Lead, at low levels, accelerates arteriolopathy and tubulointerstitial injury in chronic kidney disease

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Lead treatment was well tolerated and resulted in modest elevations in whole blood lead levels (26.4 ± 4.5 vs. 1 ± 0 μg/dl, week 16, P < 0.001). Lead treatment was associated with higher systolic blood pressure (P < 0.05) and worse renal function (creatinine clearance 1.4 ± 0.4 vs. 1.8 ± 0.5 ml/min, RK+L vs. RK, P < 0.05), and with a tendency for greater proteinuria (6.6 ± 6.1 vs. 3.6 ± 1.5 mg protein/mg creatinine, RK+L vs. RK, P = 0.08). While glomerulosclerosis tended to be worse in lead-treated rats (37.6 ± 11 vs. 28.8 ± 2.3%, RK+L vs. RK, P = 0.06), the most striking finding was the development of worse arteriolar disease (P < 0.05), peritubular capillary loss (P < 0.05), tubulointerstitial damage, and macrophage infiltration (P < 0.05) in association with significantly increased renal expression of monocyte chemoattractant protein-1 mRNA. In conclusion, lead accelerates chronic renal disease, primarily by raising blood pressure and accelerating microvascular and tubulointerstitial injury. arteriolosclerosis; interstitial inflammation; hypertension; uric acid

LEAD EXPOSURE HAS BEEN LONG associated with hypertension (25), arteriolosclerosis (12), kidney disease (14), and gout (14). Epidemiological studies in lead workers have confirmed these associations. For example, in one study, workers exposed to lead had a significantly higher prevalence of hypertension and metabolic syndrome in association with higher whole blood lead levels (81 vs. 11 μg/dl) (3). While lead toxicity was originally considered only in the presence of known sources of exposure, recent epidemiological studies have suggested that even low blood levels of lead can be associated with higher frequencies of hypertension (2, 35), hyperuricemia (11, 22, 38), and chronic kidney disease (7, 11, 28, 48) in the general population. Furthermore, a recent study reported that subjects with elevated body lead burdens and chronic renal disease can have their renal progression slowed if chelation therapy is administered (21). This suggests that even low-level lead exposure may be a significant risk factor for renal progression.

Interestingly, to our knowledge no one has examined the effect of lead ingestion in experimentally induced chronic renal disease, particularly as it relates to effects on the glomerular, vascular, tubular, and interstitial compartments. We therefore report the effect of low-level lead exposure in the rat remnant kidney model. Our primary finding is that lead can induce microvascular, inflammatory, and tubulointerstitial injury that accelerates renal disease.

METHODS

Experimental protocol. All animal protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 175–200 g, were housed in individual metabolic cages and fed a standard diet (Harlan Teklad, Madison, WI). Rats were treated with lead acetate [150 ppm of lead (L) in drinking water, n = 16] for 4 wk, followed by remnant kidney (RK) surgery with continuation of lead for an additional 12 wk; control rats (n = 9) were treated similarly but did not receive lead. Lead treatment was well tolerated and resulted in modest elevations in whole blood lead levels (26.4 ± 4.5 vs. 1 ± 0 μg/dl, week 16, P < 0.001). Lead treatment was associated with higher systolic blood pressure (P < 0.05) and worse renal function (creatinine clearance 1.4 ± 0.4 vs. 1.8 ± 0.5 ml/min, RK+L vs. RK, P < 0.05), and with a tendency for greater proteinuria (6.6 ± 6.1 vs. 3.6 ± 1.5 mg protein/mg creatinine, RK+L vs. RK, P = 0.08). While glomerulosclerosis tended to be worse in lead-treated rats (37.6 ± 11 vs. 28.8 ± 2.3%, RK+L vs. RK, P = 0.06), the most striking finding was the development of worse arteriolar disease (P < 0.05), peritubular capillary loss (P < 0.05), tubulointerstitial damage, and macrophage infiltration (P < 0.05) in association with significantly increased renal expression of monocyte chemoattractant protein-1 mRNA. In conclusion, lead accelerates chronic renal disease, primarily by raising blood pressure and accelerating microvascular and tubulointerstitial injury.

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Table 1. Body weight, systolic blood pressure, serum uric acid, and renal function evaluated at 12 wk after remnant kidney surgery

<table>
<thead>
<tr>
<th></th>
<th>RK</th>
<th>RK+L</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>522±32</td>
<td>490±43.4</td>
<td>0.047</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>159±10</td>
<td>174±21.4</td>
<td>0.028</td>
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<tr>
<td>Whole blood lead, µg/dl</td>
<td>1.0±0.0</td>
<td>2.6±4.5</td>
<td>&lt;0.001</td>
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<tr>
<td>Serum uric acid, mg/dl</td>
<td>2.0±0.2</td>
<td>2.3±0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dl</td>
<td>32±4.17</td>
<td>43±9.95</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.7±0.1</td>
<td>0.8±2.0</td>
<td>0.039</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>1.81±0.5</td>
<td>1.43±0.4</td>
<td>0.041</td>
</tr>
<tr>
<td>Urine protein/creatinine, mg/mg</td>
<td>3.6±1.5</td>
<td>6.6±6.1</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are means ± SD. RK, remnant kidney group; RK+L, remnant kidney group receiving lead.

University of Washington, Seattle, WA), for interstitial fibrosis with goat anti-type III collagen (Southern Biotech, Birmingham, AL), and for peritubular capillary density with rabbit anti-thrombomodulin antibody (gift of Y. Yuzawa, Nagoya University, Nagoya, Japan) (19). Negative controls consisted of omission of the primary antibody or substitution with an irrelevant antibody.

Glomerulosclerosis was defined as the percent area of the glomerular tuft with sclerosis, calculated from a total of 50 glomeruli/animal. Quantification of immunohistochemistry staining was performed by computer imaging using an Axiosplan 2 imaging microscope (Carl Zeiss, Munich, Germany), CR5 digitalized color camera, and Zeiss AutoMeasure software (Axiovision 4.1, Carl Zeiss). For quantification of osteopontin, collagen III, and thrombomodulin staining, single-image frames (700 × 550 mm) were captured at ×100 magnification, and the mean percent positive staining of each scanned area (cortical and medullary regions) was measured (27). The ED-1 staining was scored as the number of positive cells per square millimeter. For the study of vascular morphology, cross sections of periglomerular arterioles were traced and examined morphometrically. Both inner and outer areas (µm²) in a minimum of 15 arterioles/biopsy were measured, and the outer area-inner area (designated as arterial wall thickness) was calculated. Vessels that were not sectioned transversely were excluded (36).

Monocyte chemoattractant protein-1 mRNA analysis. Kidney tissues were frozen in liquid nitrogen for RNA extraction and analysis. Total RNA was isolated using a SV Total RNA Isolation kit (Promega, Madison, WI) according to the manufacturer’s protocol, and the optical density (OD) 260/280-nm ratios were determined. Real-time PCR for monocyte chemoattractant protein-1 (MCP-1) mRNA was performed using an Opticon PCR machine (MJ Research, Waltham, MA), and a SYBR Green master mix kit (Bio-Rad Laboratories) was used for all reactions and corrected for GAPDH expression using MCP-1 and GADPH primers as previously reported (33). The PCR reaction for each kidney sample was performed in duplicate. The MCP-1/GAPDH mRNA ratio was calculated for each sample and expressed as means ± SD (33).

Statistical analysis. Continuous variables between two groups were analyzed using Student’s t-test. Significance between groups was defined as P < 0.05.

RESULTS

Baseline data before start of the experiment and before RK surgery. Both groups had similar body weight and renal function evaluated at the basal time (RK: body wt 183.4 g, BUN 12.4 mg/dl, Cr 0.3 mg/dl vs. RK+L: body wt 183.6 g, BUN 12.1 mg/dl, Cr 0.3 mg/dl) and before RK surgery (RK: body wt 446 g, BUN 15.9 mg/dl, Cr 0.46 mg/dl vs. RK+L: body wt 451 g, BUN 17.1 mg/dl, Cr 0.42 mg/dl).

Low-lead level exposure diminished body weight and aggravated hypertension hyperuricemia and renal dysfunction. Lead treatment was well tolerated, although there was a difference in body weight with control rats at the time of death (Table 1). Whole blood levels obtained at death demonstrated levels considered mildly toxic in humans (26 vs. 1 µg/dl, RK+L vs. RK alone, Table 1). Nevertheless, these levels translated into higher systolic blood pressures (at 8 wk after RK surgery, RK: 148 ± 8.6 vs. RK+L: 163 ± 10 mmHg, P < 0.001 and at 12 wk, RK: 159 ± 10 vs. RK+L: 174 ± 21.4 mmHg, P < 0.05) and higher uric acid levels compared with control rats (Table 1). Renal function (reflected by BUN levels and creatinine clearances) also was worse in the lead-treated rats. Proteinuria tended to be worse compared with controls although this did not reach significance (Table 1).

Arteriopathy and tubulointerstitial damage in rats receiving lead. The RK+L rats demonstrated a higher percentage of segmental sclerosis within glomeruli and a tendency for a higher number of sclerotic glomeruli compared with the RK group (Table 2). However, the more striking findings related to changes in the vasculature and tubulointerstitium (Fig. 1). Lead treatment was associated with significant worsening of preglomerular vascular disease, as characterized by an increase in the media-to-lumen ratio (Table 3, Fig. 2). There was also a loss of peritubular capillaries, as reflected by a reduction in thrombomodulin staining (Table 2, Fig. 3). This was associated with worse tubular injury, as reflected by osteopontin staining, by more interstitial fibrosis (type III collagen staining), and by greater macrophage infiltration in the interstitium (Table 2, Fig. 4). Additionally, the peritubular capillary density significantly correlated with the number of macrophages within the interstitium (r = -0.52, P = 0.02).

MCP-1 expression correlates with macrophage infiltration. The increase in macrophages in the tubulointerstitium in the lead-treated rats was associated with higher renal MCP-1 mRNA (MCP-1 mRNA/GAPDH, RK: 0.11 ± 0.08, RK+L: 0.19 ± 0.11; P = 0.035), and the degree of MCP-1 expression in individual biopsies positively correlated with the number of macrophages (Fig. 5).

DISCUSSION

In this study, we have examined the effect of mild, chronic lead intoxication in an experimental model of chronic renal disease. The dose of lead administered resulted in mild toxicity (26 µg/dl) and is similar to or slightly lower than the levels observed in subjects with occupational exposure (which typi-
cally are from 30 to 60 μg/dl) (3, 6, 7, 44). Nevertheless, the degree of lead poisoning was sufficient to cause higher blood pressures and to accelerate renal progression. The most impressive finding, however, related to the histological findings. Indeed, one of the major effects of lead was to cause marked worsening of microvascular injury, as characterized by arteriolar thickening and peritubular capillary loss, and this was associated with more tubular injury, greater interstitial inflammation, and more interstitial fibrosis. Furthermore, this was associated with higher renal levels of the chemokine MCP-1. Lead has commonly been thought to induce renal disease by causing direct tubular damage. Experimental lead toxicity can be associated with proximal tubular injury with characteristic intranuclear inclusions (24). Consistent with this pattern of injury, acute lead toxicity in humans is associated with Fanconi syndrome (4, 47). Interestingly, acute lead intoxication is not associated with hypertension, either experimentally (30, 41) or clinically (4, 47).

In contrast, chronic lead exposure is commonly associated with hypertension, which has been shown to be mediated by oxidants (1, 5, 9, 29). Interestingly, the renal histological findings with chronic low-level lead exposure appear to be different from that associated with acute or subacute high-dose intoxication. The principal findings demonstrated in experimental models with chronic, low-dose lead exposure appear to be the development of microvascular disease characterized by thickening of the preglomerular vessels (34) and with the development of tubulointerstitial inflammation (32). While not all studies have documented the microvascular changes with chronic, low-dose lead exposure (9), this might be because these changes were not specifically evaluated, because the method for detection may have been less sensitive (e.g., we utilized α-smooth muscle actin staining, which highlights the vascular smooth muscle cells), or because of differences in dose and duration of lead or of age and strain of rat. Human studies have also noted the strong association of chronic lead intoxication with renal arteriolosclerosis (13, 45, 46).

In this study, we have confirmed these findings and have related them to the induction of the chemokine MCP-1 in renal tissue. Importantly, we have previously reported that the development of microvascular disease and tubulointerstitial inflammation is a major mechanism for inducing salt-sensitive hypertension and that this is mediated by intrarenal oxidative

![Table 3. Vascular morphology of periglomerular arterioles](image)

![Figure 1. Lead-treated rats have worse glomerulosclerosis, renal tubular atrophy, and interstitial cell infiltration and fibrosis. A: periodic acid-Schiff staining from remnant kidney (RK) group. B: remnant kidney group receiving lead (RK+L). Magnification ×200.](image)

![Figure 2. Lead-treated rats have worse thickening of periglomerular arteriolar walls with narrowing of the arteriolar lumen as demonstrated by α-smooth muscle actin immunohistochemistry. A: RK. B: RK+L. Magnification ×630.](image)
stress and angiotensin II generation (15, 16). We have also reported that the development of preglomerular microvascular disease can be a mechanism for renal progression, as the structural changes alter renal autoregulation and favor the development of glomerular hypertension (“the Herrera hypothesis”) (17).

An interesting question is why high doses of lead do not cause hypertension or preglomerular vascular disease in contrast to lower doses. One possibility is that high doses of lead cause significant proximal tubular injury with Fanconi syndrome and hypouricemia. In contrast, lower doses of lead may trigger proximal tubular dysfunction, as characterized by decreased net excretion of urate with the development of hyper-

Fig. 3. Lead exposure aggravates peritubular capillaries loss, as reflected by a reduction in positive thrombomodulin staining. A: RK. B: RK+L. Magnification ×200.

Fig. 4. Low-lead level exposure in the RK model aggravates tubulointerstitial injury, as noted by osteopontin, collagen III, and macrophage (ED-1) expression. Magnification ×200.

Fig. 5. Monocyte chemoattractant protein-1 (MCP-1) mRNA expression was increased in lead-treated rats (A) and correlated with the number of infiltrating macrophages (B). Values are means ± SD.
uricemia (8, 23). In turn, we have found that hyperuricemia inhibits endothelial nitric oxide levels and can induce hypertension, oxidative stress, and preglomerular vascular disease and tubulointerstitial inflammation and that this is mediated in part by MCP-1 stimulation (18, 20, 26, 42). Serum uric acid levels were also higher in the lead-treated rats.

To examine this possibility, we originally included groups that received the xanthine oxidase inhibitor allopurinol. Unfortunately, the dose administered was associated with nephrotoxicity with lithiasis, in which the stones were shown to be comprised primarily of allopurinol and to a lesser extent xanthine (data not shown). Allopurinol is excreted by the kidney, and levels can increase markedly in the setting of renal failure (10). In addition, allopurinol, being a purine, can also crystallize, and there have been reports of allopurinol (or oxypurinol) crystals in skeletal muscle (43) and in the kidneys (31) of patients with hyperuricemia, and in the latter case this was associated with acute renal failure. In a previous study examining the role of hyperuricemia in the RK model, we gave the same dose of allopurinol (150 mg/l) but only for 6 wk, and hence this may have been too early to observe nephrotoxicity (19). Interestingly, however, Trachtman et al. (40) had reported nephrotoxicity with allopurinol in spontaneously hypertensive rats with normal renal function when higher doses of allopurinol were administered. Furthermore, it is interesting that, while recent studies suggest that allopurinol slows renal progression in patients with elevated creatinine and asymptomatic hyperuricemia (37, 39), that analysis of individual patients suggests in patients with elevated creatinine and asymptomatic hyperuricemia (37, 39), that analysis of individual patients suggests that some patients with higher baseline creatinines (>3.0 mg/dl) actually showed deterioration with therapy (37). This raises the possibility that allopurinol may confer nephrotoxicity in humans if high doses are administered.

These studies therefore do not determine whether the lead-associated worsening of renal progression is mediated by uric acid. Further studies will be necessary to evaluate this possibility, either with lower doses of allopurinol or with alternative nonpurine xanthine oxidase inhibitors that are unlikely to crystallize in the setting of renal dysfunction.

GRANTS
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DISCLOSURES
R. J. Johnson is a consultant on the Scientific Board of Nephromics, Inc.

REFERENCES


