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Renin angiotensin system blockade ameliorates lead nephropathy

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ABSTRACT

Lead intoxication is usually insidious and may cause a variety of complications such as kidney damage and hypertension. The role of intrarenal renin-angiotensin system (RAS) in lead-induced nephropathy has not been investigated. Adult male Sprague-Dawley rats were fed with water containing 250 ppm of lead acetate (lead group) and deionized water (control group) for 4 weeks. Another two groups started to receive intraperitoneal captopril (50 mg/kg/d) or losartan (10 mg/kg/d) after 2 weeks of lead feeding and continued for another 2 weeks. Immunoblotting was used to analyze the protein amount of intrarenal RAS components and transforming growth factor-beta (TGF-β). Compared with control group, lead exposure resulted in increased proteinuria after 2-week treatment (4.2 ± 0.9 mg/100 g *vs.* 1.8 ± 0.8 mg/100 g, $p < 0.05$) and 4-week (5.2 ± 1.7 mg/100 g, $p < 0.05$). Serum creatinine level was increased (0.40 ± 0.2 *vs.* 0.3 ±.04 mg/dL, $p < 0.05$) and calculated glomerular filtration rate (GFR) was decreased (2.68 ± 1.03 *vs.* 3.37 ± 0.11 mL/min, $p < 0.05$). Intrarenal angiotensin converting enzyme (ACE), angiotensin II (ANG II), angiotensin II type 1 receptor (AT1R) and transforming growth factor–beta (TGF-β) were upregulated in lead group. Captopril and losartan administration reduced proteinuria significantly (3.0 ± 0.50 mg/100 g of captopril and 2.7 ± 0.4 mg/100 g of losartan group) and lowered systolic blood pressure when compared with lead group. Furthermore, serum creatinine levels and GFR were improved by RAS blockade. Captopril treatment significantly reduced protein abundance of ACE, ANG II, AT1R and TGF-β. Losartan treatment also decreased ANG II and TGF-β. We concluded that lead exposure elicited intrarenal RAS activation with associated proteinuria and impaired renal function. RAS blockade was effective in alleviating lead-associated kidney injury and lowering blood pressure.

1. Introduction

Exposure to environmental and occupational nephrotoxicants is one of the important causes of kidney injury. Among various heavy metals, lead is historically recognized as a representative etiology of chronic kidney disease. Lead-based paint is the major source of intoxication but lead can also be found in drinking water, food containers and herbal remedies. Entry of lead into body is mainly through respiratory and gastrointestinal tracts [1]. In adults, approximately 90% of lead is stored in the bone [2]. There is a slow release from the bone with an estimated half life for 20–30 years [3], indicating an extended and chronic deleterious effect of lead on health.

Even at low level of lead exposure results in tubulointerstitial injury of the kidney [4]. Direct injury of proximal tubular cells with intranuclear inclusion formation has been described as the characteristic of lead nephropathy [5]. Increased oxidative stress was considered as the underlying mechanism of lead-induced kidney [6]. The role of renin angiotensin system (RAS) in lead-induced nephropathy and hypertension is still controversial. Most of the previous studies investigated plasma concentration [7,8] rather than intrarenal RAS which ultimately determines the kidney injury. Abnormal activation of RAS elicits inflammatory cascade and aggravates the process of injury [9].

In the present study, we aimed to investigate the effects of chronic lead exposure on the kidney in terms of proteinuria, renal function and intrarenal RAS. We also evaluated the effects of RAS blockade on lead-induced nephropathy.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighting 180–200 g were used for this experiment. The animals were maintained under a

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constant 12 h photoperiod at temperatures between 21 and 23 °C. The animals were allowed free access to food and water. The food contained sodium (0.33%), calcium (1.0%), and magnesium (0.16%). The animals were divided into four groups: control group, lead, captopril and losartan group. The lead group was fed with drinking water containing 250 ppm of lead acetate ($n = 12$) for 4 weeks whereas the control group were given deionized water ($n = 12$). The other two groups were administrated with captopril (50 mg/kg/d, $n = 12$) or losartan (10 mg/kg/d, $n = 12$) after 2 weeks of lead-containing water feeding. RAS blockade treatment were then continued for another 2 weeks in along with lead exposure. Body weight was measured weekly until end of study. At the end of study, 24 h urine samples were collected from the animals using individualized metabolic cages. The rats were then sacrificed and blood samples were withdrawn from the inferior vena cava for biochemical analysis. Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Kaohsiung Chang-Gung Memorial Hospital, and all animal procedures were performed according to the IACUC policy.

2.2. Biochemical assay and blood pressure measurement

Serum and urinary creatinine and uric acid levels were measured by using the SYNCHRON CX DELTA system [Beckman, Fullerton, CA, USA] according to manufacturer's operating protocol. Whole blood lead level was determined by Atomic Absorption Spectroscopy (GBC Scientific Equipment, Australia). Twenty-four hours urinary excretion of protein was presented as mg per 100 g of body weight. Urinary uric acid excretion was calculated as ratio of uric acid divided by creatinine (mg/mg). Blood pressure was measured by indirect tailcuff method (Visitech BP2000, Visitech Systems, Apex, NC, USA). Data of three consecutive measurements were recorded and then averaged for each animal.

2.3. Immunoblotting study

The protocol and procedure of immunoblotting study were described as in our previous study [10]. In brief, kidney cortical sections were frozen at −80 °C and then homogenized at 4 °C in protein lysis buffer solution, containing 20 mM Tris–HCl (pH 7.4), 0.1% sodium dodecyl sulfate, 5 m M ethylenediamine tetraacetate, 1% Triton X-100, and a protease inhibitor cocktail tablet (Roche, Penzberg, Germany). After centrifugation at 12,000*g* for 10 min at 4 °C, the protein concentrations were determined using bicinchoninic acid protein assays. The protein samples were then run on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis for angiotensin converting enzyme (ACE), angiotensin II (ANG II), angiotensin II type 1 receptor (AT1R) and TGF-β. They were then transferred to nitrocellulose membranes. β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as the internal control in this study. After washing and blocking were completed, the membrane was incubated with primary antibody of the target molecule [ACE: 1:300; Santa Cruz Biotechnology, Santa Cruz, Calif., USA; ANG II: 1:1000; Phoenix, Pharmaceuticals, Belmont, Calif., USA; AT1R: 1:1000; Santa Cruz Biotechnology, Santa Cruz, Calif., USA; TGF-β: 1:200; Santa Cruz Biotechnology, Santa Cruz, Calif., USA. β-Actin: 1:3000; Sigma Chemical Co., St. Louis, Mo., USA; GAPDH: 1:10,000, Millipore Corporation, Billerica, MA., USA) for 16 h. Further washing in 5% blocking milk was performed and the membrane was incubated with secondary antibody for 3 min. The dilution and preparation of secondary antibodies included β-Actin (1:10,000), GADPH (1:10,000), ACE (1:1000), AT1R (1:2000, peroxidase-conjugated AffiniPure goat anti-mouse IgG, all purchased from Jackson ImmunoResearch Laboratories Inc., West Grove Pa., USA) and ANG II (1:7000), TGF-β (1:8000, anti-rabbit IgG, horseradish peroxidase-linked antibody, all purchased from Cell Signaling Technology, Inc., Danvers, Mass., USA). The abundance of studied protein was then quantified by densitometric analyses. The changes in protein abundance were presented as percentages of control animal values.

2.4. Data evaluation

The biochemical values are expressed as means ± standard deviation (SD). Protein abundance is shown as means ± standard error of mean (SEM). Comparison among different groups was performed by using one way ANOVA and then examined by LSD post hoc test. A p value < 0.05 is considered statistically significant.

3. Results

Table 1 presents the body weight, daily urine amount, whole blood lead level and biochemical data of four group animals at end of experiment. The body weight was comparable but urine amount was increased in all lead exposure groups ($p < 0.05$). In lead-treated rats, whole blood lead concentration was elevated significantly than that of control group ($p < 0.005$). Serum uric acid levels were similar of all groups but urinary excretion of uric acid was significantly reduced in all lead exposed groups, especially in lead group. A significant increase of serum creatinine was found in lead group (0.40 ± 0.17 *vs.* 0.27 ± 0.04 mg/dL, $p < 0.05$) and no significant difference was noted between the two RAS blockade treatment groups and control animals.

After feeding with lead for 4 weeks, systolic blood pressure was higher than control animals (132.7 ± 6.5 *vs.* 122.0 ± 9.2 mm Hg, $p < 0.05$, Fig. 1). In RAS blockade groups, blood pressure was significantly lower than the lead group and did not differ with control animals (captopril: 126.4 ± 6.5 mm Hg; losartan: 120.9 ± 6.6 mm Hg). Daily urinary protein excretion was increased significantly after lead exposure for 2- and 4 weeks (4.17 ± 0.87 mg/100 g and 5.19 ± 1.70 mg/100 g, Fig. 2A). For the calculated GFR, a decrease of 21% in the lead group was noted compared to control group (3.37 ± 0.11 *vs.* 2.68 ± 0.23 mL/min, $p < 0.05$, Fig. 2B). We found GFR was not affected in groups treated with RAS blockade when compared to control group (captopril: 4.24 ± 0.80 mL/min; losartan: 4.46 ± 0.94 mL/min, $p > 0.05$).

A 2-fold increase in the abundance of ACE was noted than that of control group in lead group (Fig. 3A, $p < 0.05$). In captopril treatment group, abundance of ACE was diminished significantly comparing with lead group (137.2 ± 10.2 *vs.* 235.4 ± 28.2% of control, $p < 0.05$). In losartan treatment group, there was no significant difference (283.6 ± 39.4 *vs.* 235.4 ± 28.2% of control, $p > 0.05$). Lead exposure was associated with a significant increase in ANG II (178.8 ± 7.6% of control, $p < 0.05$). Administration of RAS blockade reduced ANG II abundance significantly comparing with lead group (captopril: 95.2 ± 3.0% of control; losartan: 129.4 ± 10.8% of control, both $p < 0.05$). Significant increase in AT1R was noted in lead group (173.1 ± 8.9% of control, $p < 0.05$). Captopril treatment reduced AT1R (67.1 ± 12.3% of control, $p < 0.05$) when compared with lead group. Losartan treatment did not affect the lead-treatment result (149.7 ± 19.7%, $p > 0.05$).

Lead treatment induced increased renal abundance of TGF-β (145.3 ± 7.6% of control, $p < 0.05$). With addition of RAS blockade, the amount was reduced when compared with lead group (captopril: 76.7 ± 10.5% of control; losartan: 108.6 ± 7.9% of control *vs.* 145.3 ± 7.6% of control, both $p < 0.05$).

4. Discussion

Our study clearly demonstrated that subacute lead exposure was associated with increased blood pressure, diuresis, proteinuria and