THE RELATIONSHIP BETWEEN CHRONIC LEAD ACETATE EXPOSURE AND HYPERTENSION IN RAT

Gh. Karimi[1]., *A. Khoshbaten*[2]., *A.R. Dehpour*[3]., *M. Abdollahi*[1] *and M. Sharifzadeh*[1]

(1) Department of Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

(2) Department of Physiology, Faculty of Medicine, Baghiattollah University of Medical Sciences, Tehran, Iran

(3) Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract - Effects of 28 days lead acetate (100, 500, 1000 ppm) treatment on hypertension and relationship between blood lead levels and hypertension in male Sprague-Dawley rats were studied. Results of this study showed that lead acetate treatment did not decrease body weight or water consumption, except for lead acetate (1000 ppm) and sodium acetate (500 ppm) which increased fluid consumption when compared with controls. The doses of lead acetate (100, 500, 1000ppm) used in this study, caused blood concentrations of 26.84 ± 2.23, 43.12±2.40 and 52.8±3.44 μg/dl respectively. Treatment of animals with different doses (100, 500, 1000 ppm) of lead acetate for 28 days, caused a significant change (P< 0.05) in systolic blood pressure when compared to control rats. No dose dependent correlation was observed between blood pressure elevation and blood lead levels in treated rats.

Acta Medica Iranica 37 (3): 150-154; 1999

Key Words:Lead acetate, hypertension

INTRODUCTION

Lead is recognized as an environmental and occupational hazard that has a significant impact on the health and development of many species (1). Lead poisoning is involved in the structural and functional abnormalities of multiple organ systems (2). Cardiovascular lesions such as atherosclerosis and hypertension are public problems, which have a strong interrelationship. Experimental and epidemiological studies have suggested that heavy metals including lead may be a causal factor in cardiovascular diseases (3). The relationship between lead exposure and hypertension has been a controversial issue for over a century (4). Occasional reports have suggested the existence of this association, but the available data remain inconclusive. Various epidemiological studies have shown an increased incidence of hypertension and cerebral hemorrhage among industrial lead workers, but others could not substantiate these findings (5). Cramer found no significant difference in the prevalence of diastolic hypertension in 155 workers exposed to lead who had evidence of toxicity (6). In another study diastolic blood pressure was significantly higher in the exposed group, but systolic blood pressure did not differ (7). Nonindustrial exposure to lead has also been linked to hypertension (8). Experiments in rats also have provided inconsistent results (5). Several mechanisms have been proposed to explain lead induced hypertension, including: 1) an alteration in calcium exchangibility or distribution leading to an elevation of intracelluar calcium, 2) inhibition of sodium potassium ATPase, and 3) an alteration of factores released from endothelium (9). This study was designed to investigate the relation among chronic low levels of lead exposure, blood concentration and hypertension. The rat was evaluated as an experimental model because of the ease of indirect blood pressure measurements without the need for anesthesia and the information it can provide on human lead induced hypertension (8).

MATERIALS AND METHODS

The following materials have been used in this study: Triton X-100 (TX, an alkylphenoxypolyethoxyethanol, Merck, Germany, 10% V/V), ammonium pyrrolidine dithiocarbamate (APDC, Sigma, UK, 2% W/V), methylisobutylketone (MIBK, water saturated, Merck, Germany), Heparin (sodium salt, 0.2 μg/ml, Leo pharmaceutical products, Denmark), lead standard solution for atomic absorption spectroscopy (Titrisol, 1g/l, Merck, Germany), lead acetate (Merck, Germany), sodium acetate (Merck, Germany), Apparatus : Atomic absorption spectrophotometer equipped by graphite furnace atomizer (Shimadzu, 680A, Japan), programmed electro-sphygmomanometer (Narco, PE 300, USA), physiograph (Narco, MK III-S, USA).

Adult male Sprague-Dawley rats, weighing 200-250g, were used. The animals had free access to a stock laboratory diet and water ad libitum. Following several days of acclimatization, the rats were randomly distributed into experimental and control groups. They were caged in an environment at 20-25 ° C with a light/dark cycle of 12/12 hr starting at 8:00 a.m. Animals were monitored daily for food intake and water consumption, and every week for body weight gain.

Treated animals were given three doses of lead acetate (100, 500, 1000ppm) in the drinking water for 28 days. Controls received 50, 250 and 500 ppm sodium acetate solution respectively (10). Lead acetate or sodium acetate was dissolved in distilled water.

After 28 days, rats were deeply anaesthetized with ether. The thorax was opened and blood withdrawn from the heart by syringe and collected in lead free heparinized plastic tube and stored at-18 ° C until analyzed.

The thawed whole blood were made homogenous and after adding of 1 ml 10% TX and 1 ml 2% APDC, extracted by 1.5 ml MIBK (11). The organic supernatant solution of all samples were analyzed for lead using an atomic absorption spectrophotometer equipped with graphite furnace assembly and a deuterium background corrector. Standard solution were made with control rat bloods. The blood from exposed animals was measured against a standard curve, while the blood lead levels from control animals were measured by addition of known amounts of lead solution (12).

Blood pressure monitored every week at 9 a.m. through the period of experiment (4 weeks) using tail cuff method as follows. Rats were placed in a restrainer and a cuff was placed on the tail. After 15 min acclimatization the tail cuff was inflated and released several times by sphygmomanometer. The systolic blood pressure of animals was measured consecutively 3 times using a physiograph and the mean of these 3 measurements was recorded (9).

Comparison among groups was made by analysis of variance (ANOVA) and the Newman-Keuls test. $P<0.05$ was considered significant. All statistical analysis were done by computer using PCS software.

RESULTS

As shown in table 1, lead acetate treatment did not decrease body weight or water consumption. Lead acetate (1000 ppm) and sodium acetate (500 ppm) increased fluid consumption in comparison with the control group (table 1).

Treatment of rats by different doses of lead acetate (100, 500, 1000 ppm) increased systolic blood pressure of rats from 95.8 ± 3.13, 96.6 ± 3.31 and 92.3 ± 3.72 mmHg to 124.2 ± 2.15, 122.5 ± 1.34 and 125.4 ± 1.21 respectively after 28 days (figure 1,2,3). relationship was not found between blood lead levels and hypertension. Data in these figures also show significant differences ($P< 0.01$) between systolic blood pressure of lead acetate and sodium acetate treated groups in 4th week.

Concentrations of lead in blood of control and treated rats are shown in figure 4. As shown in this figure the doses (100, 500, 1000ppm) used in this study, caused blood concentrations of 26.84 ± 2.23, 43.12 ± 2.40 and 52.8 ± 3.44 μg/dl respectively.

Table 1- Effects of lead acetate treatment (0.01%, 0.05%, 0.1%) on body weight and fluid consumption of rats during 4 weeks

Body Weight (g) / FLuid Consumption (ml)					
Treatment	Week 0 (control)	Week 1	Week 2	Week 3	Week 4
Distilled water	243 ± 2.7	249 ± 2.2/630	253 ± 2.4 /580	257 ± 2.2/555	262 ± 2.8*/540
Lead acetate (0.01%)	232.6 ± 2.4	227.3 ± 2.6/520.	230.3 ± 2.8 /595	234.3 ± 2.6/560	235.3 ± 2.4/510
Na-acetate (0.005%)	213.6 ± 3.1	216 ± 2.8/520	213.6 ± 2.6 /560	213.3 ± 2.5/560	216.3 ± 2.7/510
Lead acetate (0.05%)	224.8 ± 2.4	222.4 ± 2.5/510	226.6 ± 2.6 /470	233.6 ± 2.6/530	239.4 ± 2.9*/490
Na-acetate (0.025%)	243.6 ± 2.8	249 ± 2.6/525	255 ± 2.5 /505	266 ± 2.5/580	278 ± 2.9*/530
Lead acetate (0.1%)	246.3 ± 1.9	248.8 ± 1.7/705	253.6 ± 2.0 /505	259.8 ± 1.8/780	266.6 ± 2.4*/680
Na-acetate (0.05%)	247.7 ± 1.6	253.2 ± 2.0/690	256.7 ± 2.2 /855	262.2 ± 2.2/880	269.7 ± 2.6*/800

Data for body weight are mean ± SE.

Fluid consumption is represented as mean milliliter of fluid used by five rats in one week.

* Different from respective control (week 0) groups at $p< 0.05$

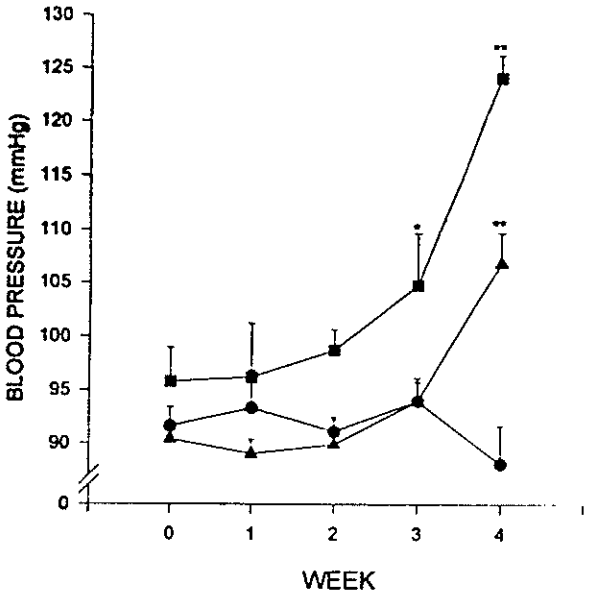

Fig. 1. Effect of lead acetate (100ppm) treatment on rat blood pressure. Animals were treated by lead acetate 100ppm (■), sodium acetate 50ppm (Δ) and distilled water (●) for 28 days.
Number of animals = 5.
** Different from control (distilled water) at P<0.01
* Different from control (distilled water) at P<0.05

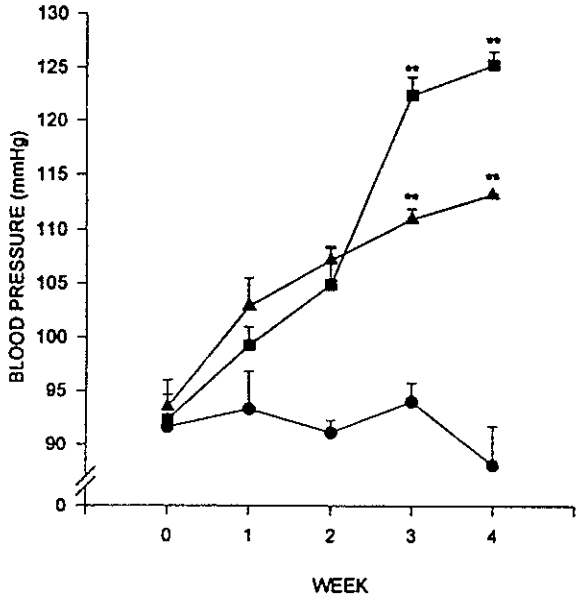

Fig. 3. Effect of lead acetate 1000ppm treatment on rat blood pressure. Animals were treated by lead acetate 1000ppm (■), sodium acetate 500ppm (Δ) and distilled water (●) for 28 days.
Number of animals = 5.
** Different from control (distilled water) at P<0.01

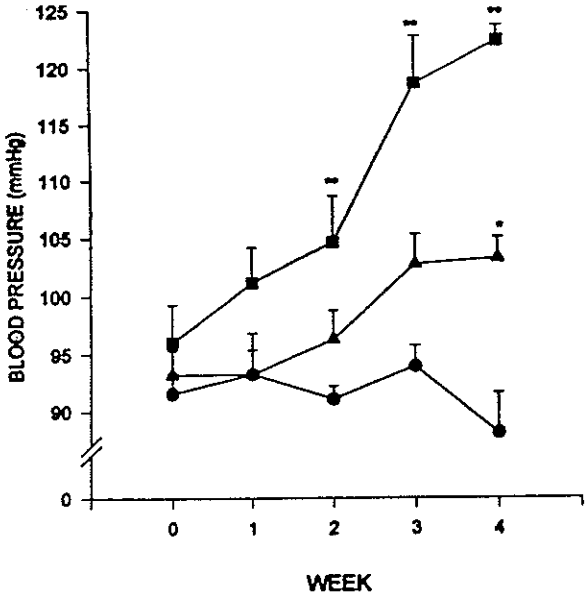

Fig. 2. Effect of lead acetate (500ppm) treatment on rat blood pressure. Animals were treated by lead acetate 500ppm (■), sodium acetate 250ppm(Δ) and distilled water (●) for 28 days. Number of animals = 5.
* Different from control (distilled water) at P < 0.05
** Different from control (distilled water) at P < 0.01

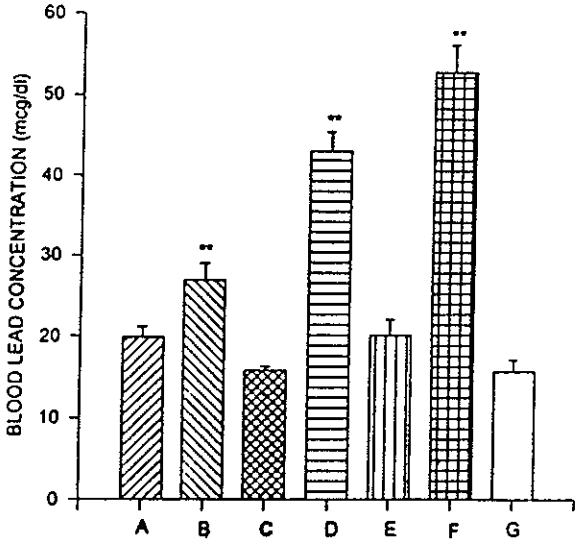

Fig. 4. Effect of different doses of lead acetate on rat blood lead concentration after 28 days.
Animals were treated by distilled water (A), lead acetate 100 ppm (B), Na-acetate 50 ppm(C), lead acetate 500ppm (D), Na-acetate 250ppm (E), lead acetate 1000ppm (F), Na-acetate 500ppm (G).
Number of animals = 5.
** Different from control (distilled water) at P<0.01

DISCUSSION

As shown in table 1, lead acetate did not significantly decrease rat body weight after 28 days of treatment. This finding is in agreement with previous studies (13,14). In one study, rats exposed to 0.5% and 1% lead acetate for 90 days, showed a significant reduction in body weight at the termination of experiment (15), but the lead concentration and duration of experiment was greater than this study. In our experiment, rats were exposed to three concentrations of lead acetate in drinking water for 28 days, causing mean blood lead levels of (26.84± 2.23, 43.12± 2.40, 52.8± 3.44 μg/dl), in treated groups by lead acetate 100, 500 and 1000ppm respectively. Low levels of lead exposure of about 100 ppm is similar to the exposure levels seen in environment. Exposure to comparable concentrations greater than 500 ppm of lead is to industrial level exposure (13). It has been reported that increase in blood pressure of rats occurs at low lead exposure levels of 0.1 to 100ppm and exposure to industrial levels does not lead to hypertension (13). This is in contrast to our data whereby 500 ppm and 1000 ppm lead acetate increased blood pressure. It is not surprising that the results have been inconsistent, because there are differences in animal races, species, routes of administration of lead acetate, duration of experiments, and the methodology for measuring blood pressure, in particular, whether or not the animals were anesthetized. Our data also showed that there is not any dose-dependent correlation between blood pressure and blood lead concentrations. Some surveys also conclude that there is no positive relationship between blood lead concentration and blood pressure, even after adjustment for confounding factors (16,17). In the present study we used sodium acetate as control and it increased blood pressure after 28 days when compared with distilled water. This effect is probably due to sodium (18). Dimercaptosuccinine acid (DMSA) an oxygen radical scavenger and lead chelating activity reversed the blood pressure elevating effects of lead acetate. This supports the idea that oxygen radicals may have a pathological role in certain forms of hypertension including those caused by lead. So it is postulated that lead but not acetate, can cause the generation of oxygen radicals and hypertension (19).

Acknowledgments

This work was supported by a grant from Deputy of the Research, Tehran University of Medical Sciences.

REFERENCES

1. Blazka M. E., Harry G. and Luster M. Effect of lead acetate on nitrite production by murine brain endothelial cell culture. Toxicol. Appl. pharmacol. 126: 191-194; 1994.

2. Tian L. and Lawrence A.D. Lead inhibits nitric oxide production in vitro by mutine splenic macrophages. Toxicol. Appl. Pharmacol. 132: 156-163; 1995.

3. Kaji T., Yamamoto C. and Sakamoto M. Effect of lead on the glycosaminoglycans metabolism of bovine aortic endothelial cells in culture. Toxicology. 68: 249-257; 1991.

4. Sharp D.S., Becher C.E. and Smith R.S. Chronic low level lead exposure: Its role in the pathogenesis of hypertension. Med. Toxicol. 2: 210-232; 1987.

5. Fine P.B., Vetrano T., Skurinick J. and Ty A. Blood pressure elevation in young dogs during low level lead exposure. Toxicol. Appl. Pharmacol. 93: 388-393; 1988.

6.Cramer, K., and Dahlberg, L. Incidence of hypertension: A follow up study over 20 years. Br. J. Ind. Med. 23: 101-104; 1966.

7. Cardozo D.S., Colacciopo S., Dal Bo C.M.R. and Guinaaim N.A. Occupational exposure to lead, kidney function tests and blood pressure. Am. J. Ind. Med. 26: 635-643; 1994.

8. Beevers D.G., Erskine E., Robertson M., Campbell B.C., Goldberg A. and Hawthorne V.M. Blood lead and hypertension. Lancet. 2: 1-3; 1976.

9. Purdy R.E., Smith J.R., Ding Y., Oveisi F. and Vaziri N.D. Lead induced hyperetension is not associated with altered vascular reactivity in vitro. Am. J. Hypertens. 10: 997-1003; 1997.

10. Abdollahi M., Dehpour A.R. and Fooladgar M. Alteration of rat submandibulary gland secretion of protein, calcium and N-acetyl - β - D-glucosaminidase activity by lead. Gen. Pharmac. 29: 675-68; 1997.

11. Zinterhofer L.J.M., Jatlow P.I. and Fappiano J. Atomic absorption determination of lead in blood and urine in the presence of EDTA. J. Lab. Clin. Med. 78: 664; 1971.

12. Carmichael N.G., Winder C. and Lewis P. Dose response relationship during perinatal lead administration in the rat: A model for the study of lead effects on brain development. Toxicology. 21: 117-128; 1981.

13. Gonick C.H., Ding Y., Bondy C.S., Ni Z. and Vaziri, D.N. Lead induced hypertension. Interplay of nitric oxide and reactive oxygen species. Hypertension. 30: 1487-1492; 1997.

14. Webb R.C., Winquist R.J. and Vander A. In vivo and in vitro effects of lead on vascular reactivity in rats. Am. J. Physiol. 10: 211-216; 1981.

15. Lal B., Murthy R.C., Anand M., Kumar R., Chanfra S.V., Tripathi O. and Srimal R.C. Cardiotoxicity and hypertension in rats after oral lead exposure. Drug. Chem. Toxicol. 14: 305-318. 1991.

16. Dolene P. Staessen JA, Lauwerys RR. and Amery A. Low level lead exposure does not increase the blood pressure in general population. J. Hypertens. 11: 589-593; 1993.

17. Staessen J., Yeoman W.B. Fletcher A.E., Marmot, M.G. Shipley M.J. and bulpit, C.J. Blood lead concentration, renal function, and blood pressure in London civil servants. Br. J. Ind. Med. 47: 442-447; 1990.

18. Nurminen M.L., Korpela R. and Vapaatalo H. Dietary factors in the pathogenesis and treatment of hypertension. Ann. Med. 30: 143-150; 1998.

19. Khalil-Manesh F., Gonick H.C., Prins B., Weber M.A., Purdy, R. and Ren, Q. Effect of chelation treatment with (DMSA) on lead related blood pressure changes. Environ. Res. 65: 86-99; 1994.