METHODS

LPO and Ethanol Biotransformation Systems in the Liver as Markers of Predisposition to Ethanol Hepatotoxicity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 12, pp. 693-696, December, 2002
Original article submitted February 20, 2001

An original experimental model for detecting organ-specific markers of predisposition to ethanol hepatotoxicity is proposed. A relationship between congenital activity of LPO processes in rat liver (before ethanol intoxication) and the type and severity of ethanol-induced damage to the liver was demonstrated using methods of mathematical modeling. It was proven that intact rats with genetically high MDA levels in the liver and more active systems of MDA generation in ascorbate- and NADPH-dependent reactions are prone to ethanol-induced damage to the liver.

Key Words: *ethanol; liver involvement; predisposition; lipid peroxidation; ethanol biotransformation*

Biochemical mechanisms responsible for human and animal predisposition to ethanol-induced damage to the liver remain still little studied. We developed an original experimental model for detecting organ-specific markers of increased sensitivity to hepatotoxic effect of ethanol. Using this model we tried to elucidate the role of LPO processes and ethanol biotransformation in the predisposition of rats to ethanol hepatotoxicity.

MATERIALS AND METHODS

Experiments were carried out on 112 random-bred male albino rats (250-300 g). Experimental (n=94) and control (n=16) animals were subjected (under ether narcosis) to partial hepatectomy (removal of the central and left lobes of the liver, 65-70% of the organ) with ligation of lobe bases [10]. After 2 months, ethanol (30% aqueous solution, daily dose 5 g/kg) was administered to experimental animals through a gastric tube for 57 days. Controls received the same volume of water.

The content of MDA, intensity of ascorbate- (Ad-LPO) and NADPH-dependent LPO (NADPH-LPO) [7], contents of diene conjugates and ketotriene ketones [13], intensity of chemiluminescence fast flash [1], and alcohol dehydrogenase [15] activity were evaluated in liver lobes removed before ethanol intoxication. Ethanol and acetaldehyde concentrations in the blood collected from the retroorbital sinus were measured by gas chromatography [5] 1 h after the first dose of ethanol. The intensity of inflammatory infiltration, hepatocyte vacuolation, destruction, and death, and fatty infiltration of the parenchyma in liver fragments were examined after ethanol intoxication by routine

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0007-4888/02/1346-0600$27.00

methods. Activities of marker enzymes of liver injury (alanine aminotransferase and γ-glutamyltransferase, AlAT and GGTP, respectively) in the plasma were measured [12].

The relationships between congenital intensity of LPO processes and ethanol biotransformation in the liver (before ethanol intoxication) and blood ethanol and acetaldehyde levels 1 h after the first ethanol dose, on the one hand, and the type and severity of subsequent liver involvement, on the other, were evaluated using correlations analysis and step-by-step multifactorial regression and dispersion analysis [2,4].

RESULTS

Parameters of LPO and ethanol metabolism in the blood and liver lobes were similar in intact rats and in the same animals 2 months after partial hepatectomy (after restoration of liver structure and function before the start of chronic ethanol intoxication). Histological structure of the liver 2 month after partial hepatectomy did not differ from normal, which attested to its complete structural and metabolic recovery.

Two of 112 rats died from unknown causes during the postoperative period; the survivors did not differ from intact animals. During forced alcoholization the animals did not gain weight and became aggressive. Some rats died, mainly from gastric hemorrhages and/or pneumonias. The animals fell asleep several minutes after ethanol administration. The duration of sleep after 2nd, 16th, 30th, and 44th doses of ethanol varied from 1 to 400 min.

The parameters of liver homogenate were as follows: MDA 0.1-0.4 nmol/mg, NADPH and Ad-LPO 0.46-1.38 and 0.49-1.98 nmol/min/mg, respectively, diene conjugates and ketotriene ketones 4.81-10.83 and 1.82-6.02 opt. dens. units/g, respectively. The intensity of chemiluminescence fast flash in the postmicrosomal fraction was 12-87 pulse/sec.

Alcohol dehydrogenase activity in the postmitochondrial fraction varied from 1.70 to 10.00 nmol/min/mg.

One hour after the first dose of ethanol its concentration in the whole blood was 13.6-95.7 nmol/liter, that of acetaldehyde 0.3-70.8 μmol/liter (no more than 20 μmol/liter in the majority of rats).

The intensity of inflammatory infiltration of the liver and hepatocyte vacuolation after chronic ethanol intoxication varied from 0.2 (very slight) to 4 (most severe) points. The degree of hepatocyte destruction and death varied from 0.33 (slight) to 4 points, degree of fatty infiltration of liver parenchyma from 0.1 (slight) to 3 (severe) points. The latter sign, together with signs of hepatocyte vacuolation, was not detected in 1 rat.

Plasma AlAT activity after chronic ethanol intoxication was 0.64-3.66 mmol/liter *vs*. 0.1-0.9 mmol/liter in the control, GGTP 6.0-50.4 μmol/min/liter (not recorded in controls).

Inflammatory infiltration of the liver parenchyma was associated with increased level of MDA in these animals before ethanol administration (Table 1). Genetically higher level of liver MDA and more active systems of its production (Ad- and NADPH-LPO) promoted hepatocyte destruction and death. Activity of liver alcohol dehydrogenase was increased in this group of animals.

High activity of plasma GGTP in rats after ethanol intoxication was associated with increased intensity of chemiluminescence fast flash in the liver of these animals before ethanol intoxication and low ethanol level in the blood (Table 1).

TABLE 1. Correlations between Parameters Characterizing the Severity of Ethanol-Induced Damage to the Liver and Intensity of LPO Processes, Ethanol and Acetaldehyde Metabolism before Ethanol Intoxication

Parameters before intoxication	Liver				Plasma	
	II	HV	HDD	FIP	AlAT	GGTP
MDA	0.42*	0.39	0.52*	0.34	-0.03	0.02
Ad-LPO	0.001	-0.26	0.44*	-0.12	-0.30	0.01
NADPH-LPO	0.20	-0.39	0.41*	-0.22	-0.35	-0.03
Diene conjugates	-0.30	-0.34	0.14	-0.28	0.12	0.62*
Ketotriene ketones	0.29	0.21	0.26	0.35	-0.08	0.16
Chemiluminescence fast flash	0.22	-0.11	0.15	-0.05	-0.32	0.62*
Alcohol dehydrogenase	0.23	-0.06	0.43*	-0.10	-0.07	-0.22
Ethanol	-0.27	0.06	-0.19	-0.08	-0.17	-0.67*
Acetaldehyde	-0.15	-0.20	-0.26	-0.35	-0.25	0.04

Note. *The most close correlations.

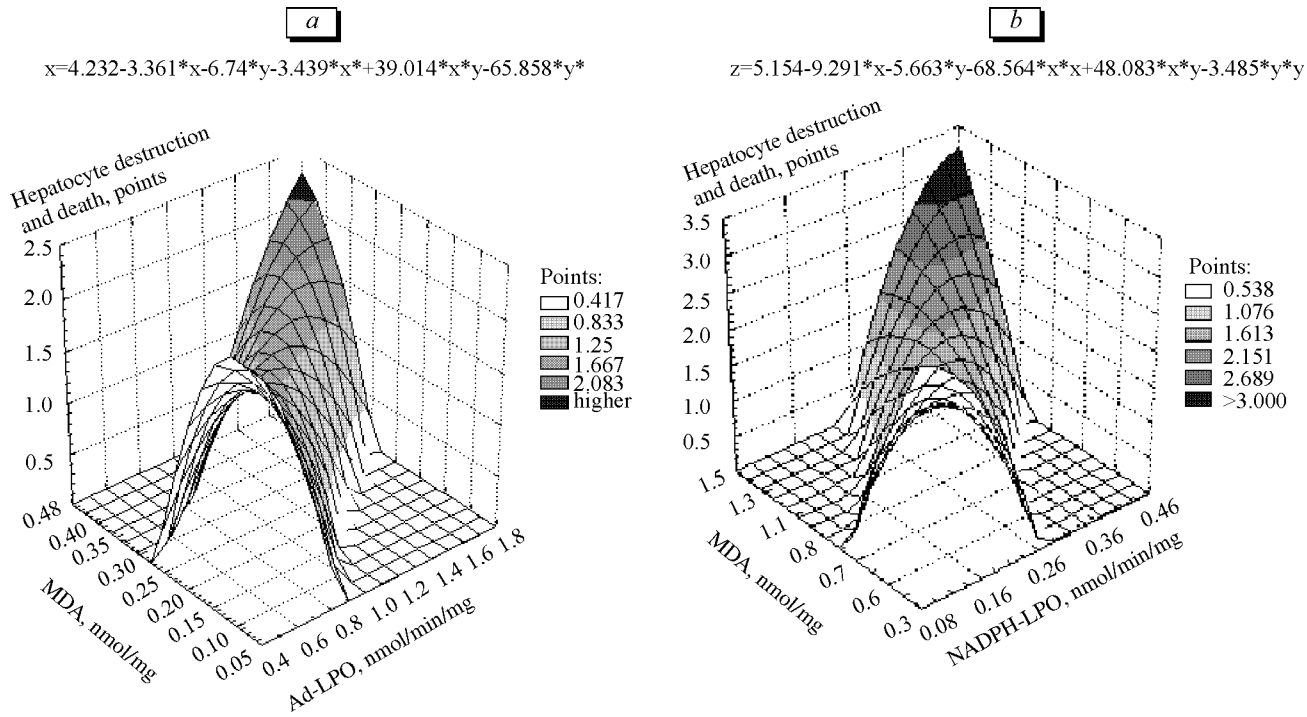

Fig. 1. Relationship between MDA content, ascorbate (Ad-, *a*) and NADPH-dependent (*b*) LPO in the liver before intoxication and severity of hepatocyte destruction and death after ethanol intoxication. II: inflammatory infiltration; HV: hepatocyte vacuolation; HDD: hepatocyte destruction and death; FIP: fatty infiltration of parenchyma.

The severity of hepatocyte destruction and death under the effect of chronic ethanol intoxication was higher in rats with initially high level of MDA in the liver and more active systems of its production via Ad- and NADPH-LPO (Fig. 1). The relationship between these parameters is described by nonlinear multiple regression equations.

The results of dispersion analysis indicate high informative qualities of these models. The models are statistically significant ($p<0.05$).

A direct correlation ($R=0.72$, $p<0.01$) between the initial biochemical parameters of the liver (before ethanol intoxication) and the type and severity of morphological changes in the liver and biochemical changes in the blood (after ethanol intoxication) was revealed. It means that (judging from the above listed morphological and biochemical parameters) predisposition to ethanol-induced damage of the liver is in 72% cases determined by congenital peculiarities of the studied biochemical processes in the liver. In 28% cases predisposition to ethanol-induced damage of the liver is caused by other unknown factors.

Hence, an original experimental model is proposed for detecting organ-specific markers of predisposition to ethanol hepatotoxicity. Our results indicate an important role of genetically determined high activity of LPO processes in rat liver (before ethanol intoxication) as a factor of predisposition to further ethanol-induced damage to the liver.

Increased activity of alcohol dehydrogenase is also a sign favoring ethanol damage to the liver, because it determines rapid transformation of ethanol into toxic acetaldehyde.

These results are in line with published reports. It was shown that ethanol intoxication is associated with LPO activation [14] at the expense of (acetaldehyde stimulates LPO and formation of superoxide by neutrophils) [6]. Moreover, ethanol induction of cytochrome P-4502E1 leads to subsequent stimulation of microsomal LPO [8]. The level of cytochrome P-4502E1 was found to be maximum in the centrolobular zone of the liver, most sensitive to hepatotoxic effect of ethanol [6].

LPO products and their metabolites induce damage to the liver by stimulating collagen production and promoting the development of cirrhosis [6]. Exhaustion of the liver antioxidant system (particularly glutathione) under the effect of ethanol aggravates these effects [9].

The approach proposed in our study can be used for the search for other markers of predisposition to ethanol-induced damage to the liver and markers of predisposition to liver injuries caused by other factors.

The study was financially supported by Belorussian Republican Foundation for Basic Research, Council of Ministers of Republic of Belarus (grant No. B96-335).

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