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Protective Effects of Selenium and Zinc on Changes in Catecholamine Levels of Brain Regions in Lead Intoxified Rat

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Abstract: Lead is a common environmental toxic element for almost all biological systems. The nervous system is the primary target for the lead exposure. In the past few years, increasing considerations have been given to investigate the interaction occurring between toxic metals and some essential metals including Se and Zn with Pb. It has been shown that some trace elements could reverse the toxicity of lead on tissue functions. In this study the protective effects of Zn and Se on lead toxicity were investigated. Results of short time study showed that, intrapritoneal administration of Pb (13.5 mg kg^{-1}) daily for 2 weeks reduced the catecholamine levels of cortex by 25, mid-Brain by 21 and cerebellum by 25.6%, respectively. Administration of the same amount of lead in combination with either Zn (0.5 mg kg^{-1}) or Se (0.4 mg kg^{-1}) reduced catecholamine levels of cortex by 8.3 and 18.3, mid-brain by 6, 10.9 and cerebellum 23, 6% respectively. Daily administration of lead alone (4 mg kg^{-1}) for 60 days reduced catecholamine level of cortex by 27.4 and mid-Brain by 47.8 and cerebellum by 39%, respectively. When the same amount of lead in combination with Zinc (0.5 mg kg^{-1}) and /or Se (0.4 mg kg^{-1}) was administration daily for 60 days, results showed that catecholamine level of cortex was reduced by 9, 20 and mid- brain by 22.6, 29 and cerebellum 25, 16%, respectively. It is concluded that lead reduced catecholamine levels in different brain regions and Zn or Se might be able to reverse this reduction and protect brain function to some extent from lead toxicity.

Key words: Protective, zinc, selenium, catecholamine, lead

INTRODUCTION

Lead (Pb), is one of the most potential toxic elements in the environment, it is a heavy metal and is known to be toxic to the central nervous system (Nour Eddine *et al*., 2005). Lead has been reported to cause oxidative stress in DNA, lipids and proteins (Smith and Cass, 2007). The toxic effects of lead might be probably due to the binding of lead to the metalothionein (MT) that is a protein rich in sulphydril groups implicated in zinc and copper homeostasis (Takeda *et al*., 2006).

Lead can interact with gastrointestinal absorption of calcium, iron, zinc and selenium. This interaction with divalent cations metabolism is the molecular bases of the toxicity of lead (Reddy *et al*., 2006).

Calcium, which is an essential nutrient, is required for cellular and physiological functions including, signal transduction and neurotransmission (Reddy *et al*., 2006). Lead is able to substitute calcium in calmodulin and alter its function (Vázquez and Pena *et al*., 2004). It has been reported that, Pb can exert an inhibitory effect on Na^+/K^+-ATPase activity that plays an original role in linking the extra cellular signals to intracellular at the neurons (Nour Eddine *et al*., 2005). Experiment is shown that oral administration of lead with selenium inhibits reduction of Na^+/K^+ATPase activity (Yallapragada *et al*., 2003).

Lead may be able to damage the brain cells and reduce the catecholamine levels (Nour Eddine *et al*., 2005).

Parkinson's disease is a neurodegenerative disorder by the selective degeneration of dopaminergic neurons located in the Substantia Nigra (SN) and this part is known to be particularly susceptible to free radical damage due to its high levels of iron and the formation of hydrogen peroxide from dopamine by monoxidase-B or autoxidation dopamine (Singh *et al*., 2007).

Glutathione (GsH) is known as one of the main antioxidant in this part and it is a protective mechanism for minimizing the oxidative damage (Schweizer *et al*., 2004). Glutathione Peroxidase (GPX) is a key enzyme in the GSH system. This enzyme consists of 4 identical subunits, each contain a few SH groups and one molecule of selenium as selenocystein. The selenium takes a direct part in the catalytic process (Schweitzer *et al*., 2004). Investigation indicates that lead not only induces

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oxidative stress but also have a high affinity for SH groups in this enzyme (Nour Eddine *et al*., 2005). The depletion in GSH levels accelerates the accumulation of oxidation free radicals and also attributed to the progressive mitochondrial dysfunction in the brain and it can impact on ATP synthesis (Singh *et al*., 2007).

Administration of selenium is shown to inhibit reduction in brain dopamine levels. Thus, the preventive effect of selenium might be due to reduction in auto oxidation of dopamine by enhancing antioxidant enzymes activity particular GPX in striatum and substantia nigra compared to the other brain regions. It is also well known that brain has poor catalase activity (Scweizer *et al*., 2004)

Catecholamines are shown to regulate motor activity in rodents (Moniri and Booth, 2006) and reduced catecholamine level has been also postulated to be involved in toxic effects of lead on the central nervous system (Devi *et al*., 2005).

In the past few years, increasing considerations have been given to the protective effects of some trace elements on toxic element actions in biological systems.

Therefore the major aim of this project was to study the protective effects of Se and Zn on lead toxicity regarding catecholamine changes of rat brain regions.

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 treatments: Male Wistar rats were kept under standard conditions. They fed on basal diet and water.

Their weights at the time of experiments were between 200-250 g.

For short time study, 4 groups (5 animals in each group) were chosen. They were injected interaperitoneally (ip), daily for 2 weeks with either Pb alone (13.5 mg kg^{-1}) or Pb in combination with Se (0.4 mg kg^{-1}) and/or Pb with Zn (0.5 mg kg^{-1}). Controls were received only distilled water. For long-term study four groups (five animals in each group) were chosen. They were injected daily ip for 60 days with either Pb alone (4 mg kg^{-1}) and/or Pb in combination with Se (0.4 mg kg^{-1}) and Pb with Zn (0.5 mg kg^{-1}). Control group was also considered for this study.

At the end of the injection times, animals were killed and their brains were carefully removed. Cerebellum, mid-Brain and brain cortex were dissected and were homogenized separately in percholoric acid 0.1 M and EDTA 0.01 M at 4°C .The homogenate was centrifuged at 10000 g for 20 min at the 4°C.

The catecholamine content of cortex, mid-brain and cerebellum was determined according to the method described elsewhere (Moshtaghie *et al*., 2004)

The fluorescence of catecholamine was measured using a spectrophotometer (Model Perkin-Elmer) with excitation and emission wavelengths of 400 and 505 nm, respectively.

Protein concentration of samples was determined by the method of Lowry (1951).

Data are presented as means±SD and t-student test was used to show the significance of the change.

The project has been carried out at department of Biochemistry in Isfahan university during 2006-2007.

RESULTS

As indicated in Table 1 in comparison with control group, lead treatment for two weeks significantly ($p<0.05$) reduced catecholamine levels in all regions of rat brain.

Lead reduced catecholamine contents of cortex, mid-brain and cerebellum by 25, 21 and 25.6% respectively which are significantly different from control values $p<0.05$.

However administration of Pb in combination with either Zn and/or Se caused the reduction of catecholamine levels of cortex 8.3 and 18.3, mid-brain by 6 and 10.9 and cerebellum by 23 and 6% respectively (Table 1).

Second series of experiment were undertaken to investigated the levels of catecholamine content of rat brain regions treated daily intrapritoneally with Pb (4 mg kg^{-1}). Data are presented in Table 2 and show that daily administration of Pb (4 mg kg^{-1}) reduced catecholamine level of cortex by 27.4, Mid-Brain by 47.8 and cerebellum by 39%, respectively. When Pb in combination with either Zn (0.5 mg kg^{-1}) and /or Se (0.4 mg kg^{-1}) was injected, there were reductions of catecholamine content in cortex by 9 and 20, mid- brain by 22.6 and 29 and cerebellum by 25 and 16%, respectively (Table 2).

Table 1: Short term protective effects of Zn and Se on the levels of rat brain catecholamines treated with Pb for two weeks

	Catecholamine levels (ng mg^{-1} protein)		
Treatments	Cortex	Mid-brain	Cerebellum
Control	60.0±2.1	12 1.0±2.9	179.0±2.7
Pb	45.0±2.9* (-25)	95.3±5.3* (-21)	133.0±4.5* (-25.6)
Pb +Zn	55.0±3.4 (-8.3)	113.7±2.7 (-6)	138.5±6.2* (-23)
Pb +Se	49.4±2.8* (-18.3)	107.7±3.4* (-10.9)	168.7±4.1 (-6)

Table 2: Long term protective effects of Zn and Se on the levels of rat brain catecholamines treated with lead for 60 days

	Catecholamine levels (ng mg^{-1} protein)		
Treatments	Cortex	Mid-Brain	Cerebellum
Control	59.0±2.2	119.0±2.2	177.0±3.1
Pb	43.0±4.7* (-27.4)	62.0±5.8* (-47.8)	108.0±3.9* (-39)
Pb +Zn	53.7±5.7 (-9)	92.7±7.4* (-22.6)	133.0±6.8* (-25)
Pb +Se	47.0±6.1* (-20)	85.0±6.1* (-29)	149.0±4.3* (-16)

The percent inhibition for value, compared to control, is shown in related brackets. *Significantly different from control ($p<0.05$)

Rats were injected daily for two weeks with Pb (13.5 mg kg^{-1}), Pb in combination with Zn (0.5 mg kg^{-1}) and /or Se (0.4 mg kg^{-1}).

Animals were killed and brain regions were prepared as mentions in the materials and methods. Data were reported as mean±SD and shows significant differences among the groups ($p<0.05$).

Rats were injected daily for 60 days with Pb (4 mg kg^{-1}), Pb in combination with Zn (0.5 mg kg^{-1}) and/or (0.4 mg kg^{-1}). Rats were killed and catecholamine levels of rat brain regions were determined. Data are showed as mean±SD.

The percent inhibition for value, compared to control, is shown in related brackets. Stars show that values are statistically significant $p<0.05$.

DISCUSSION

The protective effects of zinc and selenium on the lead toxicity upon the catecholamine levels of rat brain regions have been investigated and presented here.

Data shows that both short and long terms lead administration reduced catecholamine content of brain cortex, mid- brain and cerebellum.

This reduction is not only a dose dependent process but also depends on the duration time of lead administration (Table 1 and Table 2).

Long term (60 days) study showed that mid-brain is the most affected part by lead administration. Previous observations suggested that lead could reduce the total catecholamine levels (Smith and Cass, 2007; Devi *et al*., 2005). It was suggested that Pb and Cd impaired long-term memory and blocked learning function (Nour Eddine *et al*., 2005). When lead was administration with either Zinc and/or Se, interesting results were obtained. Thus administration of Zn reversed the lead toxicity particularly in cortex (25% changed to 8.3%) and mid-brain (21% changed to 6%), whereas Selenium exerts its effect mostly in cerebellum. It is found that the protective effects of both Zinc and Selenium on the reversal effect of lead toxicity in short and long terms are comparable.

Observations of Devi *et al*. (2005) showed that administration of lead altered the aminergic system by decreasing the Mitochondrial Monoamine Oxidase (MAO) and tyrosine hydroxylase activity and was able to reduce catecholamine levels. The observed results show that lead can induce a reduction of transmission catecholaminergic that may be either by a inhibition of the synthesis of dopamine and its acceleration auto-oxidation (Frieling *et al*., 2007) or by an inhibition of post synaptic dopamine receptors (D1, D2) (Nour Eddine *et al*., 2005; Singh *et al*., 2007). Investigation is shown that Pb can block the activation of Ca^{+2}/phospolipid dependent Protein Kinase C (PKC). The PKC isoforms are known as signal transducers in central neuron system (CNS), that roles in regulation vesicles movement and secretion in synapse.(Shang-zhixu *et al*., 2005). In agreement to our finding, Schweitzer *et al*. (2004), suggested that Selenium reduced the toxicity of several metals by forming inert Selenid complexes. Also Chen and Berry (2003) reported that Selenium supplements inhibited dopaminergic toxicity and protected neurons by oxidative stress. Selenium is known to provide protection from Reactive Oxygen Species (ROS) induce cell damage (Smith and Cass, 2007).

One of the most selenoproteins in cerebellum is selenoprotein P, it has a Metal Responsive Element (MRE) region that it was suggested to responsive to heavy metals. These regions, in conjunction with the cysteine and selenocystein content, would be predicted to confer binding to heavy metals such as mercury, nickel, cadmium, lead and silver. In fact, binding of these metals by selenoprotein P induce complexes and this protein may function to chelate heavy metals, reducing their toxicity (Chen and Berry, 2003)

On the other hand Zn has been reported to act as a neuromodulator at excitatory synapses and has a considerable role in the stress response (Patricia *et al*., 2005). Forebrain contained glutamatergic neurons, which contain free zinc ions in the vesicles of their pre-synaptic area and also higher level of zinc is available in hippocampus, amygdale and neocortex (Takeda *et al*., 2006). Investigation is shown zinc can increase ATPase, Na^{+}/K^{+}ATPase (Frederickson *et al*., 2006) in addition Zn is also necessary to mobilize defense against Reactive Oxygen Species (ROS) and H_2O_2 that induce apoptosis. (Smith and Cass, 2007; Frederickson *et al*., 2006). However it is shown that lead administration may reduce zinc uptake by these tissues and so zinc supplements may reduce lead toxicity in the brain. More investigation should be done to elucidate the exact mechanism by which the interaction between Zn and Se with Pb occurred.

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