The Influence of α-Lipoic Acid on the Toxicity of Cadmium

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Abstract. α-Lipoic acid (α-LA) is an important antioxidant drug with chelating properties In experiments performed in male mice (CD-1, Charles River) the effects of cadmium on lipid peroxidation (LP), GSH level, the activity of catalase and glutathione peroxidase (GSH-Px) in liver homogenates were studied Mice were injected with $CdCl_2$ 2 5 H_2O at a dose of 40 μmol kg^{-1} s c α-LA was administered simultaneously i p at the dose corresponding to α-LA-to-Cd molar ratio of 5 1 The experiments were completed at 24h Cadmium increased LP to 200 7% of controls This effect was prevented by α-LA treatment ($p \leq 0$ 05) GSH level was decreased to 81 7% of controls and it was not affected by α-LA GSH-Px activity diminished by Cd administration was corrected by α-LA ($p < 0$ 001) Catalase activity decreased by Cd remained unaffected The administration of α-LA alone enhanced LP and the activity of catalase As estimated by AAS, Cd content in the liver, the kidneys, the brain and the testes remained unaffected by α-LA treatment In the acute toxicity experiment, the mortality associated with cadmium was decreased by α-LA administration The results suggest that the toxicity of Cd was decreased mainly by the antioxidant activity of α-LA rather than by cadmium removal from tissues

Key words: Cadmium — α-Lipoic acid — Oxidative status

Introduction

α-Lipoic acid (α-LA) [6,8-thioctic acid, 1,2-dithiolane-3-pentanoic acid] is a naturally occurring substance It is present in all kinds of prokaryotic and eukaryotic cells and functions as a cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids α-LA is a potent antioxidant, and it has been shown to be an efficient chelator of several metals Lipoic acid β-oxidation products (bisnorlipoate and tetranorlipoate) and dihydrolipoic acid (DHLA), a reduced form of lipoic acid, contribute to the antioxidant activity of α-LA DHLA is a potent antioxidant but by virtue of its ability to chelate and reduce iron it also shows pro-oxidant activity (Biewenga et al 1997, Packer et al 1996, 1997)

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α-LA is a potent scavenger of hydroxyl radicals, singlet oxygen and hypochlorous acid but it does not scavenge hydrogen peroxide and the superoxide radical. α-LA can form stable complexes with Mn^{2+}, Zn^{2+} and Cu^{2+}. There is some evidence that it may chelate Fe^{2+}, Pb^{2+}, $Cu^{2+}$and Cd^{2+} (Müller and Menzel 1990; Packer et al. 1996; 1997).

The aim of this experiment was to study the protective effect of α-LA in acute cadmium intoxication. Our interest was focused mainly on the alteration of the oxidative status of the liver. The administration of cadmium chloride was found to increase lipid peroxidation (LP) and to deplete reduced glutathione (GSH), and to decrease glutathione peroxidase activity (GSH-Px) (Caisova and Eybl 1997; Eybl et al. 1996). The content of cadmium in the liver and several other organs was also determined.

Materials and Methods

Experiments were performed in male mice (CD-1, Charles River, 25–30 g body weight) divided into 4 groups of 6–10 animals Two groups of mice were injected with $CdCl_2$ 2 $5H_2O$ (anal Gr , Lachema, Brno, Czech Republic) at a single dose of 9 1mg/kg (40 μmol/kg) s c In one of these groups the injection of cadmium was immediately followed by the administration of α-lipoic acid i p (Sigma Chemical Co , USA)at the dose corresponding to α-LA Cd^{2+} molar ratio of 5 1 A third group was injected with α-LA alone Animals in the last group served as controls, receiving saline The experiment was completed 24 h after the drug administration The animals were killed in ether anesthesia by decapitation and the livers, kidneys, brains and testes were removed

Liver homogenates were used for further analyses Lipid peroxidation expressed as malondialdehyde (MDA) concentration was measured by the thiobarbituric acid test (Uchiyma and Mihara 1978), the level of glutathione (GSH) was determined using Ellman's reagent (Sedlak and Lindsay 1968), the activity of GSH-Px was measured according to Gunzler et al (1974), and catalase activity was determined by Aebi's method (Aebi 1972)

For cadmium determination in liver, kidneys, brain and testes, tissues were weighed, placed in platinum crucibles and dry-ashed in a muffle furnace at 460–500 °C for 18–24 h Ash was solubilized with 3 mol/l HCl Appropriately diluted samples were analyzed by graphite furnace atomic absorption spectrometry

In another experiment, pretreatment with α-LA on the acute toxicity of cadmium administered at the dose of 10 5 mg $CdCl_2$ 2 5 H_2O/kg s c was studied Mice were divided into two groups One group of animals was treated with α-LA i p 48 h and 24 h before and then simultaneously with cadmium chloride injection A single dose of α-LA corresponded to the dose of Cd^{2+} at α-LA Cd^{2+} molar ratio of 10 1 The other group received cadmium chloride only The experiment was completed on day 5 after cadmium chloride injection The results were statistically evaluated using the unpaired *t*-test and chi-square test

Results

Cadmium increased lipid peroxidation in the liver to 200.7% of controls. This effect was prevented by α-LA treatment (Fig 1). The decrease of GSH levels (to 81 7% of controls) caused by cadmium was not affected by α-LA co-administration (Fig. 2) GSH-Px activity was diminished by cadmium to 73.5% of controls. α-LA corrected

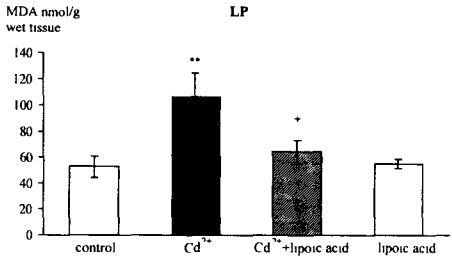

Figure 1. The level of lipid peroxidation in the liver 24h after treatment with Cd^{2+} and α-lipoic acid Data represent mean ± S E M ** Significantly different from control mice at p ≤ 0,01 + Significant difference between Cd^{2+} and Cd^{2+}+Lipoic acid groups at $p \leq 0\ 05$

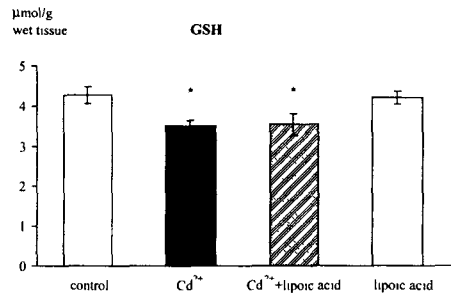

Figure 2. GSH content in the liver 24h after treatment with Cd^{2+} and α-lipoic acid Data represent mean ± S E M * Significantly different from control mice at $p \leq 0\ 05$

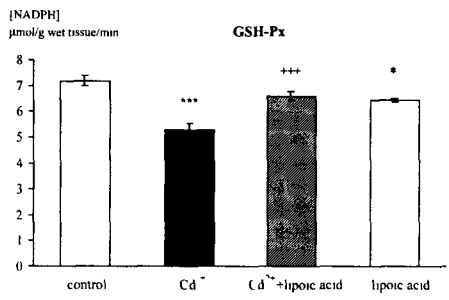

Figure 3. GSH-Px activity in the liver 24h after treatment with Cd^{2+} and α-lipoic acid Data represent mean ± S E M *** Significantly different from control mice at $p \leq 0\ 001$ * Significantly different from control mice at $p \leq 0\ 05$ +++ Significant difference between Cd^{2+} and Cd^{2+} + Lipoic acid groups at $p \leq 0\ 001$

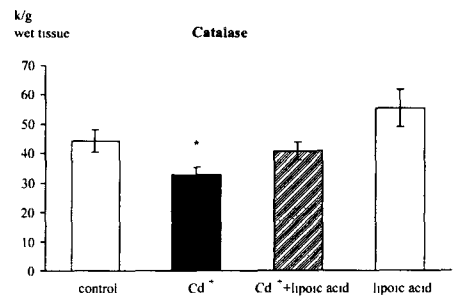

Figure 4. Catalase activity in the liver 24h after treatment with Cd^{2+} and α-lipoic acid Data represent mean ± S E M, k = rate constant (s^{-1}) * Significantly different from control mice at $p \leq 0\ 05$

this effect significantly (Fig 3). Catalase activity was decreased by cadmium to 70.8% of controls and it was not significantly affected by α-LA (Fig. 4).

The cadmium content in the liver as well as in the kidneys, the brain and the testes remained unaffected by α-LA treatment (Tab. 1).

In the acute toxicity experiment α-LA pretreatment significantly prevented the mortality of mice (Fig. 5).

Table 1. Cadmium concentration (μg/g wet tissue) 24 h after treatment with Cd^{2+} and α-lipoic acid

	Liver	Kidneys	Brain	Testes
Cd^{2+}	31 2 ± 5 5	18 68 ± 15 33	0 14 ± 0 01	0 20 ± 0.05
Cd^{2+} + lipoic acid	34 7 ± 4 5	15 34 ± 3 98	0 13 ± 0 01	0 22 ± 0 02

$\bar{x}$ ± S D , $n = 8$ in each group

Discussion

α-Lipoic acid decreases the acute toxicity of cadmium but it does not affect cadmium distribution. In similar experiments performed in our laboratory a relatively selective iron chelator deferiprone corrected cadmium induced lipid peroxidation and GSH depletion (Eybl et al. 1997) without decreasing cadmium toxicity On the other hand dithiocarbamates, the prominent cadmium antidotes, decrease cadmium toxicity as well as improve disturbed oxidative status by promoting cadmium excretion from the body The mechanism of the preventive effect of α-lipoic acid in oxidative damage seems to differ from that of dithiocarbamates and of iron chelators. We assume that the acute toxic effect of cadmium is diminished mainly due to the ability of α-LA to block cadmium pro-oxidant activity This effect is probably based on the metabolic and antioxidant action on biomembranes for which the presence of the disulfide bond in the α-lipoic acid molecule is important.

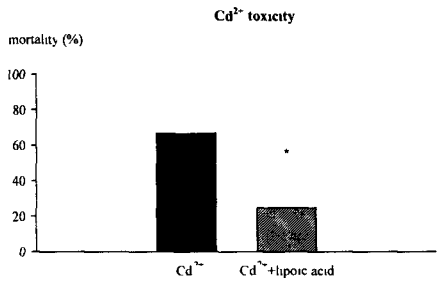

Figure 5. Mortality of male mice after treatment with Cd^{2+} and α-lipoic acid pretreatment * Significant difference between Cd^{2+} and Cd^{2+} + Lipoic acid groups at $p \leq 0\ 05$

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