Mammalian Neuro Endocrinological Changes and Oxidative Stress during Chronic Lead Intoxication

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Abstract

The dangerous effect of lead was evaluated in male mice exposed to lead acetate at dose 2000 and 4000 ppm in drinking water, as well as subjects working in fields with high lead levels. Lead exposure exhibited marked decrease in body weight, as well as in hematological parameters such as hemoglobin content, hematocrit percent, erythrocytes and leucocytes count. In addition, there was a disruptive effect on neurological and endocrinological systems reflected by alterations in T3, T4, testosterone and cortisol hormones. In contrast, there was an elevation in serotonin level, along with a reduction in the concentrations of plasma Zn and Fe in lead exposed mice and human individuals. The study showed increases in the lipid peroxidation product (MDA), accompanied with decreases in GSH and SOD in lead exposed subjects and animals. The study thus indicated that exposure to lead pollution should be avoided as it affect neuro-endocrinological systems, increases oxidative stress and decreases fertility.

Keywords: Lead acetate; Oxidative stress; Fertility; Testosterone

Introduction

The toxic effects of lead demand particular attention because of its characteristically cumulative feature associated with slow turnover and a long biological half-life [1]. It has been estimated that more than half of lead found within the atmosphere arose from the burning of tetra-ethyl lead, a compound added to gasoline to reduce engine knocking [2].

Lead exposure can arise from contact with lead based paints at home, crop enhancers (fertilizers), cosmetics, and automobiles (gasoline and disposable batteries), especially in developing countries [3]. Lead adversely affects many organs and systems in humans exposed workers, where the hematological system is one of the major targets after oxidative stress [4]. Also, lead exposure damages different vitally important systems, especially the central nervous system (CNS), after its penetration into the body with food, water and air containing the exhaust of engines that run on gasoline [5]. Lead is recognized as a dangerous neurotoxin, even at low levels of exposure [6,7] showed that oxidative stress may be an important factor in the neurotoxic effects of lead. Villeda-Hernandez et al. [8] showed that, oxidative stress can cause damage to biomolecules by free radical attachment to polyunsaturated fatty acid (PUFA) side chains in cells and this leads to lipid peroxidation which produces malondialdehyde (MDA), being toxic in nature. They also reported that, lead induced monoamine oxidase (MAO) activity and consequently affected dopamine metabolism in most of brain regions.

Lead exposure impairs several neurotransmitters systems, including acetyle-choline, dopamine, norepinephrine, and gamma-aminobutyric acid (GABA) dependent systems [9].

As special attention has been paid to health hazards of environmental pollution, the aim of the present work is to evaluate the toxic effects of lead exposure at hormonal and neurotransmitters level in mice and human.

Materials and Methods

Chemicals

Lead acetate was purchased from Sigma Company for chemicals, Egypt, and the other chemicals used were with high grade of purity.

Experimental design

Animals: Healthy Swiss male albino mice weighing 22-24 g were used. They were housed in stainless steel cages in experimental animal care center, Mansoura University. Animals were kept under good ventilation, temperature 22-24°C, and provided balanced chow diet, and water *ad libitum*. Animal husbandry and experimentation were consistent with the public health guide for the care and use of laboratory animals at National Research Council, 1996.

Mice were randomly divided into 3 groups (n=10) as follows: control group, lead acetate orally treated groups at doses (2000 ppm) and (4000 ppm), for 10 weeks. Body weight changes were recorded every week along experimentation period.

At the end of experimentation period (10 weeks) animals were sacrificed by cervical dislocation after overnight fasting, and blood samples were collected in clean non-heparinized tubes. Sera were separated by centrifugation at 3000 rpm for 15 min and stored at -20°C for biochemical analysis. Packed blood cells were obtained for lead determination. Other blood samples were collected in heparinized tubes, a part was used for blood picture determination. The other part was centrifuged at 3000 rpm for 15 min to obtain plasma for Zn and Fe as well as hormonal determinations. Brains of mice were separated, and weighed immediately, and then parts of them was frozen at -80°C for neurotransmitters estimation, other parts of brains was homogenized in ice cold saline solution and frozen at -80°C till used for biochemical analysis (GSH, MDA, SOD).

Human subjects: Male volunteers aged 20-35 years old were

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selected for the study. They have the same level of social life; and were non smokers or getting any drugs. A total of 30 male volunteers were studied. They included, control subjects (n=15), who lived away from any source of pollution as possible and Lead exposed subjects (n=15). They were exposed to lead through their work as traffic soldiers for example or near bus station, battery factories. They are working in their field through the past 3 years at least. Blood samples were collected from each of them at 1 pm ± 30 min. The study was approved according to scientific research ethics. Written informed consents were obtained from volunteers prior to the blood samples collection.

Blood samples were collected in clean tubes for hormonal and biochemical analysis, as was described for tested animals.

Biochemical analysis

Complete blood pictures were evaluated according to the method described at Dacie and Lewis [10]. MDA level was estimated according to the method of Ohkawa et al. [11]. GSH content was estimated by the method of Beutler et al. [12]. SOD activity was determined by the method of Nishikimi et al. [13].

Plasma T_3 and T_4 concentrations were estimated according described at Hollander and Shenkman [14]. Cortisol and testosterone concentrations were estimated according to Farmer and Pierce [15], and Ismail et al. [16], respectively.

Packed RBCs lead and plasma iron and zinc concentrations were determined by Atomic Absorption Spectrophotometer with air acetylene according to Zettner and Seligson [17]. Monoamines neurotransmitters content were determined by high performance liquid chromatography (HPLC) apparatus according to the method of Pagel et al. [18].

Statistical analysis

Data were analyzed using SPSS program (Statistical package for Social Science, version 17). For comparison of different mice groups, one way analysis of variance (ANOVA) was used followed by Tukey
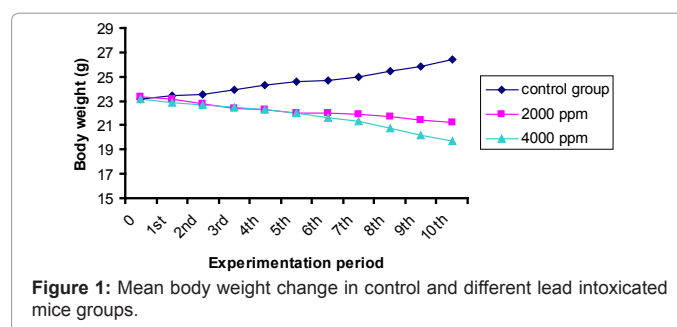

Figure 1: Mean body weight change in control and different lead intoxicated mice groups.

test, while comparison of the two human groups was carried out using independent Sample-t-test. The results were expressed as means ± SD and values of $P \leq 0.05$ were considered statistically significant.

Results

As showed in (Figure 1), there was a decrease in the body weight gain in lead intoxicated mice groups compared with control group.

Additionally, in table 1, there was a significant decrease in hemoglobin content, hematocrit percent, and RBCs in both lead treated mice groups and lead exposed human subjects compared to control ones. A significance decrease in WBCs count was also observed in the two dose (2000 and 4000 ppm) mice groups compared to control animals, however, there was no significant change in WBCs count in lead exposed human compared to control one.

Results presented in table 2 revealed significant decrease in GSH content in lead exposed human and high dose mice groups compared to normal ones, but there was significant increase in GSH content in mice treated by low dose lead compared to control group. SOD activity showed non-significant decrease in lead exposed human subjects, and significantly decrease in mice treated by high dose compared to control group. Also, there was a significant increase in MDA concentration in lead exposed human subjects and in both lead treated mice groups, compared to control group.

	Human subjects		Experimental animals		
Parameter / Group	Normal	Lead exposed	Control	Low dose exposure (2000 ppm)	High dose exposure (4000 ppm)
Hb (g/dl)	16.42 ± 1.84	10.04[a] ± 1.56	14.36 ± 0.63	11.83[a] ± 1.07	8.76[a,b] ± 0.65
Ht %	45.54 ± 3.37	41.68[a] ± 2.94	40.03 ± 3.2	38.31 ± 2.5	26.35[a,b] ± 2.4
RBCs (x10^6/mm^3)	6.20 ± 0.87	4.78[a] ± 0.51	8.47 ± 0.49	7.66[a] ± 0.68	6.04[a,b] ± 0.47
WBCs (x10^3/mm^3)	7.77 ± 1.74	7.49 ± 2.55	1.97 ± 0.64	4.98[a] ± 0.85	7.85[a,b] ± 0.64

Results are presented as means ± SD (n=8 for each mice group and 15 for human group)
a=Significant difference (P<0.05) comparing to the normal control group (mice or human)
b=Significant difference (P<0.05) comparing to 2000 ppm lead acetate (low dose) group

Table 1: Hemoglobin content, hematocrit percent, erythrocytes and leucocytes count in control and different lead exposed mice and human.

	Human subjects			Experimental animals		
Parameter / Group	Control	Lead exposed	Parameter / Group	Control	Low dose exposure (2000 ppm)	High dose exposure (4000 ppm)
MDA (nmol/dl)	18.73 ± 7.4	39.68[a] ± 2.84	MDA (nmol/g)	4.30 ± 2.18	9.52[a] ± 2.26	24.16[a,b] ± 4.13
GSH (mg/dl)	34.5 ± 7	12.6[a] ± 4.75	GSH (mg/g)	12.97 ± 0.87	24.57[a] ± 6.74	10.94[b] ± 2.77
SOD (U/dl)	5.46 ± 0.43	4.16 ± 0.22	SOD (U/g)	34.5 ± 1.6	28.5 ±1.6	21.4[a,b] ± 2.1

Results are presented as means ± SD (n=8 for each mice group and 15 for human group)
a=Significant difference (P<0.05) comparing to the normal control group (mice or human)
b=Significant difference (P<0.05) comparing to 2000 ppm lead acetate (low dose) group

Table 2: Oxidative stress biomarkers in human sera and mice brain of control and different lead exposed subject.

Table 3 recorded decrease in T3 and T4 concentrations in lead exposed human subjects, but there was an increase in lead treated mice groups compared to control group. Testosterone concentration in both lead exposed human subjects and lead treated mice groups showed significant decrease compared to normal human subjects and control mice group, however, cortisol concentration exhibited an increase in lead exposed human subjects, and a decrease in lead treated mice groups compared to control one.

Table 4 showed a significant decrease in plasma Zn and Fe levels with increasing in Pb concentrations in lead intoxicated animals and human subjects compared with normal one. We notice, a reduction in dopamine content, but an increase in serotonin content in all lead exposed groups (Table 5).

Discussion

Lead is considered the most ubiquitous toxic metal detectable in the environment and in all biological systems. In the present study, chronic lead exposure exhibited marked decrease in mice body weight gain especially high dose (Figure 1) compared to control group and this could be due to toxic effect of lead on cellular metabolism and growth rate of animals. A view which is in accordance with Gupta and Gill [19], and/or enhancing glucose synthesis by mobilization of fat [20].

The present study revealed that lead exposure caused a significant decreased RBCs count; and Hb content these results agreed with Rice-Evans [21], these result which may be due to a high affinity of lead for sulph-hydryl groups, particularly those of metallo-enzymes that impairs the biosynthesis of hemoglobin by interfering with several enzymatic steps in the heme pathway [22]. The decrease in RBCs count may also be due to lysis of RBCs by acute and high level lead absorption and/or inhibition of haem synthesis as well as decreasing red blood cell survival Levin and Goldberg [23]. Besides, prolonged lead exposure has been demonstrated to induce leucopenia, presented by decrease in WBCs count which may result from oxidative damage to the leucocytes [24].

Results in this study recorded a significant increase in MDA level after Pb exposure, which may be due to altered lipid composition of membranes due to lead exposure resulting in altered membrane integrity, permeability, and functions, so increases susceptibility to lipid peroxidation, a view which agreed with, Ahmed and Siddiqui [25]. A decrease in the level of antioxidants was also observed in both mice brain and in human blood. These results may be due to increased rate of lipid peroxidation and altered calcium and sulphydryl homeostasis. This decrease may also be attributed to enhanced level of ROS followed by oxidative stress, a view which agreed with, Abdel- Moneim et al. [26].

The observed decrease in SOD activity in Pb exposed groups may be due to the formation of superoxide radical, which possibly depleted the enzyme; this explanation is in agreement with, Cassini et al. [27]. In

Parameter \ Group	Human subjects		Experimental animals		
	Control	Lead exposed	Control	Low dose exposure (2000 ppm)	High dose exposure (4000 ppm)
T3 (ng/dl)	118.53 ± 23.54	116.64 ± 22.02	4.61 ± 1.06	5.96 ± 0.94	8.47^{a,b} ± 1.22
T4 (µg/dl)	8.29 ± 1.59	7.42 ± 1.71	49.38 ± 5.5	62.57 ± 9.01	94.47^{a,b} ± 15.87
Testosterone (ng/ml)	3.98 ± 1.38	1.64^a ± 0.65	11.5 ± 0.73	9.05^a ± 1.46	6.87^{a,b} ± 0.48
Cortisol (µg/dl)	6.07 ± 2.51	10.69^a ± 1.96	2.16 ± 0.49	1.2^a ± 0.26	0.70^{a,b} ± 0.52

Results are presented as means ± SD (n=8 for each mice group and 15 for human group)
a=Significant difference (P <0.05) comparing to the normal control group (mice or human)
b=Significant difference (P<0.05) comparing to 2000 ppm lead acetate (low dose) group

Table 3: Plasma Triiodothyronine (T3), Thyroxine (T4), Testosterone, and Cortisol hormones concentration in control and different lead exposed human and mice.

Parameter \ Group	Human subjects		Experimental animals		
	Control	Lead exposed	Control	Low dose exposure (2000 ppm)	High dose exposure (4000 ppm)
Pb (µg/g Hb)	9.83 ± 1.06	68.94^a ± 10.95	14.5 ± 2.6	23.16^a ± 2	47.6^{a,b} ± 6.5
Zn (µg/dl)	210.1 ± 19.15	88.19^a ± 18.3	768 ± 88.3	333^a ± 103.8	133^{a,b} ± 16.9
Fe (µg/dl)	63.12 ± 6.7	31.60^a ± 12.1	78.7 ± 3.5	48.5^a ± 4.4	20.8^{a,b} ± 7.3

Results are presented as means ± SD (n=8 for each mice group and 15 for human group)
a=Significant difference (P<0.05) comparing to the normal control group (mice or human)
b=Significant difference (P<0.05) comparing to2000 ppm lead acetate (low dose) group

Table 4: Lead RBCs concentration and plasma, Zn and Fe in control and different lead exposed human subjects and mice.

Parameter \ Group	Human subjects		Parameter \ Group	Experimental animals		
	Control	Lead exposed		Control	Low dose exposure (2000 ppm)	High dose exposure (4000 ppm)
Serotonin (µg/dl)	0.92 ± 0.05	1.03 ± 0.07	Serotonin (µg/g)	0.83 ± 0.1	1.09^a ± 0.08	1.24^{a,b} ± 0.06
Dopamine (µg/dl)	0.47 ± 0.04	0.25 ± 0.03	Dopamine (µg/g)	1.45 ± 0.07	0.78^a ± 0.04	0.64^{a,b} ± 0.05

Results are presented as means ± SD (n=8 for each mice group and 15 for human group)
a=Significant difference (P<0.05) comparing to the normal control group (mice or human)
b=Significant difference (P<0.05) comparing to 2000 ppm lead acetate (low dose) group

Table 5: Monoamines content in mice brains (µg/g) and human serum (µg/dl) in control and different lead exposed animals and human subjects.

addition, it seems that lead depletes cell thiol-containing antioxidants (GSH) or enzymes [28]. The neurotoxic effect of Pb may be attributed to the disturbance of pro-and antioxidative balance as reported by Bokara et al. [29]. Pb may also deteriorated cellular function decresing antioxidant via increased production of ROS, H_2O_2 and NO [30]. However, obtained increase in GSH content in low dose lead exposed mice, may be due to a protective or compensatory mechanism, wherein, lead might bind with GSH in order to reduce the toxicity Hsu [31].

Obtained decrease in serum T3 and T4 in the present study after Pb exposure, may be due to the fact that lead inhibits synthesis and release of thyroid hormone at the glandular level through affecting thyroid iodine uptake or TSH release from pituitary or TRH release from hypothalamus [32] and/or the role of Pb in damage thyroid gland [33]. In addition to the suppresses role of Pb for synthesis and release of thyroid hume at the glandular level [34]. On the other hand, the increased serum T3 and T4 in lead exposed animals may be due to altering TSH values and presumably thyroid hormones [35]. The increased serum cortisol observed in lead exposed subjects was in concern with results of Fengyuan et al. [34], and may be due to respond to oxidative stress caused by lead exposure [36].

The recorded decrease in testosterone concentrations in Pb exposed individuals, may be due to direct toxic action of lead on the testes with reducing production of testosterone, or decreasing testicular sensitivity to gonadotrophic actions [37]. The anti- androgenic activity of Pb may also lead to this result [38].

Results of the present study indicated that lead was capable of causing decrease in plasma iron concentration, this result may be due to competition between lead and iron resulting in decreased blood total iron under low or high dose of lead exposure [39]. Obtained decrease plasma zinc in Pb exposed subject may be due to the inhibition of ferrochelatase enzyme which exceeds formation of zinc protoporphyrin that excreted with urine and leads to decrease in zinc and iron concentration [40].

The decreased levels of dopamine observed following lead exposure could be the result of altered activity of biosynthetic and degradive enzymes, or altered availability of their precursor amino acid tyrosin [19]. However, the resulted increase in serotonin level may be attributed to activation of tryptophan hydroxylase.

The neurodegenerative disorders caused by Pb may lead to these neurotransmitters abnormalities [41]. The interference of Pb with dopaminergic transmission at the presynaptic level may caused this alternation [42]. The neurotransmitter production may affect interference of Pb with normal neural cell adhesion [43].

In conclusion, lead disturbs the delicate balance of micronutrients, such as Zn and Fe, which is important in the physiology of neuronal functions, these, in turn, can cause alterations of neurotransmitters such as dopamine, and serotonin, as well as affecting several indicators of oxidative stress. A disturbance in hormonal balance was also demonstrated in both mice and human individual after lead intoxication. Thus, it seems that the potential health hazards of lead poisoning still exist, and this due to lack of education regarding the precautions of workers with lead, and how to avoid Pb pollutant area.

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