Mycophenolate mofetil administration reduces renal inflammation, oxidative stress, and arterial pressure in rats with lead-induced hypertension

Yanauri Bravo,1 Yasmir Quiroz,1 Atilio Ferrebuz,2 Nosratola D. Vaziri,3 and Bernardo Rodrı́guez-Iturbe1,2

1Centro de Investigaciones Biomédicas, Instituto Venezolano de Investigaciones Científicas-Zulia, Maracaibo, and 2Renal Service Hospital Universitario Maracaibo, Venezuela; and 3Division of Nephrology and Hypertension, Departments of Medicine, University of California, Irvine, California

Submitted 19 December 2006; accepted in final form 5 June 2007

B. Rodrı́guez-Iturbe, Yanauri Bravo, Yasmir Quiroz, Atilio Ferrebuz, Nosratola D. Vaziri. Mycophenolate mofetil administration reduces renal inflammation, oxidative stress, and arterial pressure in rats with lead-induced hypertension. Am J Physiol Renal Physiol 293: F616–F623, 2007. First published June 13, 2007; doi:10.1152/ajprenal.00507.2006.—Hypertension is a likely consequence of chronic lead exposure in humans, especially in association with reduced renal function and in high risk populations. Numerous studies have demonstrated that oxidative stress plays an important role in the pathogenesis of experimental lead-induced hypertension and we have shown recently that tubulointerstitial immune cell infiltration is a feature of chronic low-dose lead exposure. Since oxidative stress, renal inflammation, and angiotensin II activity are closely linked characteristics in experimental models of hypertension, we decided to investigate whether lead-induced hypertension would be ameliorated by suppressing renal inflammation with the immunosuppressive drug mycophenolate mofetil (MMF). We studied rats exposed for 14 wk to lead acetate (100 ppm in the drinking water) that, in addition, received either MMF, 20 mg·kg⁻¹·day⁻¹ by gastric gavage (Pb.MMF group, n = 12) or vehicle (Pb group, n = 12). Control rats received MMF alone (n = 5) or neither lead nor MMF (n = 6). All rats were killed at the end of the experiment. Low-dose lead exposure resulted in mild to moderate tubular cell damage and a progressive increment in blood pressure, oxidative stress, interstitial accumulation of lymphocytes and macrophages, NF-κB activation, and increased renal angiotensin II level. The administration of MMF suppressed the tubulointerstitial accumulation of lymphocytes and macrophages and prevented the hypertension, oxidative stress, and NF-κB activation and reduced the heightened renal angiotensin content associated with chronic lead exposure. We conclude that interstitial inflammation plays an important role in lead-induced hypertension.

macrophages; lymphocytes; angiotensin II; NF-κB

Adress for reprint requests and other correspondence: B. Rodrı́guez-Iturbe, Renal Service Hospital Universitario, Ave Goajira S/N, Maracaibo, Zulia 4001-A, Venezuela.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Control groups included rats that received neither lead in the drinking water nor MMF (C group n = 6) and rats that did not receive lead but received MMF (CMMF group, n = 5). All treatments were maintained for 14 wk and at this time all rats were euthanized under diazepam-ketamine anesthesia. Kidneys were harvested for histology, immunohistochemistry, angiotensin II content, and NF-κB activation (p65).

During the 14-wk observation period the blood pressure was recorded weekly and blood and urine samples were obtained biweekly for plasma creatinine and 24-h protein excretion. Creatinine clearance was determined in five rats of each control group (C group and CMMF group) and in 7–10 rats in the lead exposed groups (Pb group and Pb.MMf group) at the end of the experiment. Potential gastrointestinal adverse effects of the MMF were evaluated by monitoring food intake and following the animals’ weights.

**Blood pressure measurements.** Systolic blood pressure was determined by tail-cuff plethysmography (IITC Life Scientific Instruments, Woodland Hills, CA). As described in previous communications (3, 41, 44, 46), animals had been preconditioned to the procedure before the experiments, and when blood pressure was determined rats were allowed to rest in the restrainer for 10–15 min before cuff inflation and release of several times and blood pressure used was the average of three determinations obtained after stabilization.

In addition, at the end of the experiment, direct intra-aortic blood pressure readings (Biosys, Vartech Medical Systems, Miami, FL) were obtained in five rats each in the Pb group and Pb.MMf group, four rats in the C group, and five rats of the C.MMf group as described in previous work (45).

**Determination of blood lead levels.** Blood lead levels were determined in six rats in the Pb group, seven rats in the Pb.MMf group, and six rats in the C group by atomic absorption spectrometry (detection limits ≥1 μg/l), during weeks 8–10 of the experiment (Departamento de Ingeniería Sanitaria y Ambiental, DISA, Facultad de Ingeniería, Universidad el Zulia, Maracaibo).

**Renal angiotensin II.** Renal angiotensin II was determined as described in previous communications (57) in the kidney of five rats in each experimental (Pb and Pb.MMf groups) and control (C and C.MMf) groups following the methodology of Durvasula et al. (12). Briefly, kidneys were removed after perfusion with cold saline solution. Renal cortex was cut in small pieces and placed in a cold (2°C) solution containing 20 mmol Tris-HCl (pH 7.40), 10 mmol/l of EDTA, 5 mmol/l EGTA, and a protease inhibitor cocktail (Roche, Molecular Biochemicals, Indianapolis, IN). Tissues were sonicated on ice and centrifuged at 16,000 g for 15 min at 2°C. Supernatants were collected in cold polypropylene tubes and stored at −20°C until determination of angiotensin II was done. Angiotensin II was determined by enzyme-linked competitive immunosorbent assay using commercially available kits (Peninsula Laboratories) after extraction in C18 Sep columns by use of a 10-point (0 to 10 ng/ml) standard curve. Intra- and interassay variations were 10 ± 8 and 28 ± 5%, respectively. Results are expressed as femtomoles per gram renal cortex. Recovery of angiotensin II added before extraction was 86 ± 12%.

**Renal p65 NF-κB.** The renal abundance of the DNA-binding p65 subunit of NF-κB was determined in nuclear extracts of the renal cortex of six kidneys from the Pb group, five kidneys from the Pb.MMf group, six kidneys from the C group and five kidneys of the C.MMf group, as described in previous communications (42, 45). Briefly, renal cortex was immediately separated from cold perfused kidneys, minced, weighted, and placed in prechilled containers. Nuclear extract was prepared commercially available kits (Active Motif, Carlsbad, CA) according to the manufacturer’s recommendations. ELISA was used to determine p65 NF-κB (TransAm NF-κB, Active Motif, Carlsbad, CA). Lower detection limits are 3 ng/ml extract. Results are given as nanograms of p65 NF-κB per gram of tissue.

**Histology and immunohistochemistry.** Light microscopy studies were done in paraffin-embedded biopsies stained with periodic acid-Schiff, hematoxylin-eosin, and trichromic stains. Severity of glomerulonephrosis was evaluated by using an index score reported in previous communications (3, 41, 44) that results from grading glomeruli in a scale from 0 (normal) to 4+ (more than 75% of the glomerular tuft) and calculating a score to the biopsy by the following formula: [(1 × n glomeruli with 1+) + (2 × n glomeruli with 2+) + (3 × n glomeruli with 3+) + (4 × n glomeruli with 4+)] × 100/total number of glomeruli examined. Tubulointerstitial damage was graded as in previous communications (3, 37, 40) according to the extent of damage (infiltration, fibrosis, tubular dilatation) in successively evaluated fields in the entire renal cortex (0 = normal, 1+ = <10%, 2+ = 10–25%, 3+ = >25–50%, 4+ = >50–75%, and 5+ = >75%). Medial thickening was evaluated in the 10–18 afferent arteriolar per biopsy by computerized image analysis of the ratio of the external circumference to internal circumference of the medial layer, as described in previous communications (3, 41, 44).

Lymphocytes (CD5-positive cells), macrophages (ED1-positive cells), angiotensin II-positive cells, and p65 NF-κB-positive cells were identified by avidin-biotin-peroxidase method. Details of the methodology in our laboratories have been published previously (37). Positive cells were evaluated within the glomeruli (per glomerular cross section) and in tubulointerstitial areas (positive cells/mm²).

All histological and immunohistological studies were done blinded as to the group in which the biopsy was obtained. Computer-assisted image analysis was done in an Olympus BX51 system and DP70 microscope digital camera with Sigma Pro (Leesburg, VA) image analysis software as in previous work (42, 45, 47).

**Antiserum.** Lymphocytes and macrophages were identified with anti-CDS and anti-ED1 monoclonal antibodies (Biosource, Camarillo, CA), respectively. Angiotensin II-positive cells were identified with anti-human angiotensin II antiserum with cross-reactivity to rat angiotensin II (Peninsula Laboratories, Belmont, CA). As described in previous communications (42, 45), specificity of the staining was tested by preincubating the antibody with human angiotensin II. Secondary antibodies with minimal cross-reactivity to rat serum proteins were purchased from Accurate Chemical and Scientific (Westbury, NY). Nonrelevant antibodies were used for negative control studies.

**Superoxide-positive cells.** Cryostat sections of the kidney biopsies were used to study intracellular superoxide production in five biopsies from the C.MMf group and six biopsies from the other groups by the cytochemical method of Briggs et al. (6) with minor modifications (35). Sections were fixed in formalin and counterstained with 1% methyl green.

**Urinary malondialdehyde determinations.** Thiobarbituric-reactive substances were determined by the method of Ohkawa et al. (38) as described previously (44, 50).

**Statistical analysis.** Statistical analyses were done by multigroup ANOVA. Statistically significant differences (P < 0.05) between groups were analyzed with Tukey-Kramer posttests. Serial changes were evaluated with repeated-measures ANOVA and changes with respect to baseline with Dunnett tests. Data are given as means ± SD.

**RESULTS**

**General data.** The body weight, blood pressure, lead blood levels, and plasma creatinine prior to and at the end of the experiment and creatinine clearance at the end of the experiment are shown in Table 1. Blood pressure was higher in the Pb group than in the other groups at the end of the study period (Table 1). To be noted, there was no significant difference in the weight gain in the experimental and control groups; in particular, the final weights in the MMF-treated (Pb.MMf group) and untreated (Pb group) rats after 14 wk of treatment were essentially similar. Blood lead levels were equally elevated in the Pb and Pb.MMf groups (Table 1).
Blood pressure. The serial determinations of systolic blood pressure are shown in Fig. 1. The mean blood pressure in the rats with lead exposure (Pb group) increased progressively and after 6 wk was significantly higher \( (P < 0.05 \text{ or } P < 0.01) \) than the blood pressure in the control groups. The administration of MMF (Pb.MMF group) prevented the increment in blood pressure. Blood pressure in the Pb.MMF group was not significantly different from that found in control rats, although the mean values were mildly but consistently above those of the control groups (Fig. 1).

Intra-aortic blood pressure determinations at the end of the experiments were consistent with the tail-cuff measurements and showed that the Pb group had significantly higher mean arterial pressure than the rest (Fig. 2).

Oxidative stress. Urinary malondialdehyde (MDA) excretion data obtained in the 4th and again in the last weeks (12th week) of the experiment are shown in Fig. 3. Lead exposure was associated with increased urinary MDA excretion at both time intervals in the Pb group. Treatment with MMF prevented the rise in urinary MDA in the Pb.MMF group which showed essentially identical values with those observed in the control group.

Data on the superoxide-positive cell counts in the renal tissues are shown in Fig. 4. Increased numbers were found in the lead-exposed rats. MMF treatment suppressed the number of superoxide-positive cells in the Pb.MMF group to values which were comparable to those observed in control animals.

NF-κB activation. Abundance of the p65 subunit of NF-κB in the nuclear extracts of renal cortical tissues in the experimental and control groups is shown in Fig. 5. The nuclear p65 NF-κB abundance was significantly increased in the Pb group, indicating heightened NF-κB activation. Administration of MMF was associated with a reduction of p65 NF-κB content in the Pb.MMF group to values comparable to those found in the control animals.

Renal angiotensin II. The renal angiotensin II data are shown in Fig. 6. The results revealed that lead exposure (Pb group) increases angiotensin II content and that MMF treatment normalizes it in the Pb.MMF group (Fig. 6A). These results are in concordance with the number of tubulointerstitial...

---

### Table 1. General data before and at the end of the experiment

<table>
<thead>
<tr>
<th></th>
<th>C ((n = 6))</th>
<th>C.MMF ((n = 5))</th>
<th>Pb ((n = 12))</th>
<th>Pb.MMF ((n = 12))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>204 ± 9.0</td>
<td>210 ± 11.0</td>
<td>202 ± 11.8</td>
<td>204 ± 8.4</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>128 ± 1.3</td>
<td>123 ± 10.3</td>
<td>158 ± 20.6*</td>
<td>139 ± 11.8</td>
</tr>
<tr>
<td>Pcr, mg/dl</td>
<td>0.37 ± 0.11</td>
<td>0.38 ± 0.11</td>
<td>0.28 ± 0.12</td>
<td>0.28 ± 0.13</td>
</tr>
<tr>
<td>Ccr, ml min⁻¹ 100 g⁻¹</td>
<td>0.75 ± 0.11</td>
<td>0.79 ± 0.21</td>
<td>0.68 ± 0.30</td>
<td>0.74 ± 0.14</td>
</tr>
<tr>
<td>Urine protein, mg/day</td>
<td>1.62 ± 1.01</td>
<td>1.52 ± 0.95</td>
<td>1.78 ± 1.29</td>
<td>1.49 ± 0.93</td>
</tr>
<tr>
<td>Pb levels, μg/dl</td>
<td>&lt;1</td>
<td>ND</td>
<td>23.7 ± 6.51</td>
<td>27.0 ± 4.93</td>
</tr>
</tbody>
</table>

Values are means ± SD. Findings before (Pre) and at the end of the experiments (Post). Pb and Pb.MMF groups received 14 wk of low-dose lead exposure (100 ppm of lead acetate in the drinking water. Pb.MMF group received in addition mycophenolate mofetil (MMF; 20 mg/kg by gastric gavage), and the Pb group received vehicle. Control groups unexposed to lead received MMF (C.MMF group) or vehicle (C group). Ccr, creatinine clearance (done in 5 rats of C and C.MMF groups and in 7–10 rats of the Pb and Pb.MMF groups); ND, not done. *\( P < 0.05 \) vs. C and C.MMF groups and \( P < 0.05 \) vs. C.MMF group.
angiotensin II-positive cells in the experimental and control groups shown in Fig. 6B. Angiotensin II-positive cells were not found inside the glomeruli.

**Histology.** Light microscopy findings were essentially confined to the tubulointerstitial areas of the kidney. Glomeruli were normal. Tubulointerstitial damage was mild to moderate, consisting of focal areas of dilatation with loss or disruption of the brush border and preservation of the integrity of basement membranes (Fig. 7). There was no interstitial fibrosis. Tubulointerstitial damage score was higher in the Pb group (1.67 \pm 0.54) than in the Pb.MMF group (0.89 \pm 0.78) and in control groups (C group = 0.4 \pm 0.5; C.MMF group = 0.6 \pm 0.5).

Appearance of afferent arterioles and thickness of their media layer (external/internal diameter) were normal and comparable in the Pb group (2.61 \pm 0.40), the Pb.MMF group (2.58 \pm 0.35) and the control groups (C group = 2.56 \pm 0.39; C.MMF group = 2.44 \pm 0.54).

**Immune cell infiltration.** There was no significant glomerular infiltration of lymphocytes or macrophages (<1 positive cell/10 glomerular cross sections) in any of the study groups. In contrast, lead-exposed animals showed significant tubulointerstitial infiltration of lymphocytes and macrophages that was prevented by MMF (Fig. 8).

**DISCUSSION**

Long-term administration of lead causes progressive nephropathy the severity of which is related to the dose and duration of exposure. Khalil-Manesh and coworkers (22, 23) have shown that exposure to high doses of lead (5,000 ppm lead acetate in the drinking water) for more than 6 mo causes tubulointerstitial inflammation followed by fibrosis, glomerulosclerosis, and azotemia, whereas 12 mo of exposure to low-dose of lead (100 ppm in the drinking water) are needed to induce only mild to moderate renal fibrosis and tubular atrophy (24). Although overt nephropathy occurs after many months of lead exposure, only 12 wk of exposure to low levels of lead resulting in blood lead levels of 10–15 \mu g/dl result in arterial hypertension (25). In humans, the clinical characteristics of lead toxicity are well known and the potential consequences of occupational exposure to lead (40) and the dose-related reduction of childhood intelligence quotient attributable to lead (51) have been extensively studied. Blood lead levels of 20–29 \mu g/dl are associated with increased all-cause and cardiovascu-

---

**Fig. 3.** Urine malondialdehyde (MDA) excretion at 4 and 14 wk in rats with chronic lead exposure (Pb group), rats with chronic lead exposure treated with MMF (Pb.MMF group) and control rats unexposed to lead with (C group) and without (C.MMF group) MMF administration. MMF treatment reduced MDA induced by lead exposure to levels comparable to those found in control rats unexposed to lead. Urinary MDA determination in the C.MMF group were only done at 12 wk. *P < 0.01 vs. control and P < 0.05 vs. Pb.MMF group; **P < 0.05 vs. the rest. Data are means ± SD.

**DISCUSSION**

Long-term administration of lead causes progressive nephropathy the severity of which is related to the dose and duration of exposure. Khalil-Manesh and coworkers (22, 23) have shown that exposure to high doses of lead (5,000 ppm lead acetate in the drinking water) for more than 6 mo causes tubulointerstitial inflammation followed by fibrosis, glomerulosclerosis, and azotemia, whereas 12 mo of exposure to low-dose of lead (100 ppm in the drinking water) are needed to induce only mild to moderate renal fibrosis and tubular atrophy (24). Although overt nephropathy occurs after many months of lead exposure, only 12 wk of exposure to low levels of lead resulting in blood lead levels of 10–15 \mu g/dl result in arterial hypertension (25). In humans, the clinical characteristics of lead toxicity are well known and the potential consequences of occupational exposure to lead (40) and the dose-related reduction of childhood intelligence quotient attributable to lead (51) have been extensively studied. Blood lead levels of 20–29 \mu g/dl are associated with increased all-cause and cardiovascu-

---

**Fig. 4.** A: superoxide-positive cells in the kidney of rats with chronic lead exposure (Pb group), rats exposed to lead treated with MMF (Pb.MMF group) and control rats unexposed to lead without (C group) and with (C.MMF group) MMF treatment. ***P < 0.001 vs. the rest. Data are means ± SD. Bottom: superoxide-positive cells in a biopsy of a rat of the Pb group (B) and in a rat from the Pb.MMF group (C). (superoxide staining with counterstaining with methyl green. Original magnification ×400).
lar mortality (27), and a recent review of the epidemiological evidence indicates that lead contributes to nephrotoxicity even at blood lead levels below 5 μg/dl, especially in populations in which other risk factors for chronic kidney disease are present (13).

The association of lead exposure to arterial hypertension in humans is increasingly recognized. Dissenting opinions notwithstanding (36, 52, 53), population studies report an association between chronic lead exposure and hypertension (1, 5, 9, 17) particularly in African Americans (64), postmenopausal women (28), selected communities of elder individuals (29), and patients with pregnancy-associated hypertension (8, 63). The widespread distribution of potential sources of lead exposure, which include lead paint (currently estimated to exist in 38 million homes in the United States) (40), drinking water circulated through lead-soldered plumbing fixtures and pipes, ceramic glazers, batteries, etc., have prompted Gonick and Behari (18) to hypothesize that lead may be a major contributory cause of essential hypertension, and a recent review of the available data concluded that there is sufficient evidence to infer causal relationship of lead exposure with hypertension (33).

The association between lead exposure and high blood pressure is well established in experimental animals and heightened sympathetic activity (56), elevated angiotensin-converting enzyme and reduced bradykinin levels (7), and upregulation of NAD(P)H oxidase with increased generation of superoxide and hydrogen peroxide (61) play a role in lead-induced hypertension. Increased generation of reactive oxygen species, elevated plasma/tissue MDA, and reduced NO production and bioavailability (37, 58, 59) have all been previously shown to be induced by chronic lead exposure.

The present studies confirmed that chronic lead exposure results in hypertension. To be noted, the tail-cuff determinations of systolic blood pressure in the Pb group had a large variability (coefficient of variation 12–14%) that contrasts with the low variance in the blood pressure data of the rest of the groups in the study (Fig. 1). It is unlikely that this represents observer error since the same observer recorded the blood pressure with the same methodology in all animal groups. Furthermore, the relatively large variance in tail-cuff blood pressure in the Pb group is in agreement with other studies that show similar or higher coefficient of variation in rats with lead-induced hypertension (61). It is possible that the increased sympathetic activity associated with chronic lead exposure (56) contributes to the variability of blood pressure measured by noninvasive method in the conscious animal. Nevertheless, blood pressure readings obtained by direct intra-aortic monitoring (Fig. 2) confirmed the results obtained with tail-cuff methodology.

![Graph](image1)

**Fig. 5.** p65 NF-κB abundance in nuclear extracts of the cortex of rats chronically exposed to lead (Pb group), rats exposed to lead treated with MMF (Pb.MMF group), and control rats unexposed to lead and given MMF (C.MMF group) or not (C group). Lead exposure increased NF-κB activation and MMF treatment reduced lead-induced activation of NF-κB to values comparable to those in control rats. *P < 0.001 vs. C group, P < 0.01 vs. C.MMF group, and P < 0.05 vs. Pb.MMF group. Data are means ± SD.

![Graph](image2)

**Fig. 6.** A: renal cortical angiotensin II (AII) content in rats with chronic lead exposure (Pb group), rats exposed to lead treated with MMF (Pb.MMF group) and control rats unexposed to lead and treated with MMF (C.MMF group) or not (C group). Lead exposure increased renal angiotensin II abundance and MMF treatment reduced lead-induced renal angiotensin II content to values comparable to those in control rats (*P < 0.001 vs. C, P < 0.01 vs. C.MMF and P < 0.05 vs. Pb.MMF). B: angiotensin II-positive cells in tubulointerstitial areas in rats of experimental and control groups identified above. Chronic lead exposure increased the number of angiotensin II-positive cells (*P < 0.001 vs. the rest) and MMF treatment to lead-exposed rats reduced the number of angiotensin II-positive cells that nevertheless was significantly higher (*P < 0.01) than those in the C and C.MMF groups. Data are means ± SD. Bottom: representative microphotographs of angiotensin II-positive cells in the biopsy of a rat of the Pb group (C) and of the Pb.MMF group (D). Avidin-biotin-peroxidase staining (original magnification ×400).
The present work also confirmed the existence of oxidative stress and, in addition, provides new evidence on the pathophysiology of lead-induced hypertension, including heightened intrarenal angiotensin II generation, NF-κB activation, and tubulointerstitial lymphocyte and macrophage infiltration. As shown in Fig. 6, increased angiotensin II content was a characteristic observed in the kidney of rats with chronic exposure to lead. It should be noted, however, that because of cross-reactivity, in addition to angiotensin II, the ELISA system detects several other angiotensin fragments. In separate investigations (15) we found that cross-reactivity with angiotensin I and angiotensin 1–7 was <0.01%, but cross-reactivity with angiotensin 3–8 and angiotensin III (H-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) was 34 and 100%, respectively. Therefore, ELISA determinations do not discriminate between angiotensin II and the latter two fragments.

The results of this study are in line with previous investigations by our group (3, 34, 41, 42, 44–46, 49) and other laboratories (4, 30, 32, 54, 55) that have provided compelling evidence for the role of renal inflammation in the pathogenesis of hypertension in several other animal models of hypertension. The accumulated evidence from these studies has established that renal immune cell infiltration, increased intrarenal angiotensin II, and oxidative stress are involved in a vicious cycle that maintains a tendency for sodium retention and thereby plays a central role in development and maintenance of hypertension (reviewed in Refs. 48, 62). In previous studies done in the postangiotensin infusion hypertension model that, like lead-induced hypertension, is associated with interstitial inflammation, oxidative stress, and increased renal angiotensin II, we have demonstrated afferent glomerular vasoconstriction and reduction of single nephron glomerular filtration rate (GFR) in cortical nephrons while the total renal GFR is normal (16). In the present studies, plasma creatinine and creatinine clearance were not significantly different among the study groups which is in agreement with studies that have reported normal GFR in chronic low-level lead exposure (10, 24). Micropuncture studies are necessary to demonstrate the existence of regional glomerular hemodynamic alterations; nevertheless, we think it probable that a tendency to sodium retention driven by preglomerular vasoconstriction and reduction of single nephron glomerular filtration rate in cortical nephrons is likely to be present in lead-induced hypertension, as in the previously cited hypertension model of increased renal angiotensin II (16), and that these changes are prevented or amelio-

![Fig. 7. Microphotograph of a biopsy of a rat with low-dose lead exposure for 14 wk, showing normal glomeruli near an area showing tubular dilatation and loss of tubular cell brush borders with intact tubular basement membrane. Normal tubulointerstitium surrounds the damaged area (periodic acid-Schiff staining, original magnification ×200).](image)

![Fig. 8. A: infiltration of lymphocytes (CD5-positive cells) and macrophages (ED1-positive cells) in tubulointerstitial areas of rats exposed chronically to lead (Pb group), rats exposed to lead treated with MMF (Pb/MMF group), and control rats unexposed to lead treated with MMF (C/MMF group) or not (C group). Lead exposure induced accumulation of lymphocytes and macrophages and MMF treatment reduced immune cell infiltration to values comparable to those in control rats. ***P < 0.001 vs. the rest. Data are means ± SD. Bottom: representative microphotograph of infiltrating macrophages (arrows) in a biopsy of a rat from the Pb group (B) not present in the renal tubulointerstitium of a rat from the Pb/MMF group (C). (Immunoperoxidase staining with anti-ED1 antibody. Original magnification ×400).](image)
rated with the use of MMF. To be noted, MMF has a variety of effects in addition to its well-known immunosuppressive activity (2), including a suppression of proliferation of glomerular mesangial cells and endothelial cells (31); yet there were no discernible lead-induced changes in the glomeruli or in arterioles that could conceivably be modified by MMF. Furthermore, the administration of MMF alone to control rats did not modify their blood pressure (Fig. 1); therefore, the effects of MMF cannot be attributed to antihypertensive effects of the drug. This is in line with other studies that have shown that MMF does not reduce blood pressure in normotensive rats (19, 21, 41, 44, 55). Nevertheless, a recent investigation (26) has found that MMF inhibits endothelial NAD(P)H oxidase activity and superoxide formation, which could contribute to the reduction in oxidative stress and improvement in hypertension in the Pb.MMF group. MMF administration is at times associated with well-recognized gastrointestinal adverse effects including diarrhea or reduced food intake that could potentially reduce the blood pressure. Therefore, we monitored food ingestion and body weight in the experimental groups. No significant side effects were observed as a result of MMF treatment, food intake was normal, and weight gain was similar to that seen in the groups that did not receive MMF (Table 1).

In earlier studies, both Khalil-Manesh et al. (24) and our group (47) showed that 12–14 wk of low-dose lead exposure resulted in tubular cell damage and interstitial immune cell infiltration while glomeruli were normal. Similar results were obtained in the present studies; therefore, hypertension in the Pb group is unrelated to structural glomerular damage. We did not find significant changes in plasma creatinine, creatinine clearance, or proteinuria (Table 1), which, as noted earlier, is consistent with studies that have shown that GFR is unchanged by low-dose lead exposure (10, 24). The importance of the renal tubulointerstitial inflammation in the development and maintenance of oxidative stress is evident from the fact that despite similar blood lead levels in the experimental groups (Pb group and Pb.MMF group, Table 1), blood pressure, oxidative stress, and renal angiotensin II activity were reduced by MMF in parallel with attenuation of tubulointerstitial inflammation. These results are in concert with recent findings in patients with essential hypertension who receive MMF for treatment of psoriasis and rheumatoid arthritis and show a significant reduction in blood pressure that parallels a decline in the urinary excretions of inflammatory cytokines and MDA (20).

In conclusion, oxidative stress, heightened intrarenal angiotensin activity, and hypertension in lead-exposed rats are associated with and causally linked to renal tubulointerstitial inflammation. The pattern observed in this model parallels that seen in several other forms of hypertension and as such appears to represent a common theme in the pathophysiology of hypertension.

ACKNOWLEDGMENTS

Data from this paper were presented in part at the 2005 Annual Congress of the American Society of Nephrology and published in abstract form (J Am Soc Nephrol 16: 163A, 2005).

GRANTS

Financial support was provided by FONACIT grant F-2005000283, Venezuela.

REFERENCES


