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Chronic Exposure to Low Doses of Lead Results in Renal Infiltration of Immune Cells, NF-κB Activation, and Overexpression of Tubulointerstitial Angiotensin II

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ABSTRACT

Chronic exposure to low doses of lead results in generation of reactive oxygen species, reduced nitric oxide availability, and arterial hypertension. The present studies were done to define if other conditions associated with oxidative stress, such as renal interstitial inflammation, nuclear factor-κB (NF-κB) activation, and cells expressing angiotensin II, are, in fact, features of low-dose lead exposure. Male Sprague–Dawley rats were randomly assigned to the lead group (*n* = 8) or the control group (*n* = 9). The lead group received 100 ppm lead acetate in the drinking water for 14 weeks. At the end of this period of time, rats were killed under general anesthesia, and the kidneys were harvested for studies. The lead-exposed group presented focal tubulointerstitial damage and highly significant increments in nitrotyrosine immune staining, lymphocyte and macrophage infiltration, angiotensin II-positive cells, and intranuclear positive staining for the p65 DNA-binding subunit of NF-κB in tubulointerstitial cells. Tubulointerstitial inflammation, cells expressing angiotensin II, and NF-κB activation are consequences of a 3-month low-dose exposure to lead and likely play a role in the development of hypertension and chronic lead nephropathy. *Antioxid. Redox Signal.* 7, 1269–1274.

INTRODUCTION

CHRONIC EXPOSURE TO LEAD results in structural and functional changes of the kidneys. The first few months of lead administration are characterized by renal hypertrophy and hyperfiltration that correlate with blood lead levels (16); however, after 6–9 months of high doses of lead in the drinking water (5,000 mg/L lead acetate), there are severe tubulointerstitial inflammation and scarring, and azotemia develops (12). Glomerulosclerosis and interstitial fibrosis are observed after 12 months of high-dose lead exposure (13).

Low-dose lead exposure (100 mg/L in the drinking water) is reported to produce no major renal histological changes until 12 months of exposure, when mild tubular atrophy and interstitial fibrosis are present (14). Nevertheless, low-dose lead exposure, associated with plasma lead levels of ~10–15 μg/dl, maintained for only 12 weeks results in arterial hypertension in experimental animals, a finding that may be relevant to the reported association between hypertension and environmental lead exposure (11, 31).

Studies from several laboratories, including ours, have disclosed aspects of the pathophysiology of lead-induced hypertension. Some investigations have demonstrated increased plasma norepinephrine, reduced β-adrenergic receptor activity, and reduced vasodilatory response in vascular tissue (4, 33). Other studies have shown increased plasma angiotensin-converting enzyme and kininase II activities in association with elevated plasma angiotensin II and reduced bradykinin levels (3). We have explored the role of oxidative stress and showed that reduced nitric oxide (NO) availability resulting

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from sequestration of NO by reactive oxygen species (ROS) plays a key role in the pathogenesis of lead-induced hypertension (9). Several months of lead exposure induce a marked increase in plasma and tissue lipid peroxidation, as shown by increment in malondialdehyde content, and a reduction in stable NO metabolites (34). Increased NO consumption is the result of a high rate of superoxide generation (6, 7) associated with up-regulation of NAD(P) oxidase (37) and compensatory up-regulation of NO synthase expression in the vessels and in the kidney (35). The significance of the role of oxidative stress in the pathogenesis of lead-induced hypertension has been made evident by the demonstration that several antioxidant compounds, such as desmethyltirilazad, dimethylthiourea, superoxide dismutase, tempol, and vitamin E, ameliorate NO consumption and improve or correct of hypertension (5, 34, 35, 37).

Recent studies have emphasized the relationship between oxidative stress, renal infiltration of immune cells, and tubulointerstitial overexpression of angiotensin II in the pathogenesis of hypertension (for review, see 28). First, infiltration of macrophages and lymphocytes in the kidney is a constant feature in experimental models of hypertension (25); second, in many of these models, the intensity of the tubulointerstitial immune infiltrate is correlated with the oxidative stress, with the number of renal tubular cells as well as infiltrating cells expressing angiotensin II, and with the severity of the hypertension (1, 20, 26). Finally, a reduction of the immune infiltrate with immunosuppressive treatment results in a decrease of the renal oxidative stress and angiotensin II-positive cells and in prevention or improvement of the hypertension (1, 19, 20, 26); alternatively, reduction of the oxidative stress by melatonin administration (19) and by an antioxidant-rich diet (27) ameliorates both hypertension and the renal interstitial inflammation.

Little attention has been given to the possibility that interstitial inflammation and renal overexpression of angiotensin II could be present in the early months of low-dose lead exposure, when histology is relatively unaltered but, nevertheless, hypertension and oxidative stress are well established (5, 6, 9, 34, 35, 37). The complex interrelationship between inflammation, generation of ROS, and angiotensin activity makes this possibility likely (24, 32, 38, 39), as it also appears likely that proinflammatory transcription factors would be increased in the early months of lead-induced, ROS-driven hypertension (8, 10, 17, 29, 30).

These combined events in tubulointerstitial areas may not only contribute to the hypertension, which persists many months after lead is stopped (12, 15), but, in addition, constitute a final common pathway toward the irreversible scarring that characterizes severe lead nephropathy. Therefore, the present immunohistological studies were done to explore these aspects and their possible association with oxidative stress, as evaluated by nitrotyrosine abundance.

MATERIALS AND METHODS

Animals

Studies were done in male Sprague–Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN, U.S.A.) with an average mass of 200 g. Animals were housed in climate-controlled animal facilities with 12-h light and dark cycles and fed regular rat chow *ad libitum*. Rats were randomly assigned to the control group (n = 9) or the lead group (n = 8). The latter group was given drinking water containing 100 ppm lead acetate for 14 weeks, and the control group received regular water. At the end of 14 weeks, all the animals were euthanized under general anesthesia (pentobarbital, 50 mg/kg intraperitoneally), and the kidneys were harvested for histochemical and immunohistological studies.

Histology and immunohistology

Paraffin-embedded, 3–4-μm sections fixed in 10% buffered formalin (American Master Tech Scientific, Inc., Lodi, CA, U.S.A.) were used for studies. Light microscopy was used to study renal histology using hematoxylin and eosin, trichrome, and periodic acid Schiff (PAS) stainings. Glomerular sclerosis was evaluated in a minimum of 40 glomeruli (range 40–82) per biopsy using a score described by Raij *et al*. (22). In this score, the individual glomerulus is graded from 0 (normal) to 4+ (>75% sclerosis), and the final glomerular sclerosis index of each biopsy is calculated from these values in percent values so that the score range extends from 0 (normal) to 400 (all glomeruli present a 4+ sclerosis), as described in detail in a previous communication (20). Tubulointerstitial injury was evaluated in the entire cortical and subcortical areas in each biopsy section using a previously described score that ranged from 0 (normal) to 5+ (>75% of the tubulointerstitial areas in cortical and juxtamedullary regions presented infiltration, tubular dilatation, or fibrosis) (20, 23).

Thickness in the afferent arterioles was evaluated by computerized image analysis by the ratio of the external circumference/internal circumference of the medial layer of the afferent arterioles as described in previous communications (1, 20). Eight to 15 afferent arterioles were examined in each biopsy.

Indirect immunohistology was used to evaluate infiltration of lymphocytes (CD5-positive cells) and macrophages (ED1-positive cells) in the kidney by immunoperoxidase methodology as reported in previous investigations (1, 17, 21, 27). Cellular counts in the glomeruli were expressed as positive cells per glomerular cross section and in tubulointerstitial areas as positive cells/mm^2.

Renal nitrotyrosine staining was evaluated with computer image analysis as described in previous communications (1, 17, 20, 25). Positive and negative controls were included with each evaluation Specificity of the nitrotyrosine staining was tested by preabsorption of the antisera with 3-nitrotyrosine, which eliminated the corresponding histological staining.

Intracellular (nuclear) staining for p65 nuclear factor-κB (NF-κB) was done by immunoperoxidase technique as described previously (1, 19, 21).

Antisera

Monoclonal antibodies were used to identify lymphocytes (anti-CD5 clone MRCOX19; Biosource, Camarillo, CA, U.S.A.) and macrophages (anti-ED1; Harlan Bioproducts, Indianapolis, IN, U.S.A.). Secondary rat anti-mouse and donkey anti-rabbit antibodies with minimal cross-reactivity to rat serum proteins were obtained from Accurate Chemical and Scientific Co. (Westbury, NY, U.S.A.). Angiotensin II-positive cells

were identified with rabbit anti-human angiotensin II antisera (Peninsula Laboratories, Belmont, CA, U.S.A.) with cross-reactivity to rat angiotensin II (1, 19, 20, 26, 27). The p65 DNA-binding subunit of NF-κB in the kidney sections was identified with the corresponding antibody (Zymed Laboratories, Inc., San Francisco, CA, U.S.A.) and nitrotyrosine-positive staining was determined using anti-nitrotyrosine monoclonal antibody (Biomol Research Laboratories, Philadelphia, PA, U.S.A.).

Statistical analysis

Differences between groups were evaluated by ANOVA and Tukey post-tests. p values < 0.05 (two-tailed) were considered significant. Values shown are means ± SD.

RESULTS

Earlier studies have demonstrated that administration of 0.01% lead acetate in the drinking water for 12 weeks results in increased blood lead levels and systolic blood pressure in the rats (5–7, 9, 34, 37). Oxidative stress, as evaluated by nitrotyrosine staining was increased sevenfold by lead exposure ($p < 0.001$; Fig. 1).

Light microscopy studies

Light microscopy revealed no sclerotic glomeruli and, in general, normal tubulointerstitial areas. Focal areas of tubular dilatation and tubular cell damage consisting of loss and disruption of brush border without loss of tubular basement membrane were found (Fig. 2). The tubulointerstitial damage score was 1.50 ± 0.53 [$p < 0.001$ versus control (0.44 ± 0.25)]. Focal tubulointerstitial cellular infiltration was occasionally present, but no significant thickening or scarring of tubulointerstitial areas was observed. Afferent arterioles had normal appearance, and the thickness of the media was similar in the lead-exposed group (2.59 ± 0.32 external/internal ratio) and in the control group (2.56 ± 0.27, p = NS).

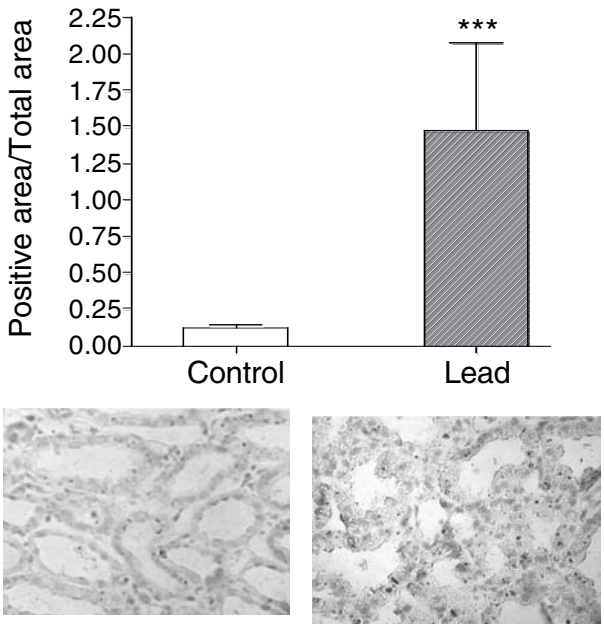

FIG. 1. Significant increment of nitrotyrosine staining, representing increased tyrosine abundance in proximal tubular cells after 14 weeks of lead exposure (*$p < 0.001$ versus control).** Immunoperoxidase staining, ×20.

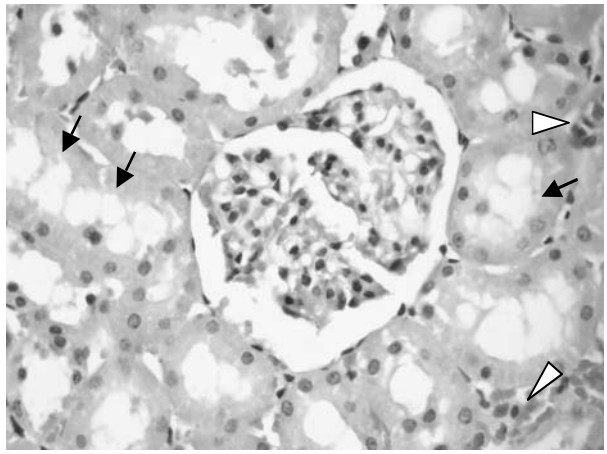

FIG. 2. Light microscopy of a kidney biopsy taken after 14 weeks of low-dose lead exposure (see text). Essentially normal glomeruli with focal loss of brush border and mild alterations in proximal tubular cells (black arrows) and small foci of infiltrating cells (white arrowheads) are seen. PAS staining, ×40.

Interstitial cell infiltration

Lead administration induced interstitial infiltration of lymphocytes ($p < 0.001$; Fig. 3) and macrophages ($p < 0.001$; Fig. 4). Angiotensin-positive cells in tubulointerstitial areas, corresponding to both tubular cells and infiltrating cells, were similarly increased by experimental lead intoxication ($p < 0.001$ versus control group; Fig. 5).

NF-κB

Cells expressing activated NF-κB, as determined by intranuclear positive staining for the p65 DNA-binding subunit, were increased in the tubulointerstitial areas of the lead exposed group ($p < 0.001$ versus control group; Fig. 6). The cells showing intranuclear p65 NF-κB were proximal tubular cells as well as infiltrating cells (Fig. 6).

DISCUSSION

The relationship between oxidative stress and hypertension has been well established in other models of hypertension in which a variety of antioxidant treatments reduce the blood pressure levels (for review, see 28). We selected a period of 14 weeks of low-dose lead exposure because we have demonstrated in previous studies that the administration of 100 ppm lead acetate in the drinking water during this period of time results in significant oxidative stress and hypertension.

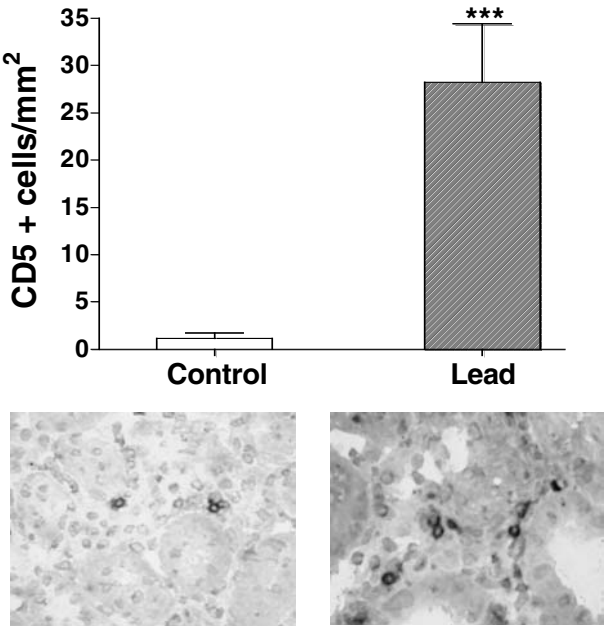

FIG. 3. Tubulointerstitial lymphocyte infiltration, shown as CD5-positive cells, is present in the low-dose lead exposure group (*$p < 0.001$ versus control group).** Immunoperoxidase staining, ×40.

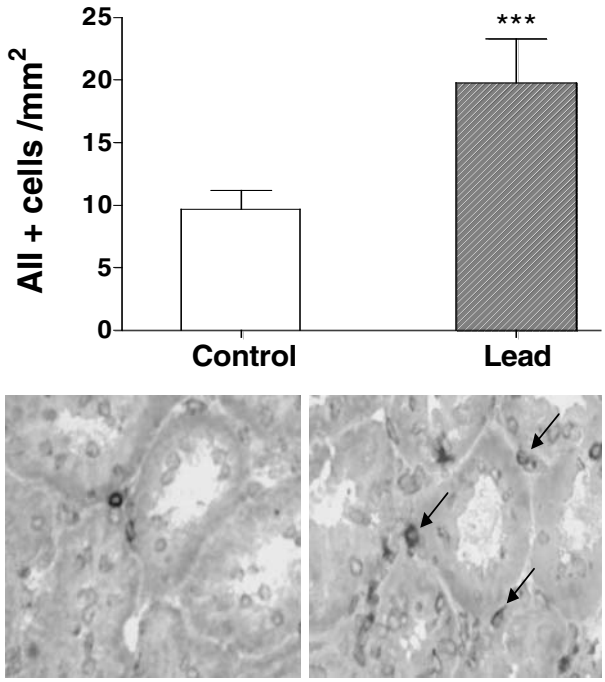

FIG. 5. Angiotensin II-positive cells are increased in tubulointerstitial areas of the lead-exposed group (*$p < 0.001$ versus control).** Most of the angiotensin II (AII)-positive cells in the microphotograph are infiltrating cells (arrows). Immunoperoxidase staining, ×40.

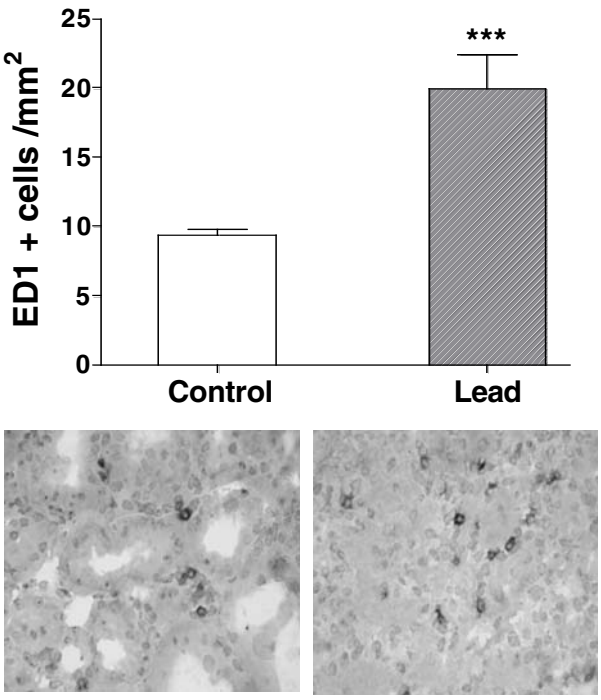

FIG. 4. Macrophage infiltration (ED1-positive cells) in tubulointerstitial areas is significantly increased by 14 weeks of low-dose lead exposure (*$p < 0.001$ versus control group).** Immunoperoxidase staining, ×40.

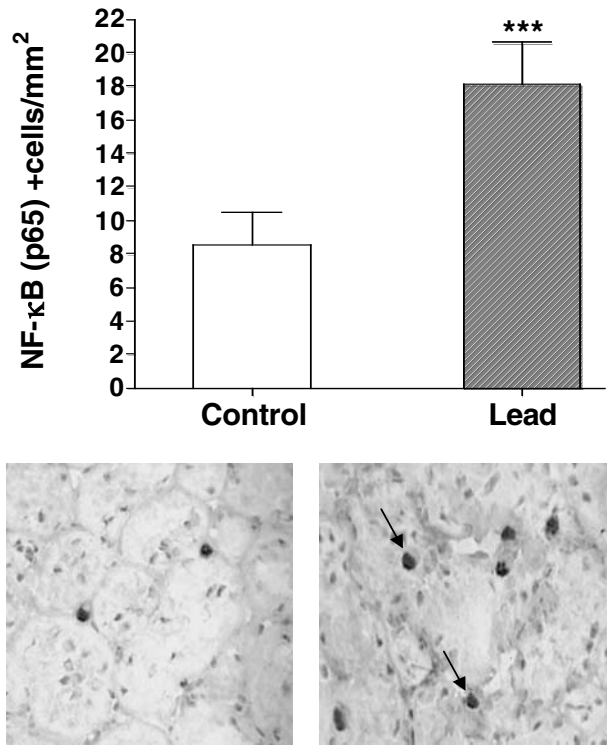

FIG. 6. The number of tubulointerstitial cells showing positive intranuclear staining for NF-κB (p65 subunit) is increased by 14 weeks of low-dose lead exposure (*$p < 0.001$ versus control).** Immunoperoxidase staining, ×40.

The lead-exposed rats used in this study exhibited severalfold increment in nitrotyrosine staining in the proximal tubular cells. This finding is consistent with our earlier investigations that showed that plasma, brain, heart, liver, and kidney nitrotyrosine abundance, determined by western blot analysis, was significantly increased after 12 weeks of lead exposure (36). Increased nitrotyrosine abundance results from the interaction of NO, ROS, and proteins, and its accumulation is an indirect indication of oxidative stress that is mainly due to increased superoxide and hydroxyl radical generation (6, 7).

Light microscopy findings were restricted to focal tubular cell damage and interstitial inflammatory infiltration. The tubulointerstitial damage score was increased in the lead-exposed group, but the findings, in general, were extended to <20% of the tubulointerstitial areas. There was no evidence of interstitial fibrosis or glomerulosclerosis. These findings are in agreement with the reports of Khalil-Manesh *et al*. (12, 16) who found that 9–12 months of lead exposure were required to have evidence of chronic irreversible renal damage.

The central findings of this investigation are the demonstration that, despite nearly normal histological appearance by light microscopy, 4 months of low-dose lead exposure induces infiltration of lymphocytes and macrophages, increased numbers of proximal tubular cells and infiltrating cells showing intranuclear p65 NF-κB, and angiotensin II expression in tubular and infiltrating cells. Likely, these findings are causally related to the oxidative stress and may play a role in the chronic nephropathy that results after 9 months of high-dose lead exposure (12, 13).

The accumulation of lymphocytes and macrophages in the interstitial areas of the kidney is not surprising because interstitial inflammation is a recognized feature of oxidative stress that improves as a result of treatment with antioxidants (27). Another potential cause of cellular infiltration is increased angiotensin II activity. Angiotensin II activity can promote interstitial inflammation not only as a result of its capacity to generate oxidative stress mediated by NAD(P)H oxidase (24), but also because it stimulates the activation of proinflammatory NF-κB transcription factor (8, 29). The latter effect has been shown to be involved in several models of hypertension (1, 21). Intrarenal angiotensin II activity is likely elevated in this model because there is an increment in angiotensin II-positive cells, as it has been shown to be present in other models of hypertension (28). The relationship between increased angiotensin and oxidative stress has been emphasized in double transgenic rats harboring both human renin and angiotensinogen genes in which antioxidant treatment with lipoic acid improves hypertension and angiotensin II-induced end-organ damage (18).

In previous investigations, we have shown that 20–30% of the infiltrating macrophages stain positive for angiotensin II in other experimental models of hypertension (28). Interstitial inflammation, oxidative stress, and intrarenal angiotensin II activity most likely act in concert to promote nephropathy and hypertension with chronic lead exposure. As hypertension induced by lead exposure is sustained for as long as 6 months after lead administration has been stopped (12, 15), it is likely that these features support one another and eventually result in nephron loss, thereby participating in the development and maintenance of hypertension (2).

In summary, a 12-week exposure to lead results in only minor changes in renal histology; nevertheless, it is associated with oxidative stress and interstitial renal inflammation. These features likely play a pathogenetic role in the development of chronic lead nephropathy.

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ABBREVIATIONS

NF-κB, nuclear factor-κB; NO, nitric oxide; PAS, periodic acid Schiff; ROS, reactive oxygen species.

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