Research in Pharmaceutical Sciences 2 (2006) 80-84
Received: Oct 2006
Accepted: Feb 2007

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Isfahan University of Medical Sciences and Health Services

Original Article

Protective effects of selenium and zinc on the brain acetyl cholinesterase activity in lead intoxified rat

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Abstract

Lead is a common toxic agent which impairs various organs functions of the body. The basis of this toxicity is believed to be the negative influence on enzymatic system of cells. In the past few years, increasing consideration has been given to interactions occurring between lead and some essential metals including Se and Zn. Based on the finding that some trace elements could reverse the toxicity of lead on tissue functions this work was performed to study the protective effects of Zn and Se against lead toxicity. Thus changes in acetyl cholinesterase (AchE) activity in brain regions were investigated in different conditions. Four groups of rats were chosen. In acute study, i.p. administration of Pb (13.5 mg/kg) for 2 weeks reduced the activity of AchE in the cortex by 28.7% but Pb in presence of Zn (0.5 mg/kg) and Se (0.4 mg/kg) inhibited AchE activity only by 11.5% and 25%, respectively, such inhibition in mid-brain were 44.7%, 18.2%, 32% and in the cerebellum 48.2%, 38.4% and 12.0%. In chronic study, animals were administered Pb alone (4 mg/kg) or in the presence of Se or Zn for 60 days. Following these treatments, AchE activities in the cortex were reduced by 41.5%, 22.0% and 39.6%, respectively. These inhibitions in mid-brain were 56.0%, 37.6%, 54.5% and in cerebellum 46.0%, 45.0%, 32.6%. It is concluded that lead had toxic effect on AchE activity in different brain regions and Zn and Se could reverse this inhibition to some extent.

Keywords: Acetyl cholinesterase; Selenium; Zinc; Lead

INTRODUCTION

Lead is a ubiquitous environmental and industrial pollutant that has been detected in almost all biological systems. Many investigations have been performed about the physiological, biochemical, and behavioral effects of this toxic element in animals including disorders of central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidney, liver and reproductive systems (1). Especially in this regard developing brain has been shown to be more susceptible to the neurotoxic effects of lead. Lead has also been shown to cause oxidative stress on DNA, lipids, proteins and other metabolic elements (2). The toxic effect of lead may be due to the binding to the metalothionein (MT) that is rich in sulphydril groups implicated in zinc and copper homeostasis (1). Observations are shown that administration of lead reduced gastrointestinal absorption of calcium, iron, zinc and selenium (3). This interaction with divalent cations metabolism is the molecular bases of toxicity of this element (3). Calcium has been shown to be required for physiological functions including signal

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transduction and neurotransmission which is also affected by lead. In fact lead can compete with Ca^{2+} for calcium receptors, which are coupled with second messenger function of this ion and may account for its disruption of cholinergic function and alterations in other transmitter systems. Lead exposure mainly affects cholinergic system by reducing acetylcholine release, uptake and turnover rates (4). Lead also has been shown to reduce enzyme activity including AchE and Na^+/K^+ATPase activity, although the exact mechanism by which the enzyme activity is altered remains to be cleared but lead probably changes the binding kinetics of Na^+/K^+ATPase activity that plays an original role in linking the extracellular signals to intracellular at the neurons and so affects cholinergic transmission (5). Investigation is shown lead not only induce oxidative stress but also has a high affinity for free-SH groups in proteins and enzymes including catalase, glutathione peroxidase and glutathione system (1). The reduction of activity of these enzymes, accelerate the accumulation of oxidation free radicals and also attributed to progressive mitochondrial dysfunction in the brain and it can impact on ATP synthesis (2).

In the past few years, increasing consideration has been given to the interactions occurring in the organism between toxic and essential metals. So, one can use a beneficial or less harmful element to combat damages induced by toxic elements. In this regard Se and Zn have been shown to reverse some toxicity of lead in experimental animals (6, 7). Selenium and zinc are two elements that are essential for normal metabolic reactions. Selenium seems to reduce the toxicity of several metals probably by forming inert selenid complexes. Selenium is also known to provide protection from reactive oxygen species (ROS) -induced cell damages (8).

Another essential element with beneficial effects is zinc. Zinc is shown to act as a neuromodulator at excitatory synapses and has a considerable role in the stress response (9). It is well known that Zn and Pb compete for similar binding site on a metallothionine like transport protein (1). The presence of Zn reduces absorption of Pb from gastrointestinal tract (10). The major aim of this study was to investigate the protective effects of Se and Zn on lead toxicity regarding AchE activity changes in different regions of rat brain.

MATERIALS AND METHODS

All chemicals used in this study were of reagent grade and obtained from Sigma chemical company (Germany). Deionized water was used throughout the experiments.

Animals and Treatment

Male Wistar rats were kept under standard conditions and fed on basal diet and water. Their weights at the time of experiments were between 200-250 g. To study the acute effects of Pb, four groups (five animals in each) were chosen. Animals were injected i.p. for two weeks with Pb alone (13.5 mg/kg), Pb + Se (0.4 mg/kg) and Pb + Zn (0.5 mg/kg). The fourth group considered as control receiving only distilled water.

Chronic study was performed by choosing another four groups (five animals in each). They were injected for 60 days with Pb alone (4 mg/kg), or Pb + Se (0.4 mg/kg) and Pb + Zn (0.5 mg/kg). Control group was also considered for this study. At the end of the injection periods, animals were killed and their brains were carefully removed. Cerebellum, mid-brain and cortex were dissected and were homogenized separately in 0.1 M phosphate buffer (pH 7.8) containing triton 1% at 4 °C. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C. The total AchE activity was estimated by the method reported by Ellman et al.

(11). Protein concentration of the samples was measured by the method of Lowry et al. (12).
Data are presented as means ± SD and Student's t-test was used to show the significance of the changes.

RESULTS

It was shown that the acute dose of lead significantly decreased AchE activity in all brain regions in comparison with control group (Table 1). As indicated in this Table 1, lead inhibited AchE activity in cortex, mid-brain and cerebellum by 28.7, 44.7 and 48.7 percent, respectively, which are significantly different from control values.
Results also showed that Zn and Se protected the enzyme against the toxic effects of Pb to some extent. The interesting observation is that Zn in mid-brain and Se in cerebellum are more effective in this respect, the finding which will be discussed later. The results obtained from chronic studies are shown in Table 2. As indicated in this table, lead again inhibited AchE activity in different parts of the brain and Zn and Se reversed this inhibition.

DISCUSSION

The protective effects of zinc and selenium on lead toxicity upon AchE activity in rat brain regions have been shown. Preliminary results indicated that both short and long-term administration of lead reduced AchE activity in cortex, mid-brain and cerebellum.

This reduction is not only a dose dependent process but also depends on the duration time of lead exposure. Although it

Table 1. The protective effect of Zn (0.5 mg/kg) and Se (0.4 mg/kg) against the acute dose of Pb (13.5 mg/kg) in two weeks.

Regions	Acetycholinesterase activity (µmol Acetic Acid/mg $_{pr}$/min)			
	Control	Pb	Pb + Zn	Pb + Se
Cortex	8.14 ± 0.3	5.80 ± 0.2* (-28.7%)	7.20 ± 0.2 (-11.5%)	6.10 ± 0.3* (-25.1%)
Mid-brain	13.64 ± 0.3	7.54 ± 0.5* (-44.7%)	11.16 ± 0.3 (-18.2%)	9.20 ± 0.5* (-32.3%)
Cerebellum	15.44 ± 0.3	8.00 ± 0.6* (-48.2%)	9.50 ± 0.2* (-38.4%)	13.59 ± 0.6 (-12.2%)

Data are expressed as mean ± SD of 5 experiments. Percent inhibition, compared with control values are shown in brackets. *P<0.05 compared with control groups.

Table 2. The chronic effect of Pb (4 mg/kg for 60 days) on AchE activity in three different parts of the brain and the protective effect of Zn and Se.

Regions	Acetycholinesterase activity (µmol Acetic Acid/mg $_{pr}$/min)			
	Control	Pb	Pb + Zn	Pb + Se
Cortex	7.60 ± 0.2	4.44 ± 0.3* (-41.5%)	5.90 ± 0.4* (-22.1%)	4.59 ± 0.3* (-39.6%)
Mid-brain	14.60 ± 0.4	6.41 ± 0.3* (-56.1%)	9.10 ± 0.5* (-37.6%)	6.64 ± 0.5* (-54.5%)
Cerebellum	15.60 ± 0.3	7.70 ± 0.4* (-51.2%)	8.50 ± 0. 4* (-45.2%)	10.5 ± 0.3* (-32.6%)

Data are expressed as mean ± SD of 5 experiments. Percent inhibition, compared with control values are shown in brackets. *P<0.05 compared with control groups.

binding could induce this reduction but it may explained on the basis of previous is not well understood that how lead molecular studies. It is proposed that AchE has two distinct sites, an acylation site (A-site) at the base of the active site and a peripheral site (P-site) near the enzyme surface (13). The role of the P-site in catalysis has been suggested to serves an intermediate binding site for cationic ligands which proceed to the A-site. P-site binding also can lead to allosteric activity changes of the acylation step so that the whole rate of the reaction is affected. Binding of lead to the P-site of the enzyme molecule may cause the consequent conformational changes of the enzyme and may be for enzyme inhibition (14).

Studies demonstrate that inhibition of catalase, glutathione peroxidase by lead, accelerates aggregation of free radicals and induce changes in electrical charge. This may be the reason for the inhibition of AchE activity (3). Our observations have indicated that lead induced the most reduction in AchE activity in cerebellum and mid-brain.

Long-term (60 days) exposure showed that mid-brain is the most affected part. Pervious observation suggested that lead could reduce AchE activity in cerebellum and hippocampus of developing rat (3). Lead also is shown to block nicotinic receptors (15). Nicotinic receptors form a heterogeneous family of ion channels and they are expressed in many regions of the central nervous system and peripheral nervous system. These receptors functions are also modulated by some other divalent cations including Ca^{+2}, Zn^{+2}, Mg^{+2}, Cd^{+2} and Pb^{+2} (16).

When lead was administrated with either zinc and/or selenium, interesting results were obtained. Administration of Zn reversed lead toxicity particularly in cortex and mid-brain whereas selenium exerts its effect mostly in cerebellum. Different concentrations of selenium in various brain regions have been reported and it has been shown that the gray matter has higher selenium levels and the most concentrated region in this regard was found to be cerebellum (17). The higher level of Se in cerebellum and the probable presence of selenoproteins in high concentration that why Se is more effective to reverse the toxic effect of lead on cerebellum AchE activity.

One of the most selenoproteins in cerebellum is selenoprotein P (Sel P). It has a metal responsive element (MRE) region that is suggested to be responsive to heavy metals. These regions, in conjunction with the cysteine and selenocystein content, are predicted to confer binding to heavy metals such as mercury, nickel, cadmium, lead and silver. In fact, binding of these metals by Sel-P induce complexes and thus protein may function to chelate heavy metals, reducing their toxicity (18).

Pervious reports showed that lead inhibited Na^{+}/K^{+}ATPase activity but when lead is orally administered with selenium no inhibition is observed (19). It is well known that Na^{+}/K^{+}ATPase is important for neurotransmission and the release of neurotransmitters (7).

Previous observations show that Se administration with Hg (20) and As (21) reduces or reverse the inhibition on AchE activity caused by these elements. Another essential element with beneficial effects is zinc. Zinc is shown to act as a neuromodulator at excitatory synapses and has a considerable role in the stress response.

It has also been found that the brain has the highest zinc content with respect to other organs of the body and total brain zinc concentration is about micromolar range (9). Previous studies revealed that the forebrain represents a subgroup of excitatory glutamatergic neurons containing free zinc ions that are located in the vesicles of their pre-synaptic area (22). These cells are called zinc-enriched neurons (ZEN). Higher zinc concentrations

are found in certain forebrain regions, including hippocampus, amygdala and neocortex. One of the most important functions of ZEN is response to oxidative stress. Zinc can increase ATPase, Na^+/K^+ATPase activity, in addition Zn is also necessary to mobilize defense against reactive oxygen species and H_2O_2 that induce apoptosis (23). However it is shown that lead administration may reduce zinc uptake by these tissues and so zinc supplementation may reduce this lead toxicity in the brain. The higher level of Zn in neocortex and mid- brain may be a good explanation that why Zn is more effective to reverse the toxic effects of lead on AchE activity presented in these regions.

REFERENCES

1. Ping-chi H, Yueliang M. Antioxidant nutrients and lead toxicity. Toxicology. 2002;180:33-44.
2. Smith M, Cass W. Oxidative stress and dopamine depletion in an intrastriatal 6-Hdroxydoamine model of Parkinson's disease. J Neurosci. 2007;144:1057-1066.
3. Gottipolu R, Bhuvanes W, Chellu S. Developmental lead Neurotoxicity. Neurotoxicology. 2006;3:18-29.
4. Prasanthi R, Reddy G. Calcium or Zinc supplementation reduced lead toxicity. Nutr Res. 2006;26:537-545.
5. Yallapragada P, Butler J, Kumar B. *In vitro* effect of lead on Na^+, K^+- ATPase activity in different regions of adult rat brain. Drug Chem Toxicol. 2003;26:117-124.
6. Patricia S, Brocard A, Pandolf P. Antioxidant defenses and lipid peroxidation in the cerebral, cortex and Malathion and/or Zinc chloride. Toxicology. 2005;207:283-291.
7. Nehru B, Dua R, Lyer A. Effect of selenium on lead induced alterations in rat brains. Biol Trace Elem Res. 1997;57:251-8.
8. Santamaria A, Sanchez A, Roman B. Protective effects of antioxidant selenium on quinolinic acid induces neurotoxicity in rat. J Neurochem. 2003;86:479-488.
9. Yosef M, Hendy H, Demerdash F, Elagamy E. Dietary Zinc deficiency induced changes in the activity of enzymes and the levels of free radicals, lipid and protein electrophoretic behavior in growing rats. Toxicology. 2002;175:223-234.
10. Cerklewski F, Forbes R. Influence of dietary zinc on lead toxicity in the rat. J Nutr. 1976;106:689-96.
11. Ellman G, Coutney D, Andres V. A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochem Pharmacol. 1961;7:88-89
12. Lowry O, Rosembrough N, Farral R. Protein measurement with Foline regent. J Biol Chem. 1951;193:265-275.
13. Joseph L, Jaime T, Emami S. Measuring carbamoylation and decarbamoylation rate constants by continuous assay of AchE. J Chem Bio Interact. 2005;157:384-385.
14. Thomars R, Podleski N. Similarities between active sites of acetyl choline receptors and acetyl cholinesterase tested with quinolinum ions. Biochemistry. 1966;50:1033-1039.
15. Zhou M, Suszkiw J. Nicotinic attenuates spatial learning deficits induce in the rat by prenatal lead exposure. Brain Res. 2004;999:142-7.
16. Gotti C, Clementi F. Neuronal Nicotinic receptors. Prog Neurobiol. 2004;74:363-396.
17. Rayman P. The importance of selenium to human health. Lancet. 2004;356:233-41.
18. Chen J, Berry J, Marla M. Selenium and selenoproteins in the brain and brain disease. J Neurochem. 2003;86:1-12.
19. Nehru B, Lyer A. Effect of selenium on lead-induced neurotoxicity in different brain regions of adult rats. J Environ Pathol Toxicl Oncol. 1994;13:265-8
20. Dermerdash E. Effect of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver and bloods of rat. Sci Health. 2001;36:489-499.
21. Roy S, Chattorj A, Bhattacharya S. Arsenic induces changes in optic tactal histoarchitecture and acetyl choline esterase–acetyl choline profile. Amelioration by selenium. Comp Biochem Physiol. 2006;144:16-24.
22. Mocchegiani E, Bertoni C. Brain, aging and neurodegenration. Role Zinc ion availability. J Prog Neurobiol. 2005;75:367-390.
23. Fredeickson C, Rengarajan B, Masaalha R, Frederickson C, Zeng X.