in xenobiotic transformation in the liver during deoxycholic acid (DCA) intoxication. Heptral, α-tocopherol, ursofalk, and cordiaminum were used to correct metabolic dysfunction.

MATERIALS AND METHODS

Experiments were performed on 62 male rats weighing 200-240 g. DCA was administered through a gastric tube (i.g., 250 mg/kg/day in starch slurry) for 8 days. Heptral (100 mg/kg), cordiaminum (50 mg/kg), and vitamin E (50 mg/kg) were injected intraperitoneally (i.p.); ursofalk was administered i.g. (100 mg/kg/day in the starch slurry). Control rats received equivalent amounts of DCA or starch slurry (i.g.) and 0.85% NaCl (i.p.). Twenty-four hours after the last injection, the animals were decapitated and liver microsomal fractions were isolated. The concentration of proteins and the content of cytochromes b_5 and P450 were measured. The rates of NADPH and NADH oxidation, aminopyrine demethylation, and aniline p-hydroxylation and activities of NADPH-cytochrome P450 and NADH-cytochrome b_5 oxidoreductases, uridine 5'-diphosphoglucuronyl transferase (UDP-glucuronosyltransferase), glutathione S-transferase, and uridine-5'-diphosphoglucose dehydrogenase (UDP-glucose dehydrogenase) were estimated by the method described elsewhere [3]. The urinary excretion of glucuronic acid [14] and activity of alanine transaminase (ALT) in the serum [5] were estimated.

RESULTS

Activity of ALT in rat serum increased on day 8 after the start of DCA administration, which indicated impaired permeability of hepatocyte plasma membranes.

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Microsomal monooxygenase system was inhibited by 23-66% (contents of cytochromes P450 and b_5, activities of NADPH-cytochrome P450 and NADH-cytochrome b_5 reductases, and rates of NADPH oxidation, aminopyrine demethylation, and aniline p-hydroxylation decreased). Microsomal glutathione S-transferase activity was reduced by 31%, while activities of cytosolic 1-chloro-2,4-dinitrobenzene and bromosulfophthalein glutathione S-transferases remained practically unchanged (Table 1).

DCA also inhibited glucuronidation of xenobiotics, which was confirmed by reduced activities of UDP-glucuronosyltransferase and UDP-glucose dehydrogenase, enhanced urinary excretion of nonconjugated glucuronic acid, and decreased ratio between conjugated and total glucuronic acids (Table 1).

Heptral produced no considerable effects on the permeability of hepatocyte plasma membranes in rats with DCA intoxication. Activities of the monooxygenase and glucuronidation systems in the liver slightly increased. The content of cytochrome b_5, activity of UDP-glucuronosyltransferase, urinary excretion of nonconjugated glucuronic acid, and the ratio between conjugated and total glucuronic acids returned to norma (Table 1).

α-Tocopherol normalized the content of cytochrome b_5, activities of NADPH-cytochrome P450 and NADH-cytochrome b_5 reductases, microsomal glutathione S-transferase and UDP-glucuronosyltransferase, and UDP-glucose dehydrogenase, urinary excretion of nonconjugated glucuronic acid, and the ratio between conjugated and total glucuronic acids. α-Tocopherol reduced serum ALT activity by 33% compared to that in untreated animals. NADH-cytochrome b_5 reductase activity and the rate of aminopyrine N-demethylation increased by 20 and 117%, respectively (Table 1).

Ursofalk normalized ALT activity and inhibited the monooxygenase (contents of cytochromes P450 and b_5, activities of cytochrome reductases, and rates of NADPH oxidation and aniline p-hydroxylation), glutathione conjugation (activity of microsomal glutathione S-transferase), and glucuronidation systems (activities of UDP-glucuronosyltransferase and UDP-glucose dehydrogenase, urinary excretion of nonconjugated glucuronic acid, and the ratio between conjugated and total glucuronic acids in the urine). Ursofalk lowered ALT activity (by 46%) and increased the contents of cytochromes P450 and b_5 (by 100 and 66%, respectively), activity of NADH-cytochrome b_5 reductase (by 54%), and the rates of NADPH oxidation, aminopyrine N-demethylation, and aniline p-hydroxylation (by 64, 146, and 104%, respectively) compared with those in untreated animals. Ursofalk also reduced urinary excretion of nonconjugated glucuronic acid and increased the ratio between conjugated and total glucuronic acids (Table 1).

Cordiaminum normalized the rate of NADPH oxidation, contents of cytochromes P450 and b_5, activities of cytochrome reductases, microsomal glutathione S-transferase, and UDP-glucose dehydrogenase, and urinary excretion of nonconjugated glucuronic acid in rats with DCA intoxication. In animals receiving cordiaminum, the rate of aminopyrine N-demethylation, activity of UDP-glucuronosyltransferase, urinary excretion of nonconjugated glucuronic acid, and the ratio between conjugated and total glucuronic acids were 13-90% higher than in intact rats (Table 1).

Cordiaminum increased the contents of cytochromes P450 and b_5 (by 103 and 83%, respectively), cytochrome reductase activities (by 20 and 55%, respectively), rates of NADPH oxidation, aminopyrine N-demethylation, and aniline p-hydroxylation (by 69, 286, and 75%, respectively), activities of microsomal 1-chloro-2,4-dinitrobenzene and cytosolic 1-chloro-2,4-dinitrobenzene and bromosulfophthalein glutathione S-transferases (by 64, 72, and 38%, respectively), and UDP-glucuronosyltransferase and UDP-glucose dehydrogenase (by 99 and 40%, respectively) compared with those in untreated rats with DCA intoxication. The urinary excretion of nonconjugated glucuronic acid decreased by 47%, while excretion of conjugated glucuronic acid and the ratio between conjugated and total glucuronic acids were 64 and 24% higher, respectively, than in untreated rats (Table 1).

The elevation of serum ALT activity and suppression of the monooxygenase, glucuronosyltransferase, and glutathione S-transferase systems in the liver of rats with DCA intoxication attest to hepatotoxicity of this compound. This fact is also confirmed by published data. S. Shefer *et al.* [12] reported inflammation of hepatic triads, proliferation of bile ducts, hepatocyte necrosis, and polymorphism of hepatocyte nuclei in rats fed a DCA-enriched diet (0.5% food weight) for 2 weeks. It was shown that hydrophilic cholic acid is not hepatotoxic. Furthermore, direct inhibitory effects of DCA on activity of hepatic monooxygenase system were observed *in vitro* [7].

Intensification of LPO processes probably plays a role in the mechanisms of increased permeability of hepatocyte plasma membranes and suppression of enzyme systems involved in biotransformation of xenobiotics in the liver of rats with DCA intoxication. This assumption agrees with previous data on elevated MDA content in the liver of rats fed a DCA enriched diet rich [12]. *In vitro* experiments showed that DCA activated LPO in liver microsoms [8].

Cell membrane structures with localized enzymes are the main targets for reactive peroxidation compounds. This is confirmed by selective inhibition of

membrane-bound enzymes with DCA, which was demonstrated by comparing glutathione S-transferase activities (Table 1). Disturbances in hepatocyte plasma membrane permeability and the release of ALT from cells with elevation of its plasma activity can be interpreted in a similar manner.

Apart from LPO activation, DCA-induced enzyme inhibition can be realized via other mechanisms. DCA binds to type I cytochrome P450 binding sites and is oxidized by the enzyme, thus competing with other substrates, which decreases metabolism of many xenobiotics [2]. Furthermore, cytochrome P450 induces the formation of DCA hydroxyderivatives, which possess detergent properties and destroy substrate-binding sites in the enzyme molecule. In this case, the ability of hemoprotein to react with substrates and its catalytic activity considerably decrease. Hydroxylation of the cholesterol steroid nucleus is also impaired, which causes the accumulation of cytotoxic mono- and dihydroxyderivates of bile acids in hepatocytes [2, 4,10]. In high or low (in the case of prolonged exposure) concentrations, these compounds produce detergent effects on membranes of hepatocyte endoplasmic reticulum. This leads to the dissociation of membrane enzyme complexes, conversion of cytochrome P450 into catalytically inactive cytochrome P420, and destruction of substrate-binding sites in the enzyme molecule.

TABLE 1. Effects of DCA Intoxication and Various Methods of Its Correction on ALT Activity and Functions of Monooxygenase, Glucuronidation, and Glutathione Conjugation Systems ($M \pm m$)

Parameter	NaCl	DCA				
		+NaCl	+heptral	+vitamin E	+ursofalk	+cordiaminum
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+
NADH-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 glutathione 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 BSF/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-glucuronosyltransferase, 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*+
nonconjugated	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; BSL: bromosulfophthalein. $p<0.05$: *compared to rats receiving NaCl; +compared to rats receiving DCA+NaCl.

DCA, as well as phospholipase, attacks membrane phospholipids and increases the permeability of hepatocyte plasma membranes. In addition, DCA inhibits biogenesis of endoplasmic reticulum membranes. Therefore, cytochrome P450 not incorporated into new membrane is washed from cells out or inhibited [2].

Heptral produced no considerable effect on enzyme activities in rats with DCA intoxication.

Membrane-protective effects of α-tocopherol were more pronounced in hepatocyte plasma membranes (as estimated from ALT activity) and less pronounced in the endoplasmic reticulum. These data are confirmed by experiments of E. Serbinova *et al.* [11] showing that α-tocopherol (but not 2,2,5,7,8-pentamethylchromanol) is ineffective against LPO-induced damage to cytochrome P450. The mechanism of this selective antioxidant influence of α-tocopherol on various cell membranes is still unclear.

Unlike α-tocopherol, ursofalk produced membrane-stabilizing effects on hepatocyte plasma membranes (decrease in ALT release into the blood) and endoplasmic reticulum (normalization of microsomal oxidation, glucuronidation, and glutathione conjugation enzymes systems suppressed with DCA). It is known that nontoxic hydrophilic ursodeoxycholic acid (ursofalk, 1% food weight) prevents DCA-induced cholestasis and symptoms of hepatotoxicity in rats (elevated content of bile acids in the serum, inflammation of hepatic triads, proliferation of bile ducts, hepatocyte necrosis, and polymorphism of hepatocyte nuclei) [12].

Enzyme-normalizing effects of ursofalk during DCA intoxication are probably realized via the following mechanisms: the decrease in concentrations of DCA and other cytotoxic cholates due to the inhibition of their absorption in the small intestine; stimulation of bile acids transport; formation of nontoxic mixed micelles consisting of ursofalk and hydrophobic cholates [13]; and incorporation of ursofalk into biological membranes followed by stabilization of their conformation.

The ability of cordiaminum to restore activity of microsomal oxidation, glucuronidation, and glutathione conjugation enzyme systems during DCA intoxication is due to its enzyme-activating (substrate induction of cytochrome P450 and elevation of NADPH level) and antioxidant properties [1,6,9].

Our results indicate that DCA inhibits membrane-bound enzyme systems of microsomal oxidation, glucuronidation, and glutathione conjugation of xenobiotics and disturbs the integrity of hepatocyte plasma membranes. Heptral produced no considerable protective effects. a-Tocopherol protected hepatocyte plasma membrane, but exhibited a less pronounced effect on xenobiotic biotransformation. Ursofalk normalized the permeability of plasma membranes and improved oxidation, glucuronidation, and glutathione conjugation of xenobiotics. Cordiaminum stimulated drug metabolism in the liver.

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