Inhibition and Regulation of Antioxidant Protection, Microsomal Oxidation, and Xenobiotic Glucuronidation in Cholestatic Rats

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An 8-day biliary obstruction in rats caused by ligation of the common bile duct is accompanied by an increase in bilirubin blood content and alanine aminotransferase activity. The hepatic xenobiotic-metabolizing activity (intensities of microsomal oxidation and glucuronidation) decreases in parallel with the inhibition of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase). A comparative study of Heptral, Ursofalk, Essentiale, and Cordiamin (nikethamide) showed that Cordiamin displays the most significant enzyme-stabilizing activity.

Key Words: *liver; cholestasis; biotransformation of xenobiotics; antioxidant system*

Cholestasis is accompanied by a decrease in the drug-metabolizing capacity of the liver and activation of free-radical processes, which aggravates intoxication [7]. The search for new methods for correcting the impaired hepatic xenobiotic-metabolizing capacity and lipid peroxidation (LPO) in cholestasis is an important problem of modern hepatology.

We studied activities of the monooxygenase, glucuronidase, and antioxidant systems in cholestatic rats and the protective effects of widely used preparations Heptral (S-adenosyl-L-methionine), Ursofalk (ursodeoxycholic acid), Essentiale (the main component is phosphatidylcholine dilinoleoyl) and Cordiamin (nikethamide, stimulates biotransformation of xenobiotics in the liver and possessing an antioxidation effect in intact animals).

MATERIALS AND METHODS

Experiments were performed on 118 male rats weighing 150-200 g. Cholestasis was induced by ligation of the common bile duct under ether anesthesia. Heptral (100 mg/kg intraperitoneally), Ursofalk (100 mg/kg intraGAtrically), Essentiale (1 ml/kg intraperitoneally), and Cordiamin (50 mg/kg intraperitoneally) were administered for 5 (under chloral hydrate anesthesia) or 7 days postoperation. Control rats were subjected to laparotomy with (or without) ligation of the common bile duct and received intraperitoneal injections of 0.85% NaCl. Monooxygenase activity was measured *in vivo* by the antipyrine test. Glucuronidation was analyzed *in vivo* by the duration of chloral hydrate narcosis and urinary excretion of glucuronic acid (GA). Activity of the monooxygenase system was determined *in vitro* by the rates of NADPH oxidation and N-demethylation of aminopyrine and ethylmorphine. Function of the glucuronidation system was *in vitro* assayed by measuring UDP-glucuronosyltransferase and UDP-glucose dehydrogenase activities in the microsomal and cytosolic fractions of the liver, respectively [2]. The antioxidant system was studied by measuring activities of catalase, superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase, the content of reduced glutathione (RG) in the liver, and the concentrations of carotenes, retinols, and toco-

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pherols in the plasma [6]. The severity of structural and functional hepatic damages was determined by measuring plasma bilirubin and alanine aminotransferase activity (the traditionally used indices) [4].

RESULTS

Plasma contents of total, inbound, and bound bilirubin increased by 7.3, 3.3, and 2.4 times, respectively, 8 days after ligation of the common bile duct. The activity of alanine aminotransferase increased by 2.4 times. Urine contents of total and bound GA increased by 1.6 and 1.5 times, respectively. This was probably due to the suppression of their biliary excretion. The ratio between bound and total GA, which reflects the intensity of glucuronidation of endogenous substances, decreased. The duration of chloral hydrate narcosis in cholestatic rats increased by 34%. The rate of antipyrine elimination decreased (Table 1).

Heptral normalized the duration of chloral hydrate narcosis and the ratio bound to total GA. The duration of chloral hydrate narcosis tended normalize under the effect of Ursofalk. Ursofalk increased urinary excretion of total and bound GA and their ratio compared with those in untreated cholestatic animals (Table 1).

Essentiale normalized the bound to total GA ratio in the urine and the content of antipyrine in the blood of cholestatic rats. Cordiamin normalized the duration of chloral hydrate narcosis. Chloral hydrate was administered 12 h after the last injection of Cordiamin. Therefore, the analeptic effects of Cordiamin can be excluded under these conditions. Plasma content of antipyrine in cholestatic rats treated with Cordiamin decreased. However, the excretion of total and bound GA and their ratio increased in these animals.

Since antipyrine and chloral hydrate are the substrates for cytochrome P-450 monooxygenase and UDP-glucuronosyltransferase systems, respectively, the increase in the blood content of antipyrine and the duration of chloral hydrate narcosis and the decrease in the bound GA/total GA ratio in the urine are due to inhibition of these enzyme systems in the liver of cholestatic rats (Table 2).

Eight days after ligation of the common bile duct, the rates of NADPH oxidation and N-demethylation of aminopyrine and ethylmorphine in the hepatic microsomal fraction decreased by 52%, 55%, and 73%, respectively. UDP-glucuronosyltransferase activity and the rate of biosynthesis of its substrate, UDP-glucuronic acid (activity of UDP-glucose dehydrogenase), decreased by 28% and 36%, respectively (Table 2).

Heptral normalized UDP-glucuronosyltransferase and UDP-glucose dehydrogenase activities. Cordiamin accelerates NADPH oxidation and N-demethylation of aminopyrine and ethylmorphine by 88%, 59%, and 77%, respectively. Activities of UDP-glucuronosyltransferase and UDP-glucose dehydrogenase increased by 82% and 26%, respectively, compared with those in untreated cholestatic animals (Table 2).

Exhaustion of the hepatic antioxidant systems due to LPO activation by bile acids probably underlies the disturbances of hepatic xenobiotic-metabolizing function in cholestasis [13]. We showed that biliary obstruction in rats for 8 days induced considerable changes in the nonenzymatic antioxidant protection systems in hepatic cells, while antioxidant enzymes were inhibited. Blood contents of carotenes, retinols, and tocopherols and urine RG content were similar to those in sham-operated animals (Table 3). The concentration of RG in cholestatic rats was constant because activity of glutathione reductase was unchanged. During this period, activities of catalase, SOD, and glutathione peroxidase decreased by 48%, 26%, and 24%, respectively. Heptral increased the contents of RG (due to activation of glutathione reductase) and carotenes. Ursofalk increased activity of glutathione

TABLE 1. Effects of Preparations on the Duration of Chloral Hydrate Narcosis, Urinary Excretion of GA, and Plasma Content of Antipyrine in Cholestatic Rats (*M±m*)

Indices	Sham operation NaCl	Ligation of the common bile duct				
		NaCl	Heptral	Ursofalk	Essentiale	Cordiaminum
Chloral hydrate narcosis, min	109.90±7.19	147.80±7.30*	132.10±8.60	141.10±15.70	169.25±13.42*	96.40±9.30+
GA, mg/portion						
total	3.33±0.23	5.42±0.28*	5.79±0.30*	7.05±0.48*+	5.28±0.62*	6.87±0.23*+
bound	2.53±0.18	3.80±0.28*	3.98±0.19*	4.99±0.33*+	4.56±0.63*	4.90±0.18+
bound/total	0.75±0.01	0.68±0.03*	0.72±0.02	0.75±0.02+	0.72±0.02	0.75±0.01+
Antipyrine, μg/ml plasma	15.52±0.25	18.50±0.82*	17.25±0.50*	17.24±0.42*	15.94±0.37+	14.38±0.35*+

Note. Here and in Tables 2 and 3: *$p<0.05$ compared with sham-operated rats; +$p<0.05$ compared with cholestatic rats injected with NaCl.

TABLE 2. Effects of Heptral and Cordiamin on Activity of Hepatic Monooxygenase and Glucuronidation Systems in Cholestatic Rats (*M±m*)

Indices, nmol/min/mg	Sham operation NaCl	Ligation of the common bile duct		
		NaCl	Heptral	Cordiaminum
NADPH oxidation	4.99±0.39	2.40±0.26*	2.85±0.26*	4.50±0.51+
N-Demethylation				
aminopyrine	9.67±0.54	4.32±0.39*	4.36±0.60*	6.87±1.13*+
ethylmorphine	10.46±1.09	2.79±0.36*	2.12±0.17*	4.95±0.85*+
UDP-glucuronosyltransferase	7.11±0.75	5.11±0.61*	8.31±1.25+	9.29±0.56*+
UDP-glucose dehydrogenase	11.46±0.74	7.28±0.50*	10.23±0.61+	9.15±0.71*+

peroxidase and the content of RG. Essentiale increased hepatic content of tocopherols in cholestatic animals. Cordiamin displayed the highest antioxidant activity in cholestatic rats. Table 3 shows that Cordiamin increased catalase and glutathione reductase activities by 40% and 27%, respectively and the contents of RG and tocopherols by 49% and 33%, respectively.

Cholestasis decreased the xenobiotic-metabolizing capacity of rat liver (Tables 1 and 2). This is confirmed by *in vivo* (inhibition of aspirin elimination, prolongation of the pharmacological effects of chloral hydrate, and a decrease in the bound GA/total GA ratio) and *in vitro* (inhibition of the monooxygenase and glucuronidase systems in the hepatic microsomal fraction) studies [3]. Activation of LPO [14] and exhaustion of the antioxidant enzyme systems [13] in cells are the possible mechanisms of the damage to the hepatocyte plasma membrane (judging from plasma alanine aminotransferase activity) and inhibition of oxidation and conjugation of substances with GA in cholestasis. The normalizing effect of Heptral on the intensity of hepatic glucuronidation in cholestasis is probably due to its key role in transmethylation [10], antioxidant properties [8], and the ability to normalize microsomal membrane fluidity [11].

Hydrophobic bile acids destroying cell membranes are the major hepatotoxic agents in cholestasis [12], hydrophilic bile acids (for example, ursodeoxycholic acid) have hepatoprotective properties [9]. Our results are consistent with this fact. The protective properties of hydrophilic compounds are probably due to their membrane stabilizing effects.

The ability of Essentiale to normalize microsomal oxidation in cholestasis is probably associated with reparative properties of phosphatidylcholine. Moreover, this substance plays an important role in elec-

Table 3. Effects of Preparations on Hepatic Antioxidant System in Cholestatic Rats (*M±m*)

Indices	Sham operation NaCl	Ligation of the common bile duct				
		NaCl	heptral	ursofalk	essentiale	cordiaminum
Catalase, μmol/min/g	51.37±0.76	27.13±5.02*	28.16±7.00*	31.06±9.10*	31.06±4.94*	37.98±11.90*+
SOD, U/min/mg	2.11±0.12	1.56±0.12*	1.47±0.13*	1.66±0.12*	1.52±0.08*	1.46±0.22*
Glutathione peroxidase, μmol/min/mg	6.50±0.12	4.97±0.23*	5.45±0.17*	5.72±0.24*+	5.19±0.36*	4.73±0.23*
Glutathione reductase, nmol/min/mg	37.00±1.58	39.64±1.65	44.26±2.15*	42.50±2.22	43.30±2.30*	46.84±1.48*+
RG, μmol/g	4.75±0.22	5.29±0.43	6.50±0.26*+	6.64±0.30*+	5.94±0.16*	7.08±0.28*+
Tocopherols, mg/100 ml	0.67±0.04	0.66±0.03	0.74±0.03	0.70±0.05	0.83±0.06+	0.89±0.10*+
Retinols, μg/100 ml	24.84±3.07	21.24±2.81	19.11±3.29	20.70±3.13	27.09±2.83	26.28±4.07
Carotenes, μg/100 ml	11.20±3.08	7.68±1.51	16.46±3.41+	9.87±3.26	7.49±2.55	12.0±2.65

tron transport in the endoplasmic reticulum of hepatocytes and contributes to normal functioning of the plasma membrane enzymes [5]. The Cordiamin-induced increase in the activities of the oxidation and glucuronidation systems in cholestatic rats is probably due to its enzyme-activating and antioxidant properties [1].

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