Elevated Uric Acid Increases Blood Pressure in the Rat by a Novel Crystal-Independent Mechanism


Abstract—An elevation in circulating serum uric acid is strongly associated with the development of hypertension and renal disease, but whether uric acid has a causal role or whether it simply indicates patients at risk for these complications remains controversial. We tested the hypothesis that uric acid may have a causal role in the development of hypertension and renal disease by examining the effects of mild hyperuricemia in rats. Mild hyperuricemia was induced in rats by providing a uricase inhibitor (oxonic acid) in the diet. Hyperuricemic rats developed elevated blood pressure after 3 weeks, whereas control rats remained normotensive. The development of hypertension was prevented by concurrent treatment with either a xanthine oxidase inhibitor (allopurinol) or a uricosuric agent (benziodarone), both of which lowered uric acid levels. Blood pressure could also be lowered by reducing uric acid levels with either allopurinol or oxonic acid withdrawal. A direct relationship was found between blood pressure and uric acid (r=0.75, n=69), with a 10–mm Hg blood pressure increase for each 0.03-mmol/L (0.5-mg/dL) incremental rise in serum uric acid. The kidneys were devoid of urate crystals and were normal by light microscopy. However, immunohistochemical stains documented an ischemic type of injury with collagen deposition, macrophage infiltration, and an increase in tubular expression of osteopontin. Hyperuricemic rats also exhibited an increase in juxtaglomerular renin and a decrease in macula densa neuronal NO synthase. Both the renal injury and hypertension were reduced by treatment with enalapril or L-arginine. In conclusion, mild hyperuricemia causes hypertension and renal injury in the rat via a crystal-independent mechanism, with stimulation of the renin-angiotensin system and inhibition of neuronal NO synthase. (Hypertension. 2001;38:1101-1106.)

Key Words: uric acid ■ hypertension, renal ■ renin-angiotensin system ■ nitric oxide

Uric acid is a purine metabolite that in most mammals is degraded by the hepatic enzyme uricase to allantoin. However, mutations in the uricase gene occurred during primate development, with the consequence that humans have relatively higher levels of serum uric acid.1 An elevation in serum uric acid has been associated with an increased risk for the development of hypertension.2–4 and 25% to 50% of hypertensive individuals are hyperuricemic.4 Hyperuricemia also confers increased risk for cardiovascular mortality, especially in women.5,6 Despite the clinical and epidemiological evidence, many authorities do not consider an elevated uric acid to be a true cardiovascular risk factor, because patients with hyperuricemia often have other well-established risk factors for cardiovascular disease, such as hypertension, renal disease, obesity, dyslipidemia, and insulin resistance.6 Several studies have found that an elevated uric acid level is an independent risk factor for cardiovascular disease after controlling for the contribution of established risk factors by multivariate analyses; however, other studies, including the recent Framingham analysis,8 have not. The lack of a mechanism by which uric acid can cause cardiovascular disease, coupled with the inconclusive clinical and epidemiological data, has left the issue unresolved.

In consideration of this controversy, it is important to note that no animal model has previously been found suitable for studying the effects of mild hyperuricemia. Several previous groups had reported that hyperuricemia could be induced in rats by feeding them the uricase inhibitor oxonic acid (OA).9 In most studies, uric acid supplements were also added to the diet, resulting in a 6- to 10-fold increase in serum uric acid levels with consequent marked uricosuria and acute renal failure secondary to obstruction of the renal tubules with urate crystals.9 Targeted deletion of the uricase gene in mice also results in marked hyperuricemia, intrarenal urate crystal deposition, and renal failure.10 Although these latter models mimic the acute urate nephropathy syndrome observed after chemotherapy in some patients with cancer (tumor lysis syndrome), they are inappropriate models for the mild hyperuricemia observed in patients with cardiovascular disease.
The present study aimed to develop a model of mild hyperuricemia that did not result in intrarenal urate crystal deposition for the purpose of directly examining whether uric acid can modulate blood pressure (BP) or cause renal injury.

Methods
In pilot studies, we compared the classic model of hyperuricemia induced by OA and uric acid supplements with hyperuricemia induced by OA alone on serum uric acid levels and renal histology. Rats treated with 2% OA with 3% uric acid supplements developed marked hyperuricemia (7- to 8-fold increase over control levels), with renal injury and intratubular crystal deposition. In contrast, rats treated with 2% OA in the absence of uric acid supplements developed mild hyperuricemia (1.5- to 2-fold increase in serum uric acid), with preserved renal architecture and the absence of intrarenal urate crystals (Figure 1A and 1B). Therefore, we used the 2%-OA diet to examine the effect of mild hyperuricemia on kidney function and BP.

Experimental Design
Male 200- to 250-g Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif) were used in all experiments, and BP, serum uric acid, and blood urea nitrogen (BUN) were measured at multiple time points. Experiments were approved by the University of Washington animal care committee.

Experiment I
Initial studies (experiment I) examined the effects of mild hyperuricemia in rats on a normal-sodium diet. Rats were placed on a normal-sodium (NaCl 0.26%) diet with or without 2% OA (Ziegler Bros) for 7 weeks, when histological studies were performed (n=6 rats per group).

Experiment II
Experiment II examined the effect of mild hyperuricemia in the presence of modest salt restriction and the effect of allopurinol to prevent these changes. Rats were placed on a low-salt (LS) diet (NaCl 0.125%) according to the following groups: OA alone (n=30), LS control (n=18) for 7 weeks, and OA+LS+allopurinol (150 mg/L in drinking water) (n=6) for 7 weeks, at which point 5 or 6 animals in each group were euthanized.

Experiment III
Experiment III examined the effect of mild hyperuricemia in the presence of modest salt restriction and the effect of the uricosuric agent benziodarone on BP. Groups were followed for 7 weeks and consisted of OA+LS (n=5), OA+LS+benziodarone (n=5), and LS alone (n=5). The benziodarone dose was initiated at 10 mg/kg per day in the drinking water (adjusted according to the water intake) and was increased at the end of the second week to 15 mg/kg per day because the original dose was not sufficient to lower serum uric acid levels.

Experiment IV
Experiment IV was performed to determine the effect of lowering uric acid on BP and renal histology in previously hyperuricemic animals. Rats were placed on the LS/OA diet for 7 weeks to induce chronic hyperuricemia (see experiment II) and then were divided into various groups (matched for serum uric acid and BP) either to have the uric acid lowered with allopurinol or OA withdrawn or to continue the LS/OA diet. A control group included rats on the LS diet alone for 11 weeks, at which time, all the animals were euthanized.

Experiment V
Experiment V examined the effect of treatment with an ACE inhibitor or with L-arginine in rats with OA-induced hyperuricemia. Rats were placed on an LS diet, LS/OA diet, LS/OA diet plus enalapril (1 mg/kg per day in drinking water and adjusted according to water intake), or LS/OA diet plus L-arginine (1% in drinking water) (n=5 rats in LS/OA group and 6 rats each in LS, enalapril, and L-arginine groups).

Evaluation
In all studies, systolic BP (SBP) was measured by a tail-cuff sphygmomanometer with use of an automated system with photoelectric sensor (IITC, Life Sciences) that has been shown to correlate with intra-arterial BP measurements. All animals were preconditioned for BP measurements 1 week before each experiment. Serum uric acid was measured by a carbonic phosphotungstate method. Serum BUN was measured by a standard kit (Sigma Chemical Co).
Renal Histology
Renal biopsies were fixed in methyl Carnoy’s fixative, 10% formalin, or 100% ethanol and embedded in paraffin. The presence of urate crystals was evaluated by staining 4-μm ethanol-fixed sections with de Galantha and modified von Kossa stains. Kidney tissue from rats with acute uric acid nephropathy, induced by OA and uric acid administration, was used as a positive control.9 Light microscopy was performed in 4-μm sections of methyl Carnoy’s fixed tissue stained with periodic acid–Schiff reagent. Methyl Carnoy’s or formalin-fixed tissue sections were analyzed by indirect immunoperoxidase11 staining with the following primary antibodies: OP199, a goat anti-mouse osteopontin (OPN; gift of C. Giachelli, University of Washington, Seattle); ED-1, a mouse anti-rat macrophage antibody (Serotec); goat anti-human type III collagen (Southern Biotechnology Associates Inc); mouse anti-human renin antibody (Sanofi Recherche); and rabbit anti-rat neuronal NO synthase (NOS1) (Transduction Laboratories).

Quantification of Morphology
All quantification was performed with the researchers blinded to the experimental conditions. The tubular expression of OPN, a sensitive marker of tubulointerstitial injury, was calculated as the percentage of renal cortex occupied by OPN-positive tubules, as previously described.11 The interstitial deposition of collagen type III was calculated as the percentage of renal cortex occupied by collagen III, noted by immunostaining, by computer image analysis. The mean number of interstitial macrophages (ED-1 + cells) in each biopsy was calculated by counting the total number of positive interstitial cortical cells in 20 sequentially selected 0.25-mm² grids at ×200 magnification. Renin expression was quantified by the number of glomeruli with positive staining for juxtaglomerular renin by using a minimum of 100 glomeruli in each biopsy, a method that has previously been shown to correlate with changes in tissue renin content.13 NOS1 was quantified by counting the number of positive macula densa cells stained with anti-NOS1 by using a minimum of 100 glomeruli per biopsy.14

Statistical Analysis
Values are expressed as mean±SE. Statistical significance (P<0.05) was evaluated by ANOVA with appropriate correction for multiple comparisons (Fisher protected least significant difference). The relationship between variables was assessed by Pearson correlation analysis.

Results
Hyperuricemia Induces BP Elevation
Mild hyperuricemia was induced in rats on a normal salt diet by supplementing the diet with 2% OA (experiment I).
Baseline SBP in both groups showed no difference before initiation of the diet (SBP, 114.5 ± 16.1 versus 122.2 ± 14.9 mm Hg, OA versus controls, respectively; P = NS). However, SBP was significantly increased in OA-treated rats at 4 weeks (SBP, 142.9 ± 12.5 versus 125.4 ± 14.9 mm Hg, OA versus controls, respectively; P < 0.05), and this difference persisted until euthanasia at week 7 (SBP, 141.8 ± 19.2 versus 130.3 ± 14.6 mm Hg, OA versus controls, respectively; P < 0.05). Furthermore, the increase in BP showed a direct correlation with uric acid levels (r = 0.7, P < 0.001; n = 12).

The effect of OA on BP elevation was more profound in rats placed on modest salt restriction (LS = 0.125% NaCl diet) (experiment II). As shown in Figure 1, control rats placed on a salt-restricted diet had a gradual decline in BP during the study period. In contrast, hyperuricemic rats on the LS diet showed a significant increase in BP, with a difference of nearly 40 mm Hg between groups at the final time point (7 weeks).

The observation that OA-treated rats developed an elevation in BP does not prove that uric acid is responsible for the development of hypertension, because it is possible that the OA could be causing the BP elevation independent of its ability to raise uric acid. To determine whether the elevation in BP was because of the hyperuricemia and not a nonspecific effect of the OA, additional studies (experiments II and III) were performed in which rats on the LS/OA diet received either the xanthine oxidase inhibitor, allopurinol, or a uricosuric agent, benzoarodane, in their drinking water. Allopurinol (150 mg/L) administered from the initiation of the LS/OA diet prevented the development of both the hyperuricemia and hypertension (Figure 2A and 2B). In a separate experiment, benzoarodee was administered at an initial dose of 10 mg/kg per day that failed to lower uric acid levels in OA-treated rats. However, when the benzoarodane dose was increased to 15 mg/kg per day at the end of the second week, a decrease in the uric acid level occurred, followed by a reduction in BP (Figure 2C and 2D).

When the mean BP values for weeks 4 to 7 were correlated with the mean uric acid levels for the same period, a direct correlation was found (r = 0.75, P < 0.01; n = 69, rats for experiments II and III). An increase of 0.03 mmol/L (0.5 mg/dL) in uric acid correlated with an increase of 10 mm Hg in SBP. SBP was in the hypertensive range (SBP > 140 mm Hg) at uric acid levels of 0.125 mmol/L (2.2 mg/dL) or higher (Figure 2E).

We also examined whether reducing uric acid would reduce BP in animals that were already hypertensive (experiment IV). Specifically, rats were treated with LS/OA for 7 weeks, at which time, they were either continued on OA, treated with allopurinol (150 mg/L in drinking water) with continuation of the LS/OA diet, or had the OA withdrawn from the diet. As shown in Figure 3, lowering uric acid levels either by withdrawal of OA or adding allopurinol resulted in a reduction in the BP in association with a fall in serum uric acid values (Figure 3A and 3B).

Mild Hyperuricemia Causes Renal Fibrosis
To understand the mechanism for the hypertensive effect of hyperuricemia, we carefully examined the kidneys of the
TABLE 1. Hyperuricemic Rats Develop Renal Injury

<table>
<thead>
<tr>
<th>Type III Collagen, % Area Cortex</th>
<th>ED-1, cells/mm²</th>
<th>OPN, % Area Cortex</th>
<th>BUN, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp II: renal findings at 7 wk after OA in presence/absence of AP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (LS diet)</td>
<td>5.4±0.3</td>
<td>36.6±3.6</td>
<td>0.9±0.06</td>
</tr>
<tr>
<td>OA+LS diet</td>
<td>8.8±1.5*</td>
<td>54.4±3.8*</td>
<td>1.62±0.2*</td>
</tr>
<tr>
<td>OA/LS diet+AP</td>
<td>6.2±0.6</td>
<td>41.0±1.2</td>
<td>1.17±0.15*</td>
</tr>
<tr>
<td>Exp III: effect of OA withdrawal or addition of AP at 7 wk on renal findings at 11 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (LS diet)</td>
<td>7.2±0.6</td>
<td>55.6±2.8</td>
<td>0.7±0.08</td>
</tr>
<tr>
<td>OA+LS diet</td>
<td>13.9±0.6*</td>
<td>73.0±2.8*</td>
<td>1.98±0.28*</td>
</tr>
<tr>
<td>OA withdrawal</td>
<td>8.9±1.6*†</td>
<td>66.0±3.2†</td>
<td>1.08±0.06†</td>
</tr>
<tr>
<td>OA/LS diet+AP</td>
<td>9.6±0.3*†</td>
<td>50.4±5.2†</td>
<td>0.97±0.03†</td>
</tr>
</tbody>
</table>

Exp indicates experiment; AP, allopurinol; and TI, tubulointerstitial. Values are mean±SE.

**P<0.05 vs control; †P<0.05 vs OA+LS diet.

Hyperuricemic and control animals. At 7 weeks, the renal function (measured by BUN levels) of hyperuricemic rats in experiments I and II was normal, and routine light microscopy was unremarkable (Figure 1A) and exhibited no evidence of uric acid crystal deposition (Figure 1B). However, immunohistochemical stains revealed a striped pattern of early interstitial fibrosis, with increased interstitial collagen deposition, macrophage infiltration, and increased tubular expression of OPN, a sensitive marker of tubular injury.

To evaluate the effect of reducing uric acid levels on the progression of tubulointerstitial injury, a study was performed in which allopurinol was added or OA was withdrawn after 7 weeks of LS/OA diet. These rats were followed for an additional 4 weeks before they were euthanized (experiment IV). In this experiment, the persistently hyperuricemic rats exhibited more pronounced renal fibrosis and a statistically significant increase in BUN levels (Table 1). Rats in which the hyperuricemia was corrected by either the addition of allopurinol or by the withdrawal of OA showed significantly less renal fibrosis and lower BUN levels at the end of 11 weeks (Table 1 and Figures 3C through 3H).

Hyperuricemia Activates the Renin-Angiotensin System and Inhibits Intrarenal NOS1 Expression

The “striped” fibrotic pattern of renal injury is characteristic of chronic vasoconstriction and/or ischemia, which is of interest given the observation of Messerli et al that hyperuricemia in humans is associated with renal vasoconstriction. Therefore, we examined the renal expression of 2 important vasoactive mediators, renin and NO synthase (Table 2). The percentage of glomeruli with juxtaglomerular renin staining was markedly increased in the hyperuricemic animals. There was also a direct correlation of serum uric acid with the percentage of renin-positive glomeruli, both in the studies using the LS diet (n=11, 7 weeks; r=0.7, P<0.05) and a normal salt diet (n=12, 7 weeks; r=0.6, P<0.05). Saito et al have previously reported that uric acid levels are correlated with plasma renin activity in patients with essential hypertension.

We also examined the effect of hyperuricemia on NOS1 expression in the macula densa, which is involved in regulating afferent arteriolar tone and tubuloglomerular feedback. As shown in Table 2, the number of NOS1-positive cells in the macula densa was decreased in hyperuricemic rats. The decrease in NOS1-positive cells was prevented by allopurinol treatment (Table 2).

To further document a role for these mediators, we administered enalapril, an ACE inhibitor, or l-arginine, a substrate for NO production, to the hyperuricemic rats from the outset (experiment V). Placement of rats on the LS/OA diet resulted in an ∼40 mm Hg increase in SBP levels. In contrast, the use of both l-arginine and enalapril from the beginning of the diet prevented this increase in BP (Table 3). At 7 weeks, the SBP in the l-arginine and enalapril groups averaged 25 mm Hg less than the SBP in the hyperuricemic controls (P<0.05) (Table 3). L-Arginine and enalapril also ameliorated the renal injury when tissue was examined at this time point (Table 3). This demonstrates that the hypertension and renal disease induced by hyperuricemia are dependent on both the renin-angiotensin and NO systems.

Discussion

We developed an animal model of mild hyperuricemia to determine whether uric acid could modulate BP and/or induce renal injury. Uric acid levels in most mammals are lower than in humans because of the presence of uricase, a hepatic...
enzyme that degrades uric acid to allantoin.\textsuperscript{1} By administering low doses of a uricase inhibitor, OA, in the diet, we were able to induce mild hyperuricemia (an increase of 1.5- to 2-fold in serum uric acid levels) and avoid previous models in which marked hyperuricemia occurs with intrarenal urate crystal deposition leading to acute renal failure.\textsuperscript{9,10}

Our primary finding was that an elevation in BP developed after 3 weeks of hyperuricemia. The difference in BPs between hyperuricemic and control animals was most marked in rats placed on a LS diet. The increase of uric acid and BP levels was 0.5 vs control; and \( \dot{P} < 0.05 \) vs enalapril; and \( \dot{P} < 0.05 \) vs enalapril; and \( \dot{P} < 0.05 \) vs OA-LS diet.

Several lines of evidence suggested that uric acid is responsible for the BP elevation and for the tubulointerstitial injury. First, both the increase in BP and tubulointerstitial injury in OA-treated rats were prevented by lowering uric acid levels with the xanthine oxidase inhibitor, allopurinol, and with the uricosuric agent, benzbitorazone. Second, in rats already hyperuricemic and hypertensive, lowering uric acid with allopurinol also reduced BP and prevented the progression of interstitial fibrosis (Figure 3).

The mechanism for the hypertension and renal changes in our model involves the renin-angiotensin and NO systems. A striking finding in the present study was that juxtaglomerular renin content was increased and that macula densa NOS1 expression was reduced. These changes would be expected to result in both afferent and efferent arteriolar vasoconstriction, which are characteristic findings in many models of hypertension. Furthermore, blocking angiotensin II formation with enalapril and stimulating NO synthesis with L-arginine were able to largely prevent the hypertensive and renal changes. This strongly suggests that both angiotensin II and NO are involved in the pathogenesis of the hypertension and renal disease induced by uric acid.

Thus, these studies demonstrate that mild hyperuricemia in rats induces hypertension, as well as renal injury and fibrosis, through a crystal-independent mechanism mediated in part by activation of the renin-angiotensin system and downregulation of NOS1 expression in the macula densa. Although some caution is needed in interpreting the relevance of these animal studies to human disease, these observations may provide a mechanism to account for the association of uric acid with the development of hypertension\textsuperscript{15,17} and with chronic interstitial renal disease observed in hyperuricemic patients.\textsuperscript{17}

### References

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