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Ebselen and diphenyl diselenide do not change the inhibitory effect of lead acetate on delta-aminolevulinate dehidratase

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Abstract

It is known that lead is toxic to several species of animals, and growing data support the participation of oxidative in lead toxicity. Selenium compounds, like diphenyl diselenide and Ebselen have a thiol-peroxidase like and other antioxidant properties. In this work, we determine whether these non-thiol-containing compounds with antioxidant properties could reverse the toxicity produced by Pb^{2+}. Lead acetate injection followed by injection with Ebselen or diphenyl diselenide did not change the levels of non-protein thiol groups (NPSH), whereas simultaneous treatment with lead plus Ebselen reduced NPSH levels in liver. Lead and Ebselen caused a marked reduction in TBARS level in kidney, whereas lead or selenium compounds did not change TBARS levels in brain or liver. Lead acetate inhibited, δ-aminolevulinate dehydratase (ALA-D) activity in blood, liver, kidney and brain. Selenium compounds did not change enzyme activity nor the inhibitory effect of lead acetate in kidney and liver. Ebselen reversed brain ALA-D inhibition caused by Pb^{2+}. Reactivation index for ALA-D by DTT was higher in lead-treated groups than control groups in all tissues. Lead acetate or selenium compounds did not demonstrate alteration on [^{3}H]-glutamate uptake by synaptosomes, whereas lead acetate plus Ebselen showed an increase on [^{3}H]-glutamate uptake. The results of the present study indicate that ALA-D inhibition antecedes the overproduction of reactive oxygen species, which is becoming well documented in the literature.

Keywords: Lipoperoxidation; Selenium; Organocompounds; Antioxidant; SH groups; TBARS

1. Introduction

Lead continues to persist throughout the environment and is one of the most common environmental and occupational contaminant (Tonner and Heiman, 1997). Routes of lead exposure include ingestion of contaminated drinking water, food or soil, and via inhalation of lead-contaminated dust. For children, intake of lead-containing paint is still an important font of intoxication (Godwin, 2001).

Several classes of molecular targets have been proposed to account for the symptoms associated with lead poisoning (Bouton et al., 2001). With few exceptions, these targets fall into two primary categories: proteins thatnaturally bind calcium and proteins that naturally bind zinc (Godwin, 2001). In despite that lead has only a moderate affinity for sulfhydryl groups and does not readily undergo valence changes characteristic of transition metals (Bondy and Guo, 1996), it is commonly found in cells and tissues attached to thiol-containing proteins and small molecular weight thiols (Goering, 1993; Campagna et al., 1999). Zn^{2+}-containing enzymes, particularly those containing vicinal groups, such as δ-aminolevulinate dehydratase (ALA-D) are considered classical target molecules for this metal poisoning, (Gurba et al., 1972; Trevisan et al., 1980; Goering et al., 1986; Goering, 1993; Rocha et al., 1995, 2001; Rodrigues et al., 1996; Jaffe et al., 2001). In line with this, the activity of the δ-ALA-D, commonly studied as a response test to lead and considered an important index of subcritical effect (Secchi et al., 1974; Nordberg, 1976; Meredith et al., 1978; Simmonds et al., 1995; Polo et al., 1995).

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ALA-D activity is highly sensitive to oxidants or situations associated with overproduction of free radicals (Flora et al., 2002; Folmer et al., 2002; Bolzan et al., 2002; Farina et al., 2003; Nogueira et al., 2003a; Soares et al., 2003). Accumulation of δ-aminolevulinic acid (ALA), which results from ALA-D inhibition by Pb^{2+} or other agents, can have hepatic and neurotoxic effects (Bechara, 1996; Emanuelli et al., 2003) and part of these effects results from the overproduction of reactive oxygen species, (Monteiro et al., 1991; Hermes-Lima et al., 1991; Bechara et al., 1993). In line with this, data from different laboratories have indicated that oxidative stress can be an important factor for lead toxicity in different organs (Neal et al., 1997, 1998; Gurer and Ercal, 2000). However, the quantity of studies that have evaluated simultaneously oxidative stress and ALA-D as indexes of lead toxicity in different organs are rare.

Lead is described as a neurotoxic agent, and many of its molecular and cellular targets have been identified. However, the precise mechanisms of lead neurotoxicity are not all established (Bressler and Goldstein, 1991; Stohs and Bagchi, 1995; Bressler et al., 1999). This metal can cause alterations in neurotransmitter release (Bressler and Goldstein, 1991), activation of protein kinase C (Marcovac and Goldstein, 1988) and inhibition of glutamate uptake into astrocytes (Engle and Volpe, 1990). The amino acid glutamate is a common excitatory neurotransmitter, which in excessive amounts is toxic, causing the so-called excitotoxic reaction (Greenamyre, 1986). Agents that oxide SH groups of the transporters can produce decrease of the glutamate uptake (Trotti et al., 1997; Nogueira et al., 2003a) and consequently can increase glutamate in the synaptic cleft. Although Pb^{2+} can change neurophysiologic responses to glutamate agonists (Braga et al., 1999), there are no data on literature about the simultaneous effect of lead exposure and antioxidant treatment on glutamate uptake by brain synaptosomes.

The interest in organoselenides chemistry and biochemistry has increased in the last two decades mainly due to the fact that a variety of organoselenium compounds possess antioxidant activity (Andersson et al., 1994). In fact, Ebselen (an organoselenium compound) is now been considered as pharmacological antioxidant agent for treatment of humans (Müller et al., 1984; Saito et al., 1998; Yamaguchi et al., 1998). Ebselen is a potent antioxidant agent and part of its antioxidative properties is linked to its glutathione-peroxidase like activity (Saito et al., 1998; Yamagushi et al., 1998, Rossato et al., 2002a). Other organoselenium compounds such as diphenyl diselenide also share with Ebselen both thiol-peroxidase like activity and other antioxidant properties (Wilson et al., 1989; Rossato et al., 2002b). Based on the fact that the pharmacological properties of Ebselen are related to its thiol peroxidase-like activity we have investigated the pharmacological properties of diphenyl diselenide. We observed that diselenide causes minimal toxicity when administrated acutely to mice and rats in doses that have anti-inflammatory and antinociceptive activity (Nogueira et al., 2003b).

Of particular importance, inorganic Se can protect rodents from the toxic effect of lead (Rastogi et al., 1976; Flora et al., 1983; Dhir et al., 1985; Othman and Missiry, 1998), possibly by affording additional antioxidant capacity via GPx (Flohé et al., 1973; Ursini et al., 1982; Behne and Kyriakopoulos, 1990; Bock et al., 1991). Since selenorgano compounds have antioxidant properties, we realized that they could be potential protective agents against metal intoxication.

As pointed out above, Pb^{2+} can yield toxicity by interfering with thiol-containing proteins, particularly with those containing Zn^{2+}. Furthermore, oxidative stress can be an important outcome of Pb^{2+} exposure and the use of antioxidants and thiol protecting agents as possible therapeutic approaches against lead toxicity have been considered by basic investigators (McGowan, 1989; Flora et al., 1989, 1991; Dhawan et al., 1988, 1989; Gurer and Ercal, 2000; Hsu and Guo, 2002). However, it must be emphasized that in the majority of these studies, at least one of these antioxidants used was a thiol-containing compound. Since Ebselen and other organochalcogenides, particularly diphenyl diselenide, have antioxidant properties we aimed in determine whether non-thiol-containing synthetic compounds with antioxidant properties could reverse the toxicity produced by Pb^{2+}. Of particular importance for our working hypotesis, results data from our group have indicated that Ebselen protect developing rat brain from methylmercury-induced neurotoxicity by acting as antioxidant agent (Farina et al., 2003). A number of parameters indicative of lead poisoning in blood and soft tissues, like ALA-D activity, oxidative stress, non-protein thiol groups and glutamate uptake was determined in an attempt to establish a link between ALA-D, oxidative stress and brain glutamate transport in mice.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice (2–3 months old, 25–35 g) from our own breeding colony were maintained in an air-conditioned room (22–25 °C) under natural lighting conditions, with water and food (Guabi, RS, Brazil) ad libitum. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Medicine Veterinary and Animal Science of the University of São Paulo, Brazil.

2.2. Chemicals

Glacial acetic acid, *o*-phosphoric acid, hydrochloric acid, sodium selenite, sulfuric acid, perchloric acid, ethanol, $HgCl_2$, NaCl, K_2HPO_4, KH_2PO_4, 5,5′-dithiobis-(2-nitrobenzoic acid), hydrogen peroxide, and ascorbic acid were obtained from Merck (Rio de Janeiro, RJ, Brazil), butylated hydroxytoluene, sodium dodecyl sulfate, dimethyl sulfoxide, 2,4-dinitrophenylhydrazine,