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EFFECT OF CHRONIC EXPOSURE TO LOW LEVELS OF LEAD ON RENAL FUNCTION AND RENAL ULTRASTRUCTURE IN SD RATS

[1] I. P.ODIGIE, [1] C.O. LADIPO, [1] R.R. ETTARH and [2] M.C. IZEGBU

[1]Department of Physiology, College of Medicine, University of Lagos, PMB 12003, Lagos Nigeria.
[2]Department of Morbid Anatomy, Lagos state University, College of Medicine, Ikeja Lagos Nigeria
***Email**: ipodigie@yahoo.com **Tel.** +234-803-7230-556*

Summary: Chronic exposure to lead is associated with adverse effects on renal function in laboratory animals and man. There is controversy concerning the direction of change of renal function parameters following chronic lead intoxication. The renal effects of low-dose lead exposure, as opposed to acute and pharmacological doses, require further scrutiny. In order to determine the effect of prolonged low-dose lead exposure, male Sprague Dawley rats (initial weight, 150.5±17.4g; final weight, 300.5±17.4g; n=10) administered lead acetate (100 p.p.m.) in drinking water over a period of 3 months, were investigated for renal function parameters. Treated rats had elevated blood pressures (114.4±4.2 vs. 95.7±3.5 mmHg; P<0.01). There was no significant difference in renal blood flow (3.99±0.28 vs. 4.19±0.63 ml/min; P=NS). GFR was comparable in the two groups (0.77±0.05 vs. 0.88±0.06 ml/min; P=NS). No significant difference in serum creatinine was observed (57.8±7.5 vs. 49.0±2.7 μmol/l; P=NS). Renal histology showed minimal interstitial changes in the experimental group. Interestingly, significant sodium (P<0.01); potassium (P<0.05) and chloride (P<0.05) retention were observed in the lead treated rats. Elevation of blood pressure occurred at a stage when low-level lead exposure did not alter renal function parameters appreciably. These results suggest that hypertension may be a forerunner of significant renal damage following chronic exposure to low-levels of lead and further underscore fluid and electrolyte retention as a significant mechanism responsible for elevated blood pressure in the chronic stages of lead exposure.

***Key Words**: Low-level lead, Renal function, Hypertension, Renal ultrastructure.*

Introduction

Environmental lead poisoning is an increasing health burden and chronic exposure to high levels of lead leads to adverse effects on renal function and the hematopoietic system in both animals and humans (Hotz *et al*., 1996). Lead-induced renal damage also occurs in the absence of acute intoxication so that occult lead nephropathy may not be recognized as such (Restek-Samarzija *et al*., 1997). Delay in the onset of adverse effect of lead poisoning on kidney function and blood pressure elevation are known to occur in previous lead workers (Restek-Samarzija *et al*., 1996, Restek-Samarzija & Momcilovic, 1992). Chronic accumulation of lead in the body eventually leads to impairment in renal function (Restek-Samarzija *et al*., 1996).

There is no uniformity on the direction of change of renal function parameters in chronic lead poisoning. Some studies reported a stimulatory effect of lead on renal hypertrophy and glomerular filtration rate (GFR) during lead exposure (Khalil-Manesh *et al.*, 1994) while others reported a significantly reduced glomerular filtration rate, adjusted for age (Restek-Samarzija & Momcilovic, 1992).

In animal studies, acute low-dose lead-treatment caused no significant pathological changes in rats (Khalil-Manesh *et al*., 1992). The disparity in the onset of renal function abnormalities reported in laboratory animals appear to be related to the duration and dose of lead administration. Thus Khalil-Manesh *et al.*, (1992) found no functional or pathological changes in rats when lead exposure was discontinued after 1 month. Exposure beyond 6 months led to severe tubulointerstitial disease which resulted in significantly decreased GFR with attendant increase in serum creatinine and urea nitrogen (Khalil-Manesh *et al*., 1992).

The hypothesis advanced by some workers suggesting that a high dose of lead stimulates renal cortical hypertrophy and increases GFR in the early stages which later results in a fall in GFR as a result of tubulointerstitial changes (Khalil-Manesh *et al* 1992) requires further clarification. Most

studies on the effect of lead on renal function parameters investigated high doses of lead (Restek-Samarzija *et al*., 1997; Khalil-Manesh *et al*., 1994; Restek-Samarzija *et al.,* 1992; Khalil-Manesh *et al.,* 1992; Tejani *et al.,* 1986; Aviv *et al*., 1980). The effect of exposure to low levels of lead over a prolonged period of time on renal function as opposed to acute and pharmacological exposures requires closer scrutiny.

Renal function parameters were therefore investigated in male Sprague Dawley (SD) rats exposed to low levels (100 p.p.m.) of lead acetate in drinking water over a period of 3 months in order to determine the effect of prolonged low-level lead exposure on renal function parameters.

Materials and Methods

Animal Preparation

Male SD rats which were used in the study, weighed 150.5±17.4 g (n=10) at the beginning of the experiment. The room in which the rats were kept was maintained at a light / dark cycle of 12 hours, a temperature of 28 ^{o}C to 32 ^{o}C and a relative humidity of about 60 %. The rats were allowed free access to normal rat chow. Treated rats received drinking water containing 100 parts per million (p.p.m.) of lead acetate provided in lick-proof water bottles for 3 months (Ding *et al.,* 1998) while control rats received tap water. This level of lead exposure is known to cause significant elevation of serum lead levels in the rat (Carmignani *et al*, 2000). The animals received standard institutional care throughout the period of observation.

Clearance studies

At the end of the observation period (3 months), rats were subjected to standard clearance experiments in order to establish whether or not there were significant changes in renal function parameters in the treated rats compared to controls. The animals were weighed and anaesthetized with a mixture of 1% (w/v) alpha-chloralose and 25% (w/v) urethane in normal saline at a dose of 5ml/kg intraperitonially. It has previously been reported that this preparation does not alter resting blood pressure, heart rate and cardiovascular responses for at least 3 hours after anesthesia (Adigun, 1986). A tracheostomy was performed to guarantee spontaneous breathing. A polyethylene catheter (PP50) was introduced into the left carotid artery for continuous blood pressure recording. The arterial catheter was connected to a blood pressure transducer (P23D Statham, Hato Rey, Puerto Rico) and blood pressure was recorded on a Grass Polygraph Model 7D (Grass Instruments Co., Quincy Mass. U.S.A.). Through the cannulated right femoral vein (PP50 polyethylene catheter), the rats received sterile physiological saline solution at the rate of 10 l/g.rat/hour using a digital infusion pump. The infused solution contained 154mM Na^{+} and 154mM Cl^{-}. A priming dose of inulin (16mg/kg) and para-Aminohippurate (PAH) (8mg/kg) was followed by an infusion of sterile NaCl solution containing inulin (36mg/ml) and PAH (5.8mg/ml) infused at a rate of 10lμ/g.rat/hr until steady state was reached (Fischer *et al*., 2000; Gabel *et al.,* 1996). Through a small lower abdominal incision, the urinary bladder was cannulated with a short self-retaining catheter (PP100 polyethylene tubing). The urethra was legated to guard against spontaneous voiding. The abdominal incision was closed with a ligature, which also served the purpose of fixing the bladder catheter in place. The rectal temperature of the rat was maintained at 37.0 ± 0.5^{o}C throughout the experiment.

After an equilibration period of 90 min, constancy in urine flow rate was tested. It was assumed that the rats have reached an equilibrium status when two 30 min. urine collection period yielded constant volume. Urine samples were thereafter collected in pre-weighed vials for two 30 minutes periods. Arterial blood pressure was noted on the polygraph recording and heart rate was calculated against the timer recordings of the polygraph.

Terminal blood samples were collected in heparinized bottles and blood plasma was immediately separated by centrifugation (3000 r.p.m. for 10 mins). Plasma was stored in a deep freezer until required. Plasma electrolytes and plasma creatinine were measured within 48 hours. At the end of the experiments, the body weight of the rats were compared with their initial weight and the heart and the kidneys (decapsulated in vivo) were excised and weighed immediately on a weighing balance after blotting with a commercial blotting paper.

Analytical Methods

Urine volume was determined gravimetrically in pre-weighed vials (Sardstadt) without correcting for specific gravity. Sodium and Potassium concentration in plasma and urine were determined using flame photometry and chloride was measured electrometrically.

Creatinine was determined using the Jaffe's reaction according to Popper *et al*., (1937). The glomerular filtration rate was

determined using inulin clearance according to Führ *et al*., (1955). In order to avoid overestimation of GFR that occurs when results are divided by body weight (Pollock *et al.,* 1991), functional renal data in this study are given as absolute values. The renal plasma flow (RPF) was determined using the clearance of para-aminohippurate (PAH) according to Smith *et al*., (1945). A PAH extraction ratio of 0.9 was assumed.

The American Physiological Society's guidelines for experimental animal research were adhered to in all of the experiments.

Statistical Analysis

Data analysis was done using a computer programme (SPSS 7.5 for windows). Results are given as means ± standard error of the means (SEM). The evaluation of data for statistical significance between control and experimental rats was done using unpaired Student-Newman-Keuls test. A value of $P<0.05$ was accepted as statistically significant.

Results

Lead treated rats developed significant elevation of systolic ($P<0.05$), diastolic ($P<0.05$) and mean arterial blood pressures ($P<0.01$) (table 1). The body weights of the treated rats were not significantly different from controls (table 1). Hematocrit values were comparable in both groups of rats (table 1). No significant difference in heart rate was observed under anesthesia in treated rats compared to controls (table 1).

Combined kidney urine flow rate was not significantly different in the treated rats compared to controls (6.8±1.2 vs. 5.8±0.7 µl/min). Similarly, renal blood flow (RBF) (3.99±0.28 ml/min vs. 4.19±0.63 ml/min) and glomerular filtration rate (GFR) (0.77±0.05 ml/min vs. 0.88±0.06 ml/min) were not significantly different in treated rats compared to controls. No significant difference in serum creatinine concentration was observed (57.8±7.5 µmol/l vs. 49.0±2.7 µmol/l). Cardiac weight was not different in treated rats compared to controls (0.72±0.05g vs. 0.73±0.08g). Similarly, kidney weights were comparable in the two groups (table 1).

Table 1: *Summary of Renal function parameters and organ and body weights in lead treated (100ppm) and control rats.*

P-values are lead treated rats vs. untreated controls. SBP=systolic blood pressure; DBP=diasolic blood pressure; MAP=mean arterial blood pressure; RBF=renal blood flow; GFR=glomerular filtration rate; Rt=right; Lt=left

	Lead	Control	P-Values
Body Weight (g)	300.0±20.2	301.0±30.9	P=NS
SBP (mmHg)	137.2±7.3	111.2±4.6	P<0.05
DBP (mmHg)	103.0±3.0	88.0±3.6	P<0.05
MAP (mmHg)	114.4±4.2	95.7±3.5	P<0.01
Hematocrit (%)	53.9±1.9	50.6±0.9	P=NS
Urine flow rate V (µl/min)	6.8±1.2	5.8±0.7	P=NS
RBF (ml/min)	3.99±0.28	4.19±0.63	P=NS
GFR (ml/min)	0.77±0.05	0.88±0.06	P=NS
Heart Rate (beats / min)	421.6±11.6	402.0±2.6	P=NS
Heart weight (g)	0.72±0.05	0.73±0.08	P=NS
Serum Creatinine ((µmol/l)	57.8±7.5	49.0±2.7	P=NS
Plasma Sodium (mmol/l)	148.9±1.3	143.5±0.7	P<0.01
Plasma Potassium (mmol/l)	4.57±0.27	3.66±0.18	P<0.05
Plasma Chloride (mmol/l)	102.4±1.1	97.0±1.7	P<0.05
Rt Kidney weight (g)	0.57±0.05	0.62±0.10	P=NS
Lt Kidney weight (g)	0.57±0.06	0.63±0.08	P=NS

Compared to control rats, lead treated rats had significant elevation of plasma sodium (148.9±1.3 mmol/l vs. 143.5±0.7 mmol/l; $P<0.01$), potassium (4.57±0.27 mmol/l vs. 3.66±0.18 mmol/l; $P<0.05$) and chloride (102.4±1.1 vs. 97.0±1.7 mmol/l; $P<0.05$) concentrations (Table 1). Thus chronic exposure (3 months) to low levels of lead resulted in electrolyte retention.

Discussion

The main finding in this study is that chronic exposure to low-levels of lead resulted in electrolyte retention and elevation of blood pressure occurred at a stage when low level lead exposure did not alter renal function parameters appreciably. There is overwhelming evidence in clinical studies to show that chronic, recurrent lead poisoning by causing increasing body lead load leads to impairment in renal function parameters (Restek-Samarzija *et al*., 1996). Clinical and experimental studies suggest an association between low-level lead exposure and hypertension (Fine *et al*., 1988; Vaziri *et al*., 1999), which persists during ongoing exposure (Fine *et al*., 1988).

Delayed adverse effect of occupational lead poisoning on kidney function and blood pressure are known to occur in humans (Restek-Samarzija *et al*., 1997). Among the possible mechanisms discussed in the pathogenesis of lead-induced hypertension is endothelial dysfunction, which occurs as a result of lead-induced increase in free radical production that scavenges endothelial nitric oxide (NO), leading to vascular hypertonus (Vaziri *et al*., 1999). Undoubtedly, the renal vascular endothelium is under strong influence of NO (Navar *et al*., 1996; Beierwaltes et al., 1992).

Our findings that low-level lead exposure cause an early increase in blood pressure is in agreement with those of others (Fine *et al*., 1988). Although limited exposure to lead can result in progressive renal insufficiency and hypertension (Aviv *et al*., 1980), arterial hypertension is more strongly associated with renal dysfunction in lead workers (Pinto de Almeida *et al*., 1989). In the first 4 weeks, when chronic lead exposure did not alter renal functions or extracellular fluid volume, an associated increase in the activity of the renin-angiotensin system was reported in the dog; suggesting that hypertension may be a forerunner of renal impairment in low-level lead intoxication (Fine *et al*., 1988). In the present study, we found electrolyte retention after 3 months of exposure to low-levels of lead in the rat. This suggests that fluid retention may be an additional mechanism responsible for lead-induced hypertension in the chronic stages of exposures to low levels of lead.

Normally, acute oral protein load causes a transient hyperfiltration that can reveal a loss of glomerular permselectivity properties in the early stages of renal disease (Hotz *et al*., 1996). However, test of acute protein loading was not found useful in revealing a silent glomerular filtration disturbance in lead exposed workers even though this procedure was useful in disclosing imminent renal tubular dysfunction in cadmium workers not yet showing increased microproteinuria under baseline conditions (Hotz *et al*., 1996). These evidences suggest that in the pathogenesis of low-level lead-induced renal damage, the kidney may very well be a victim of elevated blood pressure rather than the culprit. However, contradictory reports on the effect of lead on renal function parameters exist in the literature. Some studies reported a positive correlation between blood lead levels and GFR in rats; an observation that could be a reflection of the stimulatory effect of lead on renal hypertrophy and GFR (Khalil-Manesh *et al*., 1994). However, these workers administered pharmacological doses of lead. In factory workers exposed to lead poisoning, a significantly reduced glomerular filtration rate, adjusted for age was reported (Restek-Samarzija and Momcilovic , 1992). These observations were at variance with those from studies in which sustained low-dose of lead, as reported herein, were used. Prolonged low-dose lead-treatment or acute high-dose lead-treated that was discontinued after one month, failed to cause significant pathological changes in renal untrastructure in the rat (Khalil-Manesh *et al*., 1992). In addition, acute lead exposure had no effect on GFR or plasma aldosterone concentrations (Powers and Foulkes, 1985). Interestingly, prolonged exposure beyond 6 or 9 months not only resulted in severe tubulointerstitial disease but also caused a decreased GFR and an increase in serum creatinine and urea nitrogen compared to controls (Khalil-Manesh *et al*., 1992; Khalil-Manesh *et al*., 1994). Thus as opposed to low-dose lead exposure, prolonged exposure to high dose of lead in rats may initially stimulate both renal cortical hypertrophy and an increase in GFR which later results in a fall in GFR as a result of predominant adverse effects of lead on the tubulointerstitium (Khalil-Manesh *et al*., 1992). In addition, age at the time of exposure appears to be critical to the development of progressive renal damage (Tejani *et al*., 1986).

Although nephrotoxicity is a common finding following exposure to lead, the dose-response relationship in adults with occupational lead exposure and following chronic environmental pollution is not well established and information is lacking on the early nephrotoxic effects of lead in humans. By the time serum urea nitrogen and creatinine levels are elevated, renal damage may well be

advanced (Hong *et al*., 1980). In addition, routine clinical laboratory tests are insensitive for the detection of early renal effects of lead poisoning.

In conclusion, there is urgent need for further studies on the effect of environmental lead pollution on the kidneys and other body systems in our environment. It cannot be overemphasized that we may well be underestimating the effect of environmental lead pollution in our major cities in the developing countries.

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