Lowering of Lipid Peroxidation and Acute Toxicity of Bromobenzene by a Polymeric Form of Zinc-Metallothionein

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Plasma and liver contents of malonic aldehyde are studied one day after administration of bromobenzene to mice pretreated with a polymeric form of zinc-metallothionein from rat liver. It is found that zinc-metallothionein injected in a dose of 1-4 mg/kg 5-10 min prior to injection of bromobenzene (2 g/kg, about 56% of LD_{50}) markedly lowers the malonic dialdehyde level and active toxicity of this xenobiotic. Administration of a mixture modeling Zn-metallothionein (albumin, cysteine, and zinc) in a dose of 4 mg/kg has no appreciable effect on the malonic dialdehyde level raised after bromobenzene injection, and does not change its LD_{50}. It is concluded that the protective effect of exogenous zinc-metallothionein is due to its antioxidant activity, which allows for normalization of lipid peroxidation.

Key Words: *zinc-metallothionein; bromobenzene; lipid peroxidation; acute toxicity; mice*

Metallothioneins (MT) are low-molecular-weight proteins containing up to 30% cysteine and capable of binding ions of heavy metals. MT synthesis is induced by toxic influences, which raises animals' resistance to them [6]. It is thought that the antioxidant activity of MT is one of the mechanisms underlying their antitoxic effects [6]. In fact, MT preparations do inhibit lipid peroxidation (LPO) *in vitro* [12]. Lipid peroxidation in rodents is suppressed after induction of the synthesis of endogenous MT by heavy metals [9]. At the same time, little is known about the effect of exogenous MT on LPO and other biological processes *in vivo*. Zn-MT reduces the damaging effect of Cd-MT in rats [15], mitigates the acute toxicity of ethanol [1], and protects mice against radiation [2]. The aim of this study was to examine the effect of exogenous Zn-MT on plasma and liver levels of LPO products after the injection of bromobenzene, a compound inducing lipid peroxidation.

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MATERIALS AND METHODS

Experiments were performed on male (CBA×C57 Bl)F_1 mice weighing 24-26 g. All solutions were injected intraperitoneally. Purification and characterization of Zn-MT from rat liver (the protein was dissolved in the standard 10 mM Tris-HCl buffer, pH 7.4) were described elsewhere [1]. A polymeric form of Zn-MT, which is more stable in the organism, was obtained by a reported method [11]: lysine residues were cross-linked by glutaric aldehyde (Fluka), and the protein was separated from the low-molecular form on Sephadex G-75. After electrophoresis of the polymer under denaturating conditions, a wide band corresponding to a molecular weight range of 61-70 kD was obtained, which is consistent with the published data [11]. Thus, the polymeric form consisted of 8-10 Zn-MT monomers (6-7 kD) [6,11]. Mice were in-

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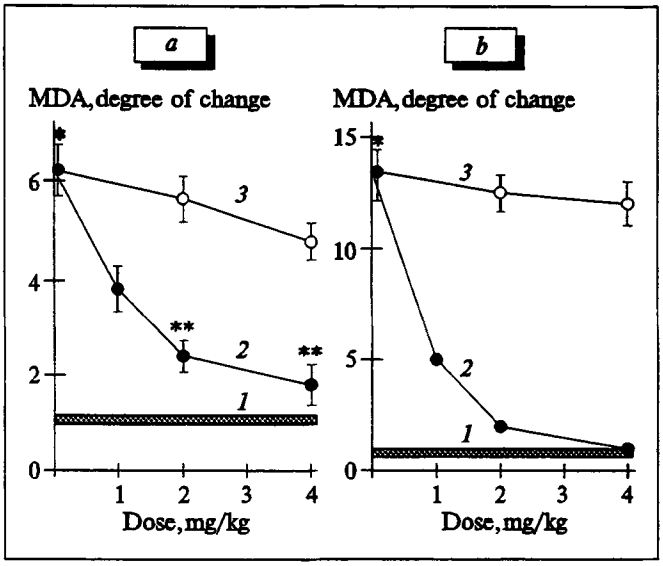

Fig. 1. Liver (*a*) and plasma (*b*) MDA content 1 day after bromobenzene injection (2 g/kg) to mice pretreated with Zn–MT. *1*) intact control; *2*) Zn–MT; *3*) mixture modeling Zn–MT. Asterisk indicates $p<0.05$ compared with intact control, two asterisks – $p<0.005$ compared with bromobenzene administration without Zn–MT.

jected with polymeric Zn-MT (in 0.2 ml standard buffer), and bromobenzene in mineral oil (0.2-0.4 ml) was injected 5-10 min later. In one control group, prior to bromobenzene injection the mice were injected with the standard buffer, while in the other control group they were administered a mixture modeling Zn-MT. This mixture (pH 7.4) contained 70% human serum albumin (Reanal), 30% cysteine chlorohydrate (Reanal), and $ZnCl_2$ (5.5% of the total content of the first two compounds) [1]. The malonic dialdehyde (MDA) content was determined in the liver homogenate and blood plasma (pooled plasma from 3-4 mice) as described [8]: proteins were precipitated with trichloroacetic acid, and the reaction with 2-thiobarbituric acid was carried out in the supernatant. Acute toxicity

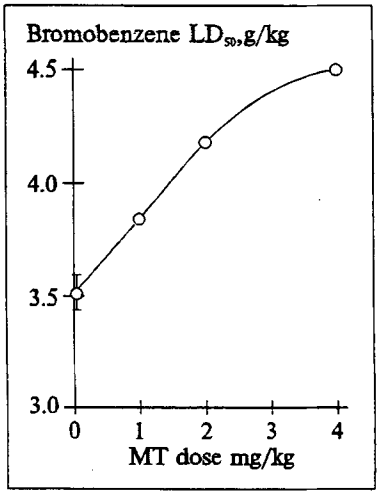

Fig. 2. Acute toxicity of bromobenzene in mice pretreated with Zn–MT.

(LD_{50}) was assessed by the method of Kerber. The protein concentration was determined by staining with Coumassie G-250 [10]. The results were analyzed using the Student-Fisher *t* test.

RESULTS

LD_{50} for bromobenzene was equal to 3.55±0.05 g/kg. In intact animals, the liver content of MDA was 0.179±0.016 nmol/mg protein in the homogenate and the plasma content was 1.5±0.1 nmol/ml. One day after administration of bromobenzene (2 g/kg), the MDA plasma and liver contents increased considerably (Fig. 1, *a, b*). There were marked changes in the protein content of the liver homogenate (82.6±6%, $p<0.05$), and therefore the observed rise of the MDA content was not due to a decrease in this parameter. In intoxicated animals, the liver mass was increased slightly (142±5%, $p<0.05$).

It is noteworthy that the increase in the MDA concentration in the plasma was higher than that in the liver (Fig. 1, *a, b*). This is probably due to the considerably lower baseline MDA level in the blood than in the liver: assuming the liver weight in intact animals to be 0.9 g and the plasma volume to be not higher than 2 ml, the total MDA content in the plasma is no more than 2% of that in the liver. The bromobenzene-induced increase in the liver MDA content leads to the release of LPO products in the blood, and the blood MDA content becomes as high as 4% of the liver MDA content.

Administration of Zn-MT markedly reduced in a dose-dependent manner the bromobenzene-induced increase in the MDA contents of the liver (Fig. 1, *a*) and the plasma (Fig. 1, *b*). The effect of the mixture modeling the Zn-MT composition was much weaker (Fig. 1, *a, b*). Administration of Zn-MT to mice resulted in LPO inhibition and a decrease of the acute toxicity of bromobenzene (Fig. 2). The modeling mixture administered in the dose corresponding to 4 mg/kg Zn-MT did not elicit this effect (after its administration LD_{50} of bromobenzene was 3.2±0.3 g/kg).

The effect of bromobenzene on LPO in the liver is due to the ability of this compound to be metabolized to peroxides which bind to reduced glutathione. As a result, the glutathione concentration in the liver drops several tens-fold, which leads to the accumulation of free radicals, intensification of LPO, and, as a consequence, liver damage [4]. It can be assumed that the mechanisms of the protective effect of Zn-MT is associated with the ability of MT to inactivate free radicals. For example, MT from rabbit liver inhibited the *in vitro* formation of hydroxyl and superoxide

radicals generated by the xanthine-xanthine oxidase system more effectively than albumin, which a higher molecular weight. All 20 cysteine residues were involved in inactivation. It should be noted that the effective cysteine concentration in MT was 20-fold lower than that contained in glutathione [13]. A high degree of DNA protection against the damage caused by hydroxyl radical was observed at 13 μM MT and 10 mM glutathione [3]. Metallothionein (4 μM) is a much more effective protector against radiolysis of dAMP than cysteine (100 μM) [5]. It is hypothesized that the extraordinary properties of MT are due to the presence of unique diamagnetic metal-thiolate clusters [3]. Similarly to glutathione, Zn-MT, replenishing the pool of groups with reduced glutathione that have been decreased by bromobenzene, binds with epoxides (bromobenzene metabolites). As a result, the toxicity of epoxides drops and their ability to bind to glutathione is weakened (by the competition mechanism), so that its level normalizes.

It should be noted that the decrease in the LPO intensity under the action Zn-MT may be associated not only with thio-groups but also with the influence of the zinc contained in MT. It has been hypothesized that the zinc inhibits LPO, blocking by competition mechanisms the effect of iron ions [12]. It is noteworthy that zinc ions have a higher reactivity in Zn-MT than in free form [14].

A tendency toward LPO normalization was also observed after administration of the modeling mixture to mice. Presumably, this is due to the presence of cysteine and zinc in it. However, the effect was much weaker than in the case of Zn-MT, and the acute toxicity of bromobenzene remained unchanged. The fact that the effective protecting doses of cysteine and zinc are equal to hundreds of milligrams per kg [7] may account for the low efficiency of the modeling mixture. It can be concluded that the ability of Zn-MT to reduce the LPO level and the acute toxicity of bromobenzenes is specific for the structure of this protein and is not due to mechanical unification of cysteine and zinc.

Thus, this study confirms the ability of MT to protect the organism not only from heavy metal ions [15], ethanol [1],and ionizing radiation [2] but also from the industrial toxin bromobenzene. Our results suggest that further investigation of MT preparations is necessary, since they show promise as protectors against various harmful agents.

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