PHARMACOLOGY AND TOXICOLOGY

Plasma Content of 2-Thiobarbituric Acid-Reactive Substances in Mice Protected with Zinc-Metallothionein against Acute Alcohol Intoxication

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The malonic dialdehyde content in murine plasma decreases considerably after 1-24 h of acute alcohol intoxication (3 g/kg intraperitoneally). Zinc-metallothionein from rat liver administered in a dose of 2 mg/kg prior to alcohol normalizes the malonic dialdehyde level, whereas a mixture modeling zinc-metallothionein (albumin, cysteine, and zinc) does not change it. A 2- to 2.5-fold increase in the malonic dialdehyde content is observed in all cases after 3 days. It is assumed that the effect of zinc-metallothionein is associated with its ability, similarly to other thiol compounds, to stimulate the metabolism of ethanol and acetaldehyde and to reduce the toxicity of the latter by forming mixed compounds.

Key Words: *thiobarbituric acid-reactive substances; blood plasma; mice; ethanol; zinc-metallothionein*

The role of lipid peroxidation (LPO) in ethanol intoxication remains unclear. There is evidence that the concentration of LPO products in animal tissues increases in alcohol intoxication [4,12,13]; however, in some studies such a rise was not revealed in either liver or plasma [4,7,10,11]. In acute intoxication the liver content of endogenous metallothioneins (MT) is elevated. Metallothioneins are low-molecular weight proteins containing up to 30% cysteine and capable of binding ions of heavy metals. An increase in their concentration is regarded as an adaptive response aimed at reducing the toxic effect [8]. Previously it was shown that exogenous Zn-MT mitigates acute toxicity of ethanol in mice [3]. The ability of MT to normalize the levels of thiol groups during LPO may account for this phenomenon [3,8]. This study is an attempt to examine the plasma LPO content in mice intoxicated with ethanol and protected with Zn-MT.

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MATERIALS AND METHODS

Experiments were performed on (CBA×C57Bl)F_1 mice weighing 25-32 g. All solutions were injected intraperitoneally. Purification and characterization of Zn-MT dissolved in standard buffer (10 mM Tris-HCl, pH 7.4) were described elsewhere [3]. In each experimental series the mice were divided into four groups. Group 1 mice were administered Zn-MT (2 mg/kg in 0.3 ml standard buffer) prior to ethanol (32% v/v in normal saline, 3 g/kg, i.e., about 1/2 LD_{50} [3]). In group 2, the mice received a mixture modeling Zn-MT [3] (0.3 ml in standard buffer, 2 mg/kg). The mixture contained 70% serum albumin, 30% cysteine chlorohydrate, and zinc chloride (5.5% of the total content of the

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first two compounds) [3]. Group 3 animals were administered 0.3 ml buffer before alcohol injection. In group 4 (control) the mice received the standard buffer (0.3 ml) and the corresponding volume of normal saline. The animals were decapitated under ether anesthesia, heparinized blood was centrifuged, and the content of 2-thiobarbituric acid (TBA)-reactive substances was determined and calculated in terms of the malonic dialdehyde (MDA) concentration. For this purpose proteins were precipitated with trichloroacetic acid (a final concentration of 9%) with subsequent centrifugation. The supernatant volume was adjusted to 2 ml (0.5-2 ml) with normal saline containing 9% trichloroacetic acid. Then we added 1.5 ml 0.9% TBA in 50% acetic acid (v/v) and $FeCl_3$ solution to a final concentration of 0.5 mM (to degrade preformed lipid hydroperoxides to MDA [15]). The mixture was heated for 20 min at 100°C, and the MDA concentration was determined according to the light absorbance of the cooled solution at 532 nm [9,15]. A direct correlation was established between the results obtained by this method and the method requiring a reaction between TBA and chloroform-methanol extract of plasma lipids ($r=+0.874$).

When protein binding to acetic aldehyde was determined, probes (final volume 0.8 ml) contained 3.5 μg [^{14}C]-acetaldehyde (specific radioactivity 5.7×10^5 cpm/μg) and 4-80 μg proteins in 10 mM Tris-HCl (pH 7.4). They were incubated for 30 min at 37°C, precipitated with 50% trichloracetic acid (0.2 ml) for 1 h, and filtered through Whatman-3MM filters. Residual radioactivity was measured in a dioxane scintillator in a Delta counter.

[^{14}C]-Acetaldehyde was from Amersham, human serum albumin and cysteine chlorohydrate were from Reanal, TBA was from Serva, and cytochrome C was from Sigma. The other reagents were of chemically pure and research grade.

The data were analyzed using the Student-Fisher *t* test.

RESULTS

In the control mice, the plasma TBA-reactive substances content (calculated for the MDA concentration) was 1.5±0.1 nmol/ml, which is consistent with published data [12,15]. Ethanol markedly reduced the MDA content 1-24 h after administration, the modeling mixture had a weak effect on alcohol intoxication, while Zn-MT increased the MDA concentration (Fig. 1). By the second day, the MDA concentration was practically the same as in the control and on day 3 it rose in all groups (Fig. 1). Injection of Zn-MT without ethanol had no effect on the MDA concentration (determinations were performed 3 h and 3 days postinjection). Presumably, there are several causes of the ethanol-induced decrease in the MDA content within a 24-h period. First, there are artifacts associated both with the effect of ethanol and acetaldehyde on the TBA-plasma reaction and with the alterations in the reaction between nonlipid substances and TBA. Thus, the decrease in the MDA content may result not from reduced LPO but rather from some other factors. However, the addition of ethanol to the plasma to a final concentration of 7% did not change its reaction with TBA, and acetaldehyde (70 mM) not only did not lower but even enhanced the release of MDA-like substances (probably due to oxidation of carbohydrates by aldehyde [9]). Sugars, pentoses, and sialic acid are nonlipid compounds reacting with TBA [9,15]. Consumption of alcohol is reported to lead to hyperglycemia [1]. Under the chosen conditions, the reaction between TBA and glucose, sucrose, and D-ribose taken in concentrations 3- to 10-fold higher than the physiological levels provided a yield of MDA-like substances ranging from 0.05 to 0.18 nmol/ml, which is considerably lower than the mean MDA content in mouse plasma. Sialic acid is present in the blood mainly as glycoprotein constituents [15]. We fractionated plasma globulins by electrophoresis. Specific staining of electrophoregrams showed that after alcohol administration the content of the carbohydrate component in plasma glycoproteins did not correlate with the plasma MDA content (data not shown). It is therefore unlikely that

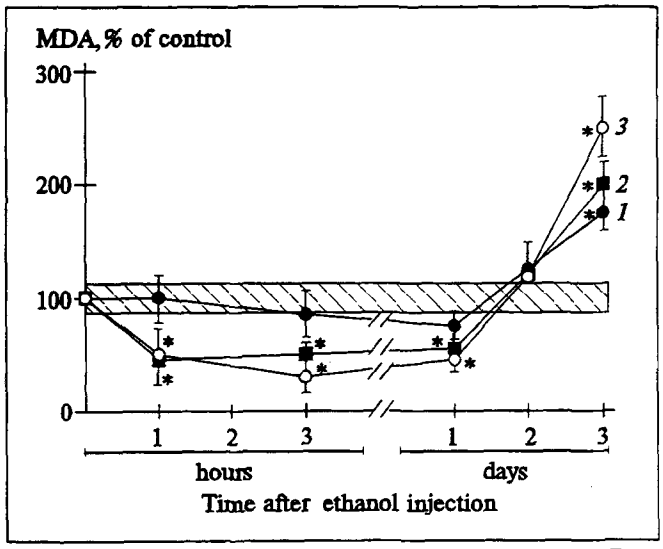

Fig. 1. Plasma MDA content in mice protected with Zn–MT and Zn–MT–modeling mixture against acute ethanol intoxication. *1*) Zn–MT + ethanol; *2*) modeling mixture + ethanol; *3*) ethanol. The shaded area is the control zone. Asterisk indicates differences statistically significant at $p<0.05$.

TABLE 1. Binding of [^{14}C]-Acetaldehyde by Various Proteins ($M \pm m$)

Protein content in probe, μg	Bound [^{14}C]-acetaldehyde, cpm
Without proteins (background)	459±26
Zn-MT	
4	540±14
8	582±16
20	649±38
40	946±18
80	1418±188
Albumin	
40	502±21
80	812±15
Cytochrome C	
8	539±19
16	561±20
40	596±7
80	764±16

the decrease in the MDA concentration after ethanol injection is due to artifacts. Presumably, the substances detected in this study appear due to degradation of primary LPO products.

It has been reported that administration of ethanol in a dose of 3 g/kg has a slight effect on the level of LPO products in the liver of rodents [4,13]. The plasma and liver MDA contents are increased 6 h after administration of 5 g/kg ethanol [12,13]. However, high ethanol doses (6-8 g/kg) do not raise the content of LPO products (including MDA) in the liver [7,10,11]. It has been shown that alcohol administered in a dose of 8 g/kg reduces the content of LPO products in rat liver. The authors attribute this phenomenon to the potential role of ethanol as a free-radical interceptor [7]. Although alterations in the plasma MDA content are not always associated with those in the liver MDA content [12], this property of ethanol may be another cause of the drop in the content of plasma LPO products. The activation of aldehyde dehydrogenase which occurs in alcohol intoxication [2] may be another reason for the reduced content of MDA, which is used as a substrate [4]. Finally, ethanol administration may induce a hypoxia-like state of the organism, due both to NADH accumulation [4,5] and to suppressed external respiration [5]. In hypoxia, LPO in the blood may be much less intense.

It is likely that normalization of the MDA plasma concentrations by Zn-MT results from the ability of Zn-MT to improve the general state of the organism by lowering the acetaldehyde toxicity.

Glutathione and other thiol compounds lower blood concentrations of ethanol and acetaldehyde by activating alcohol dehydrogenase [6] and forming stable complexes with aldehyde, which stimulates its metabolism and clearance [6,14]. Presumably, Zn-MT can form stable complexes with acetaldehyde (Table 1). Zinc-metallothionein bound much greater amounts of these toxic compounds compared with albumin and the metal-binding protein cytochrome C (Table 1). Similarly to other thiol compounds [6,14], Zn-MT lowers the blood concentration of ethanol, this diminishing its role as a free-radical interceptor. By stimulating the metabolism of ethanol and acetaldehyde, Zn-MT probably mitigates the hypoxia caused by ethanol oxidation. Eventually, all these effects can affect the oxidation processes leading to MDA formation and normalization.

The considerable increase in the MDA concentration observed on day 3 of intoxication (Fig. 1) may result not from the acute toxicity of ethanol, but rather from intoxication. Damage to the liver and to other organs, and membrane destruction (for example, due to hemolysis) may induce a release of TBA-reactive substances. Administration of Zn-MT has no effect on these consequences (Fig. 1).

On the basis of these findings it can be hypothesized that the ability of Zn-MT to reduce the acute toxicity of ethanol is not associated with its influence on LPO, but rather with the stimulation of metabolism and clearance of alcohol and acetaldehyde. It is noteworthy that the detoxicating dose of Zn-MT is lower [3] than that of other thiol compounds [6,14].

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