Full Length Research Paper

Effect of lead toxicity on coenzyme Q levels in rat tissues

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Lead is a persistent and common environmental contaminant, which chiefly plays a significant role in modern industry. Coenzyme Q acts as electron and proton carrier in mitochondria and functions as an antioxidant in its reduced form (ubiquinol). To investigate the hazardous effects of lead on the coenzyme Q level, rats were injected i.p. with lead acetate (5 mg/kg b.wt. daily for 6 weeks). Our results showed that the levels of both oxidized (ubiquinone) and reduced (ubiquinol) forms of coenzyme Q_9 and Q_{10} in serum, brain, liver and kidney of lead-treated rats are quite different depending on the organ tissue type.

Keywords: Lead, coenzyme Q, antioxidants.

INTRODUCTION

Lead, one of the oldest known metals, is also one of the most widespread toxicants, and lead poisoning remains a health threat (Hernberg, 2000). Several lines of evidence implicate high-level lead exposure as a cause of many of pathological conditions such as renal insufficiency, gout and hypertension (Wedeen et al., 1979; Batuman, 1993; Ding et al., 2000). Although different considerations were raised to explain the pathogenesis of lead toxicity, several studies suggested the primary involvement of the increased production of reactive oxygen species (ROS) observed in lead-exposed animals (Ding et al., 2000, 2001). Coenzyme Q_{10} is a component of oxidative phosphorylation in the mitochondria, which converts the energy in carbohydrates and fatty acids into ATP to drive cellular machinery (Ernster and Dallner, 1995). Coenzyme Q_{10} also functions as an antioxidant, inhibiting lipid peroxidation and scavenging free radicals. It is present in the tissues of human and rats as oxidized (ubiquinone) and reduced form (ubiquinol) which play the major role in its antioxidant activity (Ernster, 1977). Aberg et al. (1992) reported that the greater portion of Coenzyme Q in some rat and human tissues was found as ubiquinol. The deficiency of Coenzyme Q has been reported in many diseases such as diabetes (Kucharska

et al., 2000), heart failure, angina and hypertension (Mongthuong et al., 2001). Therefore, our study has been conducted to evaluate the toxic effect of lead injection on the coenzyme Q level in rat tissues.

MATERIALS AND METHODS

Materials

Lead acetate was supplied from El-Nasr Pharmaceutical Chemical Company (Cairo, Egypt), dissolved in distilled water and injected i.p in a dose of 5 mg/kg b.wt. daily for 6 weeks (Kostial et al., 1999).

Animals

Twenty-four adult male albino rats weighing 190-210 g were used in this study. The animals were obtained from the animal facility, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. The animals were maintained under standard laboratory conditions of relative humidity (55 ± 5%), temperature (25 ± 2°C), and light cycle (12 h light and dark each). They were fed standard diet pellets (El-Nasr Chemical Company, Abou-Zaabal, Cairo, Egypt), and water was provided *ad libitum*.

Experimental design

The rats were divided into two groups (12 rats/each group). The first group received standard diet and served as normal healthy control group. The other group was injected i.p. with lead acetate in a dose

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of 5 mg/kg, daily for 6 weeks and served as lead-treated group. At the end of experiment, the animals were sacrificed; serum and tissues were collected and prepared for estimation of lead by flame atomic absorption spectrometry (Kostial et al., 1999). Lipid peroxidation products were measured as thiobarbituric acid reactive substances (TBA-RS) according to the method of Ohkawa et al. (1979). Non-protein sulfhydryl group (NPSH) was measured colorimeterically at 412 nm (Koster et al., 1986).Tissue proteins were determined according to the method of Lowry et al. (1951). Percent of oxidation rate of ubiquinone (% oxidation rate of ubiquinone = Ubiquinone / Ubiquinone + Ubiquinol × 100) and total ubiquinone (Ubiquinone + Ubiquinol) were calculated according to the equation of Mabuchi et al. (2005).

Extraction of coenzyme Q from tissues

The method of Podda et al. (1999) was used to recover coenzyme Q from the tissues. One milliliter of 30% tissue homogenate (in 0.25 M sucrose) was put in a tube containing 50 μL butylated hydroxyl toluene (BHT) (1 mg/mL), to which 1 mL sodium dodecyl sulfate (SDS) (0.1 mM) was added, vortexed for 30 s, sonicated for 15 s, chilled in ice-water and vortexed for 15 s. Two ml of ethanol were added and again vortexed for 30 s, sonicated for 15 s, chilled in ice-water and vortexed for 15 s. Then, 2 mL of hexane were added. The tube was vortexed and subsequently centrifuged at 2000 rpm for 3 min. Hexane layer (1.75 mL) was transfered to another tube and evaporated under gentle nitrogen stream. The residue was dissolved in 100 μL of mobile phase, vortexed and injected into HPLC. Serum (0.2 mL) was mixed with 0.8 mL of SDS (0.1 mM). Then, 1 mL ascorbate (5 mM in 5 mM phosphate buffered saline, pH 7.4) was added and the mixture was vortexed for 1 min.

HPLC quantitation of coenzyme Q

HPLC (LDC Analytical system) was equipped with C_{18} econosil 5 micron ODS 250 x 4.6 mm column with isocratic elution pump

(constametric 4100 pump), programmable UV detector, and D-2500 computing integrator (Zhang et al., 1995).

Statistical analysis

Data are expressed as Mean ± S.E. INSTAT version 2.0 (graph pad, ISI software, Philadelphia, PA, USA, 1993) computer program was used to compute statistical analysis. Difference between means was assessed by Student t-test and statistical significance was accepted at $P \leq 0.05$.

RESULTS

Daily i.p. injection of animals with lead acetate for 6 weeks produced significant increases of lead levels in brain, serum, kidney and liver amounting to 275, 205, 152 and 126%, respectively as compared to the control healthy group (Fig. 1). Analyses of oxidative stress marker among these rats demonstrated significant elevations in TBA-RS in serum, liver, kidney and brain recording 159, 95, 192 and 153%, respectively as com-pared to control group. In addition, there were marked reduction in serum and kidney sulfhydryl group calculated as 32 and 36% in comparison with control group (Table 1). Lead

toxicity clearly diminished the level of oxidized Coenzyme Q_9 in kidney tissue only (49%) when compared with control group. The reduced coenzyme Q_9 significantly decreased in liver and kidney tissues (27 and 42% respectively). At the same time, the reduced coenzyme Q_{10} extensively decreased in liver and brain tissues (32 and 33% respectively) as compared to the corresponding tissues in the control group (Tables 2 and 3).

Serum levels of coenzyme Q_9 and Q_{10} either oxidized or reduced significantly decreased in lead-treated group as compared with control group (Figures 1 and 2). The total coenzyme Q_9 value showed a significant decrease in serum, kidney and brain tissues (32, 46 and 42% respectively) in comparison with control group. In addition, total coenzyme Q_{10} content illustrated an important decrease in liver and kidney only (30 and 30% respectively) (Table 4). Furthermore, the oxidation rate of ubiquinone Q_9 increased only in liver tissues only (17%), whereas that of coenzyme Q_{10} did not show significant changes in all studied organs (Table 5).

DISCUSSION

Lead is a persistent and common environmental contaminant. Like other commonly found persistent toxic metals e.g. mercury, lead damages cellular material and alters cellular genetics. In our present work, daily administration of lead acetate (5 mg / kg b.wt., i.p., for 6 weeks) caused lead accumulation and toxicity. This has been confirmed by a significant elevation of lead level in serum, liver, kidney and brain tissues. The observed toxicity induced by lead acetate in rats was similar to those previously reported by Kostial et al. (1999); Patra et al. (2001) and Shalan et al. (2005).

The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis) alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body (Ercal et al., 2001). In addition, lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning (Ding et al., 2000, 2001; Hsu and Guo, 2002). Oxidative stress has also been implicated in specific organs with lead-associated injury, including liver, kidney and brain tissue (Kostial et al., 1999; Patra et al., 2001). This has been confirmed by the raised level of TBA-RSs as a marker of increased free radical production in the compartments of rats injected with lead acetate in our study. In addition, the reducing equivalence like sulfhydryls decreased especially in serum and kidney tissues in the group injected with lead acetate.

Coenzyme Q functions as an electron carrier in mitochondria. However, it is also present in considerable amounts in endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes and plasma membranes (Ernster

Table 1. Thiobarbituric acid reactive substances (TBA-RS) and non-protein sulfhydryl group (NPSH) in different tissues of lead-treated rats.

Tissues	TBA-RS (µmol/l or nmol/mg protein)		NPSH (µmol/l or µmol/mg protein)	
	Control	Lead group	control	Lead group
Serum	3.99 ± 0.10	10.33 ± 0.51[*]	1308 ± 25.22	886 ± 45.97*
Liver	5.24 ± 0.36	10.23 ± 0.26[*]	25.60 ± 1.55	21.90 ± 0.32
Kidney	4.16 ± 0.12	12.14 ± 0.65[*]	70.90 ± 3.19	45.60 ± 1.58[*]
Brain	4.38 ± 0.30	11.06 ± 0.36[*]	15.54 ± 0.26	17.03 ± 1.71

*: Significantly different from control group at P ≤ 0.05 using Student t-test.

Table 2. Oxidized and reduced coenzyme Q_9 concentrations in different tissues of lead-treated rats.
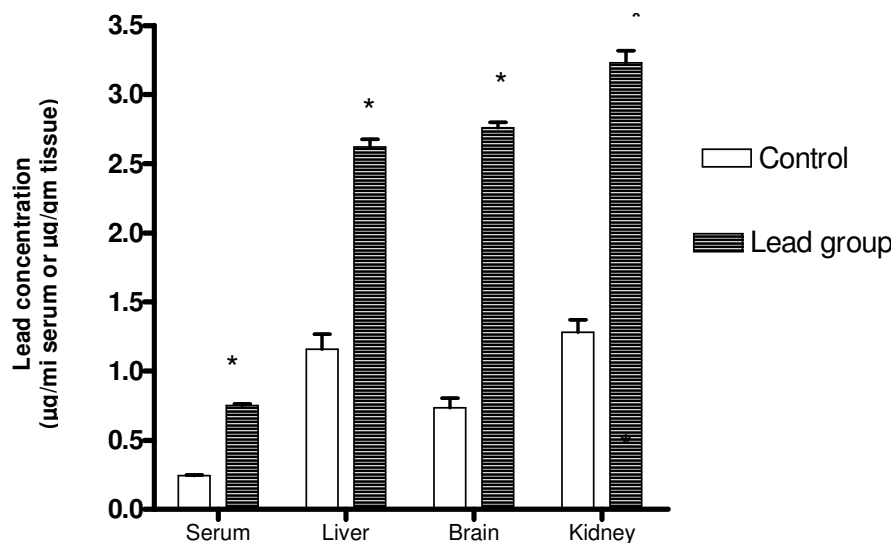
Tissues	Oxidized coenzyme Q_9 (nmol/g tissue)		Reduced coenzyme Q_9 (nmol/g tissue)	
	Control	Lead group	Control	Lead group
Liver	41.5 ± 1.82	37.3 ± 1.02	148.5 ± 12.04	108.8 ± 7.37*
Brain	10.2 ± 0.92	6.2 ± 1.2	1.7 ± 0.32	0.7 ± 0.23
Kidney	95.5 ± 10.13	48.6 ± 5.56*	61.4 ± 5.14	35.4 ± 2.99*

*: Significantly different from control group at P≤0.05 using student t-test.

Table 3. Oxidized and reduced coenzyme Q_{10} concentrations in different tissues of lead-treated rats.

Tissues	Oxidized coenzyme Q_{10} (nmol/g tissue)		Reduced coenzyme Q_{10} (nmol/g tissue)	
	Control	Lead group	Control	Lead group
Liver	6.7 ± 0.7	5.4 ± 0.3	27.3 ± 1.9	18.5 ± 1.6*
Brain	3.4 ± 0.61	2.5 ± 0.35	0.6 ± 0.035	0.4 ± 0.052*
Kidney	23.67 ± 2.52	16.67 ± 1.71	11.3 ± 1.01	7.7 ± 1.04

*: Significantly different from control group at P ≤ 0.05 using Student t-test.

Figure 1. Lead levels (mean ± SE) in serum and different tissues of lead-treated rats as compared to control. *: Significantly different from control group at P ≤ 0.05 using student t-test.
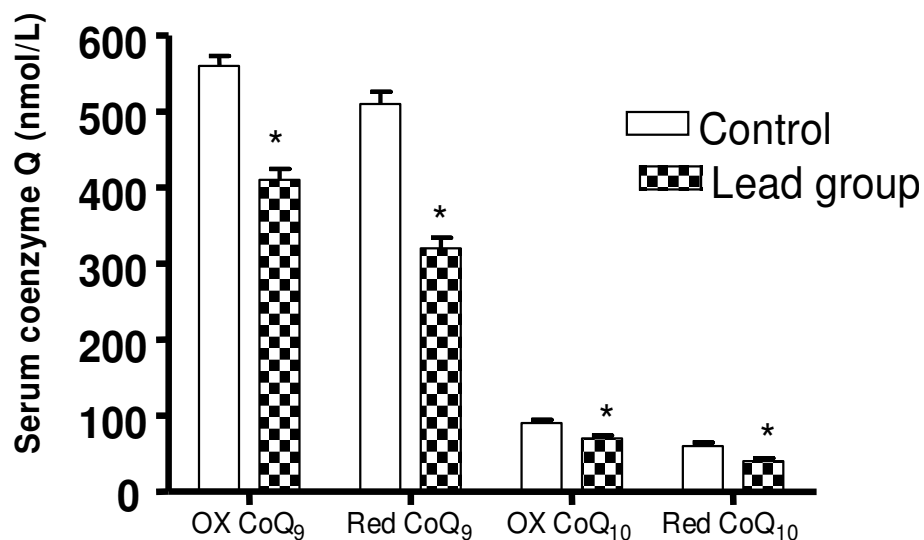
Figure 2. Oxidized (Ox) and reduced (Red) coenzyme Q_9 and Q_{10} concentrations (mean ±SE) in the serum of lead-treated rats as compared to control. *Significantly different from control group at $P \leq 0.05$ using student t-test.

Table 4. Total coenzyme Q levels in different tissues of lead-treated rats.

Tissues	Total coenzyme Q_9		Total coenzyme Q_{10}	
	Control	Lead group	control	Lead group
Serum (nmol/L)	1070 ± 3.8	730 ± 5.03[*]	150 ± 9.8	110 ± 1.5
Liver (nmol/g tissue)	189.7± 13.9	146.1 ± 6.7	34.0 ± 2.6	23.8 ± 1.4[*]
Kidney (nmol/g tissue)	156.9 ± 5.3	84.0 ± 2.7[*]	34.97 ± 1.5	24.37 ± 2.7[*]
Brain (nmol/g tissue)	11.87 ± 0.61	6.9 ± 0.98[*]	3.97 ± 0.57	2.9 ± 0.39
Total Ubiquinone = Ubiquinone + Ubiquinol				

*: Significantly different from control group at $P \leq 0.05$ using student t-test.

Table 5. Oxidation rate of ubiquinone in different tissues of lead-treated rats.

Tissues	% oxidation rate of ubiquinone Q_9		% oxidation rate ofubiquinone Q_{10}	
	Control	Lead group	control	Lead group
Serum	52.3 ± 1.0	56.1 ± 2.0	60.1 ± 1.0	63.6 ± 4.0
Liver	22.0 ± 1.0	25.7 ± 2.0*	19.5 ± 0.5	22.8 ± 2.4
Kidney	60.6 ± 4.0	57.5 ± 5.0	67.3 ± 4.0	68.6 ± 1.0
Brain	86.0 ± 4.0	88.0 ± 5.0	84.0 ± 3.0	86.0 ± 1.0

*: Significantly different from control group at $P \leq 0.05$ using student t-test.

and Dallner, 1995). This broad distribution in intracellular organelles emphasizes that coenzyme Q plays an antioxidant role (Beal et al., 1994; Crestanello et al., 1996; Hodgson and Watts, 2003; Yao-Chung et al., 2003). In addition, our results suggested that thereduced form of coenzyme Q (ubiquinol) significantly decreased in liver and brain tissues of the rats injected with lead ace-tate, while the oxidized form (ubiquinone) does not show any significant variations as compared with the healthy control group. These results may be attributed to the raised level of lead in these tissues may disrupt the activity of the enzymes responsible for the reduction of ubiquinone to ubiquinol such as cytosolic NADPH-CoQ reductase enzyme (Kishi, 1999). This explains the increased percentage oxidation rate of ubiquinone and reduced total coenzyme Q_{10} in rat liver.

All tissues contain appreciable amounts of ubiquinones, which are essential for normal cellular functions as electron and proton carrier either in the respiratory chain or as antioxidants. It has been reported that, the principal ubiquinone in rat tissues is coenzyme Q_9, accounting for 80 - 90% of the total coenzyme Q in most tissues (Aberg et al., 1992). Tissues involved in detoxification, including the liver and the kidney, have extraordinarily high concentrations of ubiquinol, perhaps to protect them from radicals escaping from P_{450}. In addition, these tissues have high concentrations of mitochondria, which could also account for their high coenzyme Q contents (Ernster and Dallner, 1995).

In conclusion, the levels of both oxidized (ubiquinone) and reduced form (ubiquinol) of coenzyme Q_9 and Q_{10} in serum, brain, liver and kidney are quite different in lead poisoning depending on the organ tissue type.

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