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2,3-Dimercaptopropanol, 2,3-dimercaptopropane-1-sulfonic acid and *meso*-2,3-dimercaptosuccinic acid increase lead-induced inhibition of δ-aminolevulinate dehydratase in vitro and ex vivo

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Abstract

We investigated the effects of dimercaprol (BAL), *meso*-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-1-propanesulphonic acid (DMPS) on human blood δ-aminolevulinate dehydratase (δ-ALA-D) activity, the most reliable indicator of lead intoxication in humans, in the presence of lead in vitro. Furthermore, we studied the effects of the chelating agents, administered subcutaneously, on δ-ALA-D activity in blood and tissues of mice submitted to sub-acute lead exposure (50 mg/kg for 15 consecutive days, subcutaneously). In vitro results demonstrated that human blood δ-ALA-D activity was significantly inhibited (62%) by lead acetate. Lead acetate (1–1000 μM) pre-incubated with human blood increased the inhibitory potency of this compound on δ-ALA-D when compared to the assay without pre-incubation (89%). Chelating agents caused a marked potentiation of δ-ALA-D inhibition induced by lead, in vitro. One of the most notable observations in the present study was the correspondence between in vitro and ex vivo effects. In fact, BAL and DMPS increase the inhibitory effect of lead on δ-ALA-D activity from mice blood. The complexes formed (lead and chelators) were more inhibitory than lead alone in kidney and liver enzyme activity, ex vivo.

Keywords: δ-ALA-D; BAL; DMPS; DMSA; Chelating agents; Lead

1. Introduction

Lead (Pb) is one of the most widely used metals in industries and in many countries exposure to lead continues to be a widespread problem. Batteries, paints and pigments, plastic, ceramic, secondary foundries and welding are the most important occupational settings. The general population may get exposed to lead due to food and water contamination, and air pollution caused by industrial emission and gasoline containing lead compound (Pande and Flora, 2002). Oxidative stress appears to be a possible mode of the molecular mechanism for lead toxicity (Adonaylo and Oteiza, 1999). Blood lead levels have been correlated with lipid peroxidation in lead-exposed workers (Ye et al., 1999). The current approved treatment for lead poisoning is to administer strong thiol-containing chelators that form an insoluble complex with lead and remove it from lead burdened tissues (Flora and Kumar, 1993). Most of these chelating agents, however, induce many side effects (Flora and Kumar, 1993; Flora et al., 1998; Nogueira et al., 2003a, 2004).

Dimercaprol (BAL) is a dithiol compound used as a therapeutic agent in the treatment of poisoning induced by several heavy metals (Klaassen, 1990). Although, BAL has the capacity to ameliorate the deleterious

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effects of metal intoxication, it has a low therapeutic index (Andersen, 1989). Despite relevant evidence of the BAL therapeutic use, obtained from in vivo and in vitro animal data, as well as, clinical accounts, its use for metal poisoning treatment has been halted by data suggesting serious neurotoxicity (Pepin et al., 1995; Nogueira et al., 2000, 2001a,b). BAL has been shown to interfere with neurotransmitter systems, including the glutamatergic and GABAergic systems (Nogueira et al., 2000, 2001a,b).

Other metal chelators, such as *meso*-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-1-propanesulphonic acid (DMPS), have also been shown to be effective for treating the toxicity induced by a number of heavy metals (Andersen, 1989; Smith et al., 2000). These compounds are more hydrophilic and less toxic than BAL (Aposhian et al., 1995; Domingo, 1995). The therapeutic mechanism underlying the action of these compounds involves promoting metal excretion from the body, thus, potential interaction between clinically employed chelating agents and endogenous metals, for instance zinc, is probable (Cantilena and Klaasen, 1982).

Endogenous metals are essential components of many enzyme systems, for instance, δ-aminolevulinate dehydratase (δ-ALA-D) is a metalloenzyme requiring zinc ions for activity (Jaffe et al., 1995). δ-ALA-D catalyses the asymmetric condensation of two molecules of δ-aminolevulinic acid (δ-ALA) to porphobilinogen in the initial steps of heme biosynthesis (Gibson et al., 1955). δ-ALA-D is a sulfhydryl containing enzyme (Gibson et al., 1955; Barnard et al., 1977) and numerous metals such as mercury (Rocha et al., 1993, 1995), lead (Rodrigues et al., 1989, 1996; Goering, 1993) and other compounds that oxidize sulfhydryl groups modified its activity (Emanuelli et al., 1996; Barbosa et al., 1998; Flora et al., 1998; Jacques-Silva et al., 2001; Flora et al., 2002). Therefore, δ-ALA-D is inhibited by substances that compete with zinc and/or that oxidize the –SH groups (Farina et al., 2002; Nogueira et al., 2003a,b; Santos et al., 2004, in press) and is linked to situations associated with oxidative stress (Folmer et al., 2002; Pande et al., 2001; Pande and Flora, 2002; Tandon et al., 2002; Soares et al., 2003).

In addition, human exposure to Pb^{2+} causes an accentuated inhibition of blood δ-ALA-D (Meredith et al., 1979; Fujita et al., 1981; Pappas et al., 1995; Polo et al., 1995; Pires et al., 2002) and is associated with an intense anemia accompanied by an increase in urinary δ-ALA excretion (Oskarsson, 1989; Duydu et al., 2001). Therefore, δ-ALA-D activity is used as one of the most reliable indicators of Pb^{2+} intoxication in humans and other animals (Meredith et al., 1979; Pappas et al., 1995).

Previously, we have reported that the complexes formed between Hg^{2+} or Cd^{2+} and DMSA or DMPS were more inhibitory than the metals or the chelating agent alone to mice hepatic δ-ALA-D activity in vitro (Nogueira et al., 2003a). Moreover, in this study the effects of BAL, DMPS and DMSA on human δ-ALA-D activity in the presence of lead in vitro were investigated. Furthermore, we extended this study to evaluate the effect of chelating agents on δ-ALA-D activity of animals exposed to lead.

2. Materials and methods

2.1. Chemicals

δ-Aminolevulinic acid (δ-ALA), *meso*-2,3-dimercaptosuccinic acid (DMSA), 2,3-dimercaptopropane 1-sulfonate (DMPS), 2,3-dimercaptopropanol (BAL) and *p*-dimethylaminobenzaldehyde were purchased from SIGMA (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. The in vitro effect of chelating agents on human blood δ-ALA-D activity in the presence of lead acetate

2.2.1. Samples

The heparinized venous blood was obtained from human volunteers from our workgroup, University Federal of Santa Maria, RS, Brazil.

2.2.2. Enzyme assay

The activity of blood δ-ALA-D was assayed according to the procedure of Berlin and Schaller (1974). The principle of the method is based on the incubation of the enzyme with excess of δ-aminolevulinic acid. The porphobilinogen, which is formed within a fixed time, is mixed with modified Ehrlich's reagent and the color developed is measured photometrically (555 nm) against a blank. Enzymatic reaction was initiated by adding the substrate (ALA) to a final concentration of 2.2 mM in a medium containing 45 mM phosphate buffer, pH 6.8 and blood samples (200 μl). The incubation was carried out for 90 min at 37 °C.

2.2.2.1. Effect of chelating agents on δ-ALA-D inhibition induced by lead. The protective effect of chelating agents on δ-ALA-D inhibition induced by lead was carried out. The blood samples were pre-incubated with BAL (10 μM), DMPS (1 μM) or DMSA (10 μM) at 37 °C for 10 min. After this time, lead acetate (1–1000 μM) was added to the reaction medium followed immediately by the addition of the substrate (δ-ALA). The incubation was carried out for 90 min at 37 °C. The concentrations of BAL, DMPS and DMSA were chosen based on Nogueira et al. (2004) because of chelators, at these concentrations, did not modify blood δ-ALA-D activity.