Relationship between the Changes of Catecholamines and Blood Pressure Induced by Exposure to Low- and High-levels of Lead in Rats

Suh-Young YOON, Kyeong-Seok YOO and Jae-Hoon, CHEONG*

Department of Pharmacy, Sahm-Yook University

Abstract – In this study, it was tested whether the changes of catecholamines and its metabolites are related with the changes of blood pressure(BP) induced by different levels of lead exposure. Adult male SD rats were exposed to lead by giving drinking water containing 50(low doses), 200 and 1,000 ppm(high doses) of lead(as lead acetate) or sodium acetate(for control groups, supplying an identical amount of acetate) for 7 or 16 weeks. The systolic BP was measured in the unanesthetized state by the tail-cuff technique. Levels of catecholamines and its metabolites in urine were measured by HPLC-ECD. Rats receiving 200 and 1,000 ppm developed an elevation of systolic BP at 3 and 7 weeks compared with week 0, but blood pressure levels at 16 weeks returned to normal. For the 50 ppm lead treated group, systolic BP increased significantly at 7 weeks and 16 weeks. The concentrations of norepinephrine and VMA in the urine of lead exposed rats changed similarly to the changes of blood pressure, but blood viscosity levels in all lead treated rats increased continuously during all lead treatment periods. This result suggests that the changes of catecholamines and its metabolites in urine by lead intoxication may influence the changes of blood pressure.

Keywords ☐ Lead intoxication, Blood pressure, Cathcholamine, Blood viscosity

INTRODUCTION

Even low levels of blood lead could be considered hazardous. Many occupational workers have been damaged by lead poisoning at high levels in the past years, but the toxicity of low levels is also a considerable risk in health problems, especially for infants and children. It is now suggested that there may be no levels of lead that do not produce a toxic effect (Needleman, 1988; Rutter, 1983).

Both epidemiological observations and experimental animal studies have reported that chronic low level lead exposure was related to hypertension. Staessen et al. reviewed 23 epidemiological studies and 21 animal studies related to low-level lead exposure and blood pressure. In epidemiological investigations, 13 surveys were performed in the general population and 10 studies were performed in occupational groups. In all 23 studies combined, a two-fold increase in blood lead concentration was associated with a 1 mm Hg rise in the systolic pressure(CI 0.4-1.6 mmHg; P= 0.002) and with a 0.6 mmHg increase in the diastolic pressure (CI 0.2-1.0 mmHg; P=0.02). Of the 21 animal studies, one was carried

out in dogs, one in pigeons and the remainder in various rat strains. In 15 studies, in which the lead doses in drinking water or food exceed 1 ppm, the association between blood pressure and exposure was found to be positive in seven, inconsistent in three, absent in four and negative in one. Of the six studies at lower exposure levels (<or=1 ppm), five found a pressor effect attributable to lead (Staessen et al., 1995).

In several of the high-dose experiments, hypertension was observed, but nephrotoxicity of lead may have contributed to its development. Moreover, in other high-dose experiments, no hypertension was observed, and in at least one experiment, the evidence suggested that lead could reduce an elevated blood pressure. In contrast, the lower dose experiments consistently demonstrated a hypertensive effect. Overall, the data suggest a biphasic dose response (Victery, 1988).

The mechanism whereby lead affects blood pressure is not known, although experimental studies have suggested several plausible possibilities. The major possible mechanisms of hypertension are calcium mediated control of vascular smooth muscle contraction and renal effects mediated through the renin-angiotensin system.

Other factors were shown in lead intoxicated people or rats

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*To whom correspondence should be addressed.

such as abnormalities in cardiac function, increased suscep-tibility to the arrhythmogenic action of norepinephrine, in-crease in the urinary excretion of catecholamine metabolites and the enhancement of adrenergic function in the central ner-vous system (Silbergeld, E. K. and Chisolm, J. J., 1976; Mic-ciolo et al., 1994).

Recently, much experimental data indicates that blood vis-cosity is a possible factor of hypertension. Hypertensive patients displayed a significantly lower erythrocyte fluidity and significantly elevated values for hematocrit, plasma and whole blood viscosity (Sandhagen et al., 1990). In the case of cold-induced hypertension, whole blood viscosity was increased 25% (Roukoyatkina et al., 1999).

In our experiment, we established three levels of lead, 50 ppm as a low level, and 200 and 1,000 ppm as high levels. And it was tested which difference of blood pressure can be caused by lead levels and, if it can be caused, whether blood viscosity or the changes of catecholamines and its metabolites in urine are related with the changes of blood pressure levels induced by different levels of lead exposure.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats(200 g) were purchased from DaeHan Laboratory Animal Research Center(Seoul, Korea). Animals were housed in groups of five and maintained in air-conditioned and temperature-controlled room ($23\pm1°C$) under controlled light (12 h light and 12 h dark), with rat chow and water *ad libitum*. Rats were exposed to lead by giving drink-ing water containing 50, 200, 1,000 ppm lead(as lead acetate) or sodium acetate(for control group, supplying an identical amount of acetate) for 3, 7 or 16 weeks.

Materials and Reagents

Folin & Ciocalteu's phenol reagent, defatted bovine serum albumin, sodium octyl sulfate, and sodium potassium tartarate were purchased from Sigma Chemicals (St. Louis, Mo, USA). Lead acetate, sodium acetate, sodium carbonate and cupric sulfate were purchased from DukSan Pure Chemicals (Ansan, Kyungkido). HPLC-acetonitrile was purchased from Baxter Scientific Products (McGaw Park, IL., USA).

All other reagents were reagent grade. Deionized double-distilled water was used in the preparation of reagents and all the experimental procedures.

For the measurement of blood pressure, the Grass model 7 polygraph (Grass instrument Co.) was used; for protein deter-mination, the UV spectrophotometer (Spectronic Ge-nesis 5, Milton Roy) was used; and for hematological para-meters, the Hema-check 5 (blood cell counter, Biochemical Systems Co.) were used.

For the measurement of catecholamine and its metabolites in urine, the HPLC-ECD system (BAS 400, Lafayette, Ind., U.S.A.) was used. The HPLC-ECD system was composed of a Pump(BAS PM-60), Electorochemical detector(BAS LC-4B), and Injection valve(Rheodyne 7175, Cotati, Calif., U.S.A.). The analytical column was Lichrosorb RP-18 col-umn(length 25cm; particle size 10 μm) which was supplied from Merck KGaA (Darmstadt, Germany), and a 3 cm Hibar precolumn was used for preventing an occlusion of the col-umn.

Blood pressure

Systolic blood pressures were measured in the unanesthe-tized state by tail-cuff technique at 0, 3, 7 and 16 weeks through the study. The animals were pre-warmed for 10 to 15 minutes on a heating pad with a surface temperature of 40°C and then transferred to a restrainer for blood pressure mea-surement (Bruce et al., 1980).

Measurement of Whole Blood Viscosity

Blood samples were collected by heart puncture at the end of experiment and used to evaluate hematological parameters. Lowry's method was used for protein determination in whole blood (Lowry et al., 1951). Bovine serum albumin was used as standard. An estimate of the whole blood viscosity(WBV) was computed by using the following formula $WBV=0.12\times HCT+0.17\times TBP-2.07$ (HCT=hematocrit level; %, TBP=total blood protein; g/dl) (Micciolo et al., 1994).

Measurement of Catecholamines and its Metabolites in Urine

Experimental animals were adapted to the living environ-ment of metabolic cages for 24 hours. Urine samples were collected for the next 24 hours from each rat. Then the urine samples were filtered through filter paper and stored at -20°C until analysis. Urinary catecholamine and its meta-bolites were measured with the HPLC-ECD system. The composi-tion of the mobile phase was 0.1 M citric acid, 0.43 mM sodium octyl sulfate, 0.06% triethylamine, 0.05 mM Na_2EDTA, 0.9% acetonitrile. Using NaOH, the combined solution was adjusted to pH 2.55. Before the acetonitrile was combined with the others, reagents were filtered through 0.22 μm (pore size) nylon membrane filters. The flow rate of the mobile phase was 1.5 mL/min. Peak identification and single point quantification were performed by comparison with the

Table I. Blood Viscosity of 50, 200 and 1000 ppm Lead-treated Groups for 7, 16 Weeks

	Control	50 ppm	200 ppm	1,000 ppm
7 weeks	5.5±0.4	6.2±0.3**	6.5±0.2**	6.5±0.3**
16 weeks	6.0±0.2	7.0±0.1*	7.1±0.4*	7.9±0.5*

Each value represents the mean±S.D. of data from 10 animals. * indicates a significant difference from control group (*: p < 0.05, **: p < 0.01).

results of standard (Ko et al., 1992; Gamache et al., 199; Peaston et al., 1996).

Statistical Analysis of data

Data was expressed as the mean±S.D.. For statistical evaluation of the data, the Newman-Keuls test was used. Differences were considered statistically significant if P< 0.05.

RESULTS

The data on systolic blood pressure at the levels of 50, 200, 1,000 ppm lead are shown in Figure 1. Rats receiving 200 and 1,000 ppm lead for 3 (121.8±6.2 mmHg, 120.9±6.6 mmHg vs 109.5±5.0 mmHg in control) and 7 weeks (120.0±2.9 mmHg, 123.8±2.9 mmHg vs 110.3±4.8 mmHg in control) developed an elevation of systolic blood pressure but blood pressure levels at 16 weeks returned to normal (114.4±4.9 mmHg, 113.1±4.9 mmHg vs 113.6±2.2 mmHg in control). 50 ppm of lead produced continuous high blood pressure at 7 (123.6± 0.9 mmHg vs 110.3±4.8 mmHg in control) and 16 weeks (126.0±0.4 mmHg vs 113.6±2.2 mmHg in control).

Table I shows the blood viscosity of each of the lead treated groups for 7 and 16 weeks. Blood viscosity levels in all lead treated rats increased continuously in all lead treatment peri-
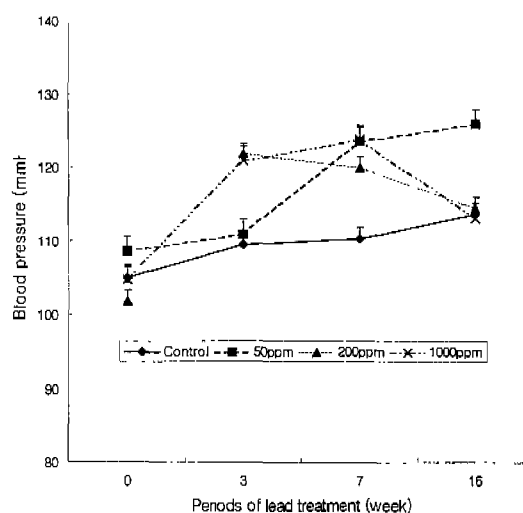
Fig. 1. Blood pressure at the level of 50, 200, 1,000 ppm lead Each symbol represents the mean±S.D.

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Table II shows catecholamines and its metabolites in urine of lead exposed rats. The concentrations of VMA in urine were significantly higher in lead treated groups (5.0±0.4 μmol/ml in 50 ppm, 8.7±1.0 μmol/ml in 200 ppm, 7.3±0.7 μmol/ml in 1,000 ppm) than that of control rats (2.9±0.3 μmol/ml) at 7 weeks, but they were decreased at 16 weeks (6.0±0.5 μmol/ml in 50 ppm, 6.0±0.3 μmol/ml in 200 ppm, 5.2±1.1 μmol/ml in 1,000 ppm vs 8.7±0.9 μmol/ml in control). The concentrations of NE in urine were also higher in 50 (25.6±28.3 μmol/ml), 200 (24.6±7.0 μmol/ml) and 1000 (25.0±12.2 μmol/ml) ppm, than that of control rats (9.6±2.7 μmol/ml) at 7 weeks. Although the concentrations of NE in urine of lead exposed rats of 200 and 1,000 ppm decreased (11.2±.1 μmol/ml, 11.6±4.9 μmol/ml respectively), it

Table II. Catecholamines and its metabolites in urine

		VMA	NE	EP	NM	DA	HVA
7 weeks	Control	2.9±0.3	9.6±2.7	2.8±3.1	43.4±18.3	3.4±3.0	0.5±0.2
	50 ppm	5.0±0.4**	25.6±28.3*	3.4±2.1	54.7±42.0	4.9±5.3	0.6±0.3
	200 ppm	8.7±1.0**	24.6±7.0*	2.0±1.2	40.5±28.5	1.1±0.7	0.5±0.2
	1,000 ppm	7.3±0.7**	25.0±12.2*	1.8±1.2	42.7±35.4	0.9±0.4	0.7±0.2
16 weeks	Control	8.7±0.9	17.9±9.0	1.2±0.8	19.6±9.8	6.5±6.7	0.7±0.3
	50 ppm	6.0±0.5**	30.0±19.7*	1.4±1.0	29.2±8.4	6.0±8.1	1.2±1.1
	200 ppm	6.0±0.3**	11.2±6.1	1.9±1.0	46.6±20.3**	1.6±1.1	0.7±0.3
	1,000 ppm	5.2±1.1**	11.6±4.9	2.0±1.2	48.3±11.7*	3.7±2.5	1.0±0.9

Each value represents the mean±S.D. of data(μmol/urine ml). VMA=vanylic mandelic acid, NE= norepinephrine, EP=epinephrine, NM=normethanephrine, DA=dopamine, HVA=homovanylic acid, * indicates a significant difference from control group (*: p<0.05, **: p<0.01).

Table III. Ratio of VMA/NE, 5-HIAA/5-HT, HVA/DA

		Ratio VMA/NE	Ratio 5-HIAA/5-HT	Ratio HVA/DA
7 weeks	Control	1133.2±488.8	33.0±10.1	1183.2±1088.0
	50 ppm	1148.1±729.0	93.9±29.6	1101.8±1174.4
	200 ppm	1294.6±354.2	140.3±39.9*	1948.5±1275.5
	1,000 ppm	1069.9±397.3	233.9±100.8**	3280.8±2145.5
16 weeks	Control	825.1±987.5	123.8±57.9	1013.6±786.6
	50 ppm	1657.5±1467.2	88.6±42.9	1504.9±2261.1
	200 ppm	2806.6 ±1277.1*	170.5±123.6	2992.3±1959.9
	1,000 ppm	2231.2±806.4*	182.4±132.3	2024.3±1922.1

Each value represents the mean±S.D. of data(μmol/urine ml). HIAA=hydroxyindole acetic acid, 5-HT=5-hydroxytryptamine, *indicates a significant difference from control group (*: p<0.05, **: p<0.01).

increased in 50 ppm (30.0±19.7 μmol/ml) compared to the control group (17.9±9.0 μmol/ml) at 16 weeks.

Table III shows the ratio of VMA/NE, 5-HIAA/5-HT, HVA/DA in lead treated rats. The ratio of VMA/NE in 50ppm lead exposed rats (1657.5±1467.2 μmol/ml) was lower than that of 200 (2806.6±1277.1 μmol/ml)and 1,000 ppm (2231.2±806.4 μmol/ml) at 16 weeks.

DISCUSSION

Rats receiving 200 and 1,000 ppm lead for 3 and 7 weeks developed an elevation of systolic blood pressure but elevated blood pressure levels returned to normal at 16 weeks.

Perry et al. have documented that as little as 1 ppm lead in the drinking water can produce hypertension (Perry et al., 1979). Aviv et al. reported that 10,000 ppm lead exposure of rats during 3 to 9 weeks of age resulted in the development of hypertension in adulthood. However, these investigators used very large doses of lead, which caused severe renal damage (Aviv et al., 1980).

In the experiment of Victery et al., lead exposure was begun in utero and rats received drinking water containing 100 and 500 ppm lead. At 3½ months of age, the rats drinking 100 ppm lead first manifested a statistically sig-nificant increase in blood pressure(152±3.7 mmHg) relative to the control group(135±5.6 mmHg). In contrast, the animals drinking 500 ppm lead tended to have lower blood pressures than did control group animals throughout the experiment, but at no time was the difference statistically significant (Victery et al., 1982).

Khalil et al. observed lead-induced hypertension with low lead(0.01%) and high lead(0.5%) administration. The results

of this study confirmed increased blood pressure in low level lead treated rats. But high levels of lead couldn't produce hypertension similar to the experiments of Victery et al. (Khalil et al., 1993). Williams et al. used 2,000 ppm of lead and they couldn't produce hypertensive rats (Williams et al., 1977). This is the biphasic effect of blood pressure in lead intoxication. It indicates that chronic exposure to low-level of lead can produce continuous hypertension, but high level temporarily increase blood pressure and return to normal (Victery, 1988).

In our study, 200 ppm of lead failed to produce continuous high blood pressure but 50 ppm of lead produced continuous high blood pressure. We presumed that the lead levels lower than 200 ppm could produce continuous hypertension and higher levels of lead couldn't produce hypertension con-tinuously.

Victery et al. indicated that hypertension-producing 100 ppm doses are still extremely large compared to human ex-posures (produce blood lead concentration: 40 μg/dl) (Victery et al., 1982). 50 ppm of lead is the more environmentally appropriate level in order to determine the validity of associating this pollutant with blood pressure effects in the human population.

Many investigators reported the association between blood pressure and blood lead level, (Elwood et al., 1988; Weiss et al., 1986; Hertz-Picciotto and Croft, J., 1993) but association between blood lead concentration and high blood pressure has not been uniformly positive (Micciolo et al., 1994; Hu et al., 1996). We investigated the relationship between blood pressure and intoxicated lead levels, and lead levels to induce continuous high blood pressure was certificated.

In this experiment, whole blood viscosity could be cal-

culated by using the following formula WBV=0.12×Hct+ 0.17×Pr−2.07 (Hct=hematocrit level; %, Pr=total protein; g/ dl) (Micciolo *et al.*, 1994). Hct and TBP in all lead-treat groups were elevated in all lead treatment periods. The change of WBV didn't correspond to the biphasic effect on blood pressure induced by lead intoxication.

Micciolo *et al.* observed increased Hct and WBV in lead intoxicated people of Northern Italy, and they concluded a weak association between WBV and lead intoxication (Micciolo *et al.*, 1994). Hense *et al.* founded a strong association between Hct and blood lead levels in a low-exposure population (Hense *et al.*, 1992). But Victery *et al.* observed lower Hct in 100 ppm rats (Victery *et al.*, 1982). From other data, some investigators found increased peripheral resistance in patients with essential hypertension, which may in part be explained by an increased blood viscosity and vascular constriction(Linde *et al.*, 1993). Diastolic and mean blood pressures were positively related to WBV and hematocrit levels (de Simone *et al.*, 1990). Hypertensive patients displayed a significantly lower ery-throcyte fluidity, elevated values for hematocrit, plasma and whole blood viscosity, as well as an aggregation tendency. It is suggested that the molecular mechanism underlying the evolution of essential hypertension are multifactorial rather than being based on a single molecular derangement. Primary events resulting in altered physico-chemical properties of the red blood cells may work in concert in the development of essential hypertension, in addition to the increased avail-ability of calcium ions and their potential role in smooth muscle contraction (Sandhagen *et al.*, 1990).

The relationship between blood viscosity and lead-induced hypertension is conflicting. In this experiment, WBV may be one of several factors which affect the development of hypertension, but isn't related to the decrease of blood pressure in 200 and 1,000 ppm at 16 weeks.

Levels of NE and VMA in urine of 200 ppm and 1,000 ppm lead exposed rats increased at 7 weeks but decreased at 16 weeks. Change patterns of NE and VMA corresponded to changes of blood pressure in lead treated groups. Levels of NE and VMA in urine of 50 ppm lead exposed rats increased at both 7 weeks and 16 weeks. They also corresponded to changes of blood pressure. The ratio of VMA/NE in 50 ppm lead exposure rats were lower than 200 and 1,000 ppm at 16 weeks.

Cells of the adrenergic system are a direct source of urinary unconjugated norepinephrine(NE) and epinephrine(EP),

whereas Dopamine(DA) originates primarily from peripheral metabolism of 3, 4-dihydroxy-phenylalanine(DOPA) in the kidney. VMA is a major catecholamine metabolite formed by the actions of catechol-O-methyltransferase and monoamine oxidase. It is excreted by the kidney and represents an average of 40-50% of the urinary excretion production of NE and EP. NE is the major source of VMA. Normetanephrine(NM) is metabolic products of NE and is formed by the action of cate-chol-O-methyl transferase without deamination (Rosano *et al.*, 1991).

Silbergend and Chisolm studied the effects of lead on the nervous system, and observed significant increases in homo-vanillic acid(HVA) and vanillylmandelic (VMA) acid in the brain and urine of lead treated mice. In children, they observed five-fold increases in the daily output of these compound (Silbergeld, E. K. and Chisolm, J. J., 1976).

This result suggests that the changes of catecholamines and its metabolites in urine by lead intoxication may influence the changes of blood pressure. The increased excretion of NE, and VMA may be related to increased activation of sympathetic nervous system. Many investigatiors studied the relationship between sympathetic activity and blood pressure (Judy *et al.*, 1976; Murphy *et al.*, 1976). Patients with primary hypertension have been identified that have elevated circulating norepinephrine levels (Dequattro *et al.*, 1975; Goldstein, 1983). Goldstein *et al.*, suggested that increased central sympathetic outflow might play a pathophysiologic role in some patients with essential hypertension (Goldstein *et al.*, 1985).

Proposed mechanisms of lead(low level)-induced hypertension may include interference of lead with ion transport across cell membranes, interactions with calcium homeostasis and calcium-mediated processes, direct vasomotor actions and the potentiation of sympathetic stimulation. Interference of lead with the balance between the renin-angiotensin-aldosterone and the kallikrein-kinin systems and impairment of renal function are unlikely to be implicated (Staessen *et al.*, 1995).

Khalil *et al.* reported that the path to development of hypertension in low lead rats may be through an increase in the concentration of the vasoconstrictor hormone, endothelins 3(ET-3), and a decrease in the vasodilator hormone, end-othe-lium-derived relaxing facor(EDRF). High levels of lead exposure did not result in hypertension, perhaps because concentrations of ET-1, ET-3 and cGMP concentrations were coordinately and significantly decreased at 12 months (Khalil

et al., 1993).

Future research should study on the mechanism of catecholamine changes in urine induced by lead poisoning and relation factors between catecholamine and blood pressure.

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