Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia

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Departments of 1Nephrology and 2Pathology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico; 3Department of Medicine/Nephrology, University of Florida, Gainesville, Florida; and 4Renal Service and Laboratory, Hospital Universitario and Instituto de Investigaciones Biomédicas, Maracaibo, Venezuela

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Sánchez-Lozada LG, Soto V, Tapia E, Avila-Casado C, Sautín YY, Nakagawa T, Franco M, Rodríguez-Iturbe B, Johnson RJ. Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia. Am J Physiol Renal Physiol 295: F1134–F1141, 2008. First published August 13, 2008; doi:10.1152/ajprenal.00104.2008.—Endothelial dysfunction is a characteristic feature during the renal damage induced by mild hyperuricemia. The mechanism by which uric acid reduces the bioavailability of intrarenal nitric oxide is not known. We tested the hypothesis that oxidative stress might contribute to the endothelial dysfunction and glomerular hemodynamic changes that occur with hyperuricemia. Hyperuricemia was induced in Sprague-Dawley rats by administration of the uricase inhibitor, oxonic acid (750 mg/kg per day). The superoxide scavenger, tempol (15 mg/kg per day), or placebo was administered simultaneously with the oxonic acid. All groups were evaluated throughout a 5-wk period. Kidneys were fixed by perfusion and afferent arteriolar morphology, and tubulointerstitial 3-nitrotyrosine, 4-hydroxynonenal, NOX-4 subunit of renal NADPH-oxidase, and angiotensin II were quantified. Hyperuricemia induced intrarenal oxidative stress, increased expression of NOX-4 and angiotensin II, and decreased nitric oxide bioavailability, systemic hypertension, renal vasoconstriction, and afferent arteriolar pathology. Tempol treatment reversed the systemic and renal alterations induced by hyperuricemia despite equivalent hyperuricemia. Moreover, because tempol prevented the development of preglomerular damage and decreased blood pressure, glomerular pressure was maintained at normal values as well. Mild hyperuricemia induced by uricase inhibition causes intrarenal oxidative stress, which contributes to the development of the systemic hypertension and the renal abnormalities induced by increased uric acid. Scavenging of the superoxide anion in this setting attenuates the adverse effects induced by hyperuricemia.

REACTIVE OXYGEN SPECIES (ROS) play an important pathophysiological role in the development of hypertension and renal disease (43, 56, 58). The kidney and the vasculature are rich sources of NADPH oxidase-derived ROS, which under pathological conditions decrease the bioavailability of nitric oxide (NO), thus contributing to renal dysfunction and vascular damage (12, 49, 57). Superoxide can react with NO that can deplete the endothelium of this valuable vasodilator; in the process, peroxynitrite is generated, and that by itself is implicated in oxidative and nitrosating reactions (28). Moreover, because the kinetics and thermodynamics of the reaction between superoxide and NO are particularly favorable, the formation of peroxynitrite is inevitable in vivo (46).

On the other hand, uric acid (UA) and ascorbate (vitamin C) are considered the two major antioxidants in plasma (42). Furthermore, the ability of UA to preferentially react with peroxynitrite has been shown to improve certain neurological conditions associated with increased oxidative stress (16, 22, 41). However, the reaction of UA with peroxynitrite can also generate radicals, consistent with the ability of UA to become prooxidative under various circumstances (38). In addition, a frequent finding in humans is a remarkable association of hyperuricemia with hypertension, obesity/metabolic syndrome, kidney disease, and cardiovascular disease (17, 18, 50, 59).

Recent studies (21, 34) revealed that hyperuricemic rats have low plasma NO and suppressed urinary excretion of the final products of NO metabolism (NO2−/NO3−); these abnormalities in endothelial function were in association with renal vasoconstriction, glomerular and arterial hypertension, and preglomerular arteriolopathy. Stimulation of NO synthesis by L-arginine markedly increased urinary NO2−/NO3− (suggesting an increase in endothelial NO production), lowered systemic blood pressure, relieved cortical vasoconstriction, and prevented glomerular hypertension and preglomerular arteriolopathy (34). The mechanism by which UA reduces the availability of intrarenal NO is not known; however, increased oxidative stress could be implicated (39).

In effect, there is accruing evidence that part of the intracellular detrimental effects induced by UA are mediated by oxidative stress; for example, urate-induced monocyte chemotactrant protein 1 (MCP-1) expression in vascular smooth muscle cells was attenuated by antioxidants (19). More recently, we reported (39) that adipocyte differentiation was associated with increased uptake of UA and ROS accumulation; in this setting, increased concentrations of UA in the culture media induced a further increase in intracellular ROS production mediated by activation of NADPH oxidase. Intracellular NADPH oxidase-mediated oxidative stress induced activation of MAP kinases p38 and ERK1/2, an increase in protein nitrosylation and lipid oxidation, and a decrease in NO bioavailability (39). These effects were prevented by probenecid and benz bromarone (urate transport blockers), antioxidants, and NADPH oxidase inhibitors (39). In addition, Corry et al. (6) recently reported that UA induces oxidative stress and a depletion of intracellular NO in rat vascular smooth muscle...
cells. The reduction of intracellular NO in human vascular (endothelial and vascular smooth muscle cells) has also been reported (20).

We therefore studied the possible contribution of oxidative stress induced by experimental mild hyperuricemia on the development of arterial hypertension and the glomerular hemodynamic changes, as well as the renal microvascular alterations observed in this model. To test this hypothesis, we treated oxonic acid (OA) dosed rats with the superoxide dismutase mimetic tempol and evaluated the glomerular hemodynamics and the microvascular renal damage. In addition, changes in the expression of the markers of oxidative stress (3-NT) and 4-hydroxynonenal (4-HNE) and protein expression of NADPH oxidase subunit NOX-4 and angiotensin II (AII) were quantified.

MATERIALS AND METHODS

Three groups of male Sprague-Dawley rats were studied for a total period of 5 wk. To produce hyperuricemia, 14 animals received regular diet and OA (Sigma, St. Louis, MO) at a dose of 750 mg/kg body wt daily by gastric gavage. Seven rats were given Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1 oxyl; catalog no. 176141, Sigma-Aldrich), a stable membrane-permeable superoxide dismutase mimetic (15 mg/kg per day by gastric gavage), simultaneously with OA. An additional group of seven normal rats was studied as normal controls. Experiments were performed in accordance to the Mexican Federal Regulation for animal experimentation and care (NOM-062-ZOO-2001) and were approved by Bioethics and Investigation Committee of Instituto Nacional de Cardiologia Ignacio Chavez.

Measurements. Systolic blood pressure (SBP) was measured in conscious rats by tail cuff sphygmomanometer (XBP-1000; Kent Scientific, Torrington, CT). All animals were preconditioned for blood pressure measurements 1 wk before each experiment. Plasma UA was measured by phosphahtungstic acid method (Uricostat, Wiener, Argentina) in all animals at baseline and at the end of 4 wk. In urine samples taken during micropuncture, the excretion of the final products of NO metabolism (NO3-/NO2-) was measured. Samples were first incubated with Escherichia coli nitrate reductase to convert the NO3- to NO2-, as described previously (3, 14). After incubation, total NO2- was measured using the Griess reagent. Known concentrations of NaNO2 and NaNO3 were used as standards in each assay. Data were corrected by whole glomerular filtration rate (GFR) and are expressed as nmol/ml.

Micropuncture. At the end of 5 wk, animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and placed on a thermostated table to maintain body temperature at 37°C. Trachea, jugular veins, femoral arteries, and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50, and PE-10). The left kidney was exposed, placed in a Lucite holder, sealed with agar, and covered with Ringer's (catalog no. 06-284; Oxis International, Portland, OR); rabbit anti-rat NOX-4 polyclonal antibodies (catalog no. ab60940; Abcam, Cambridge, MA); and rabbit anti-4-HNE monoclonal antibodies (catalog no. 24325; Oxis International, Portland, OR); rabbit anti-4-HNE monoclonal antibodies (catalog no. ab60940; Abcam, Cambridge, MA); and rabbit anti-3-NT monoclonal antibodies (catalog no. 06-284; Upstate, Lake Placid, NY); mouse anti-4-HNE monoclonal antibodies (catalog no. 24325; Oxis International, Portland, OR); rabbit anti-3-NT monoclonal antibodies (catalog no. ab60940; Abcam, Cambridge, MA); and rabbit anti-4-HNE monoclonal antibodies (catalog no. T-4007; Bachem, Torrance, CA). In brief, 3-NT and 4-HNE, as AII were quantified by immunohistochemistry and morphometry using the following antibodies: rabbit anti-3-NT polyclonal antibodies (catalog no. 06-284; Upstate, Lake Placid, NY); mouse anti-4-HNE monoclonal antibodies (catalog no. 24325; Oxis International, Portland, OR); rabbit anti-rat NOX-4 polyclonal antibodies (catalog no. ab60940; Abcam, Cambridge, MA); and rabbit anti-3-NT monoclonal antibodies (catalog no. T-4007; Bachem, Torrance, CA). Whole glomerular filtration rate (GFR) was calculated using the following formula:

\[
GFR = \frac{(U \times V)}{P}.
\]

Where U is the polyfructosan concentration in urine, V is urine flow rate, and P is the polyfructosan concentration in plasma. The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of tubular polyfructosan was measured by microfluorometric method of Vurek and Pegram (53). Single-nephron glomerular filtration rate (SNGFR) was calculated using the formula:

\[
\text{SNGFR} = \frac{\text{TF}/P_{\text{prf}} \times V}{\text{TF} + P_{\text{prf}}},
\]

where \(\text{TF}/P_{\text{prf}}\) is the ratio of concentration of polyfructosan in tubular fluid (TF) and plasma (P), and V is the tubular flow rate that is obtained by timing the collection of tubular fluid (4). Protein concentration in afferent and efferent samples was determined according to the method of Viets et al. (52). MAP, GFR, glomerular capillary hydrostatic pressure (PGC), single-nephron plasma flow (QA), afferent (AR), efferent (ER), and total resistances, and ultrafiltration coefficient \((K_t)\) were calculated using equations previously reported (4).

Statistical analysis. Values were expressed as means ± SE. Differences between groups were evaluated by ANOVA with appropriate correction for multiple comparisons (Bonferroni). The relation between variables was assessed by correlation analysis.

RESULTS

General parameters. Body weight was comparable in all groups of rats at the end of the study [normal (N) = 333 ± 6 g; OA = 353 ± 17 g; and OA + Tempol = 350 ± 7 g], suggesting that tempol treatment did not affect food consumption. After 5 wk of OA administration, rats developed hyperuricemia (N = 1.2 ± 0.20 mg/dl; OA = 3.33 ± 0.20 mg/dl; P <
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Fig. 1. Effect of antioxidant therapy with tempol to oxonic acid (OA)-treated rats on uric acid, blood pressure, glomerular filtration rate (GFR), urinary excretion of nitric oxide (NO) metabolites (NO2⁻/NO3⁻), single nephron GFR, and glomerular pressure.

0.001 vs. N, Fig. 1) and systemic hypertension (N = 122 ± 2.2 mmHg; OA = 149 ± 4.7 mmHg; P < 0.001 vs. N, Fig. 1) compared with normal control rats (34). The group that received tempol had similar values of UA as the OA control group at the end of Week 4 (UA = 2.96 ± 0.24 mg/dl, P < 0.001 vs. N, P = ns vs. OA, Fig. 1); however, SBP values were significantly lower relative to hyperuricemic rats (131 ± 2.1 mmHg, P < 0.01 vs. OA, Fig. 1).

Glomerular hemodynamics. Urinary excretion of NO2⁻/NO3⁻ corrected for whole GFR is shown in Fig. 1. In OA group, the excretion of NO stable end products was suppressed compared with normal control rats (N = 5.1 ± 0.6 nmol/ml GFR; OA = 0.30 ± 0.1 nmol/ml GFR, P < 0.001 vs. N, Fig. 1) (34). Chronic treatment with tempol maintained urinary excretion of NO2⁻/NO3⁻ in values equivalent to the normal group (5.0 ± 1.5, P < 0.01 vs. OA, Fig. 1).

In the hyperuricemic (OA) group, MAP was significantly higher (N = 123 ± 1 mmHg; OA = 149 ± 2 mmHg; P < 0.001), whereas whole GFR tended to be lower compared with normal control animals (N = 0.84 ± 0.1 ml/min; OA + V: 0.66 ± 0.1 ml/min; P = ns) (34). Scavenging of superoxide with tempol partially prevented the rise of MAP (136 ± 5 mmHg, P < 0.01 vs. OA and N) and preserved whole GFR in normal values (0.78 ± 0.1 ml/min).

Hyperuricemic rats developed cortical vasoconstriction as indicated by reduced single nephron GFR (Table 1, Fig. 1) and ultrafiltration coefficient (Table 1); glomerular plasma flow tended to be lower, although it did not reach statistical significance (Table 1) (34). AR and ER increased significantly because of hyperuricemia as well (Table 1) (34). In addition, despite cortical vasoconstriction, hyperuricemic rats suffered glomerular hypertension (Table 1, Fig. 1) (34).

Treatment with tempol prevented glomerular hemodynamic alterations induced by hyperuricemia; single nephron GFR (Table 1, Fig. 1), glomerular plasma flow, ultrafiltration coefficient, afferent and efferent arteriolar resistances (Table 1), and glomerular pressure (Table 1, Fig. 1) remained at normal values.

Afferent arteriole morphology, markers of intrarenal oxidative stress, and NOX-4 and AII renal expression. Figs. 2 and 3 show the results of pregglomerular arteriolar morphological evaluation and tubulointerstitial markers of oxidative stress. OA-induced hyperuricemia was associated with arteriolar wall thickening (34). Chronic administration of tempol maintained pregglomerular vessel morphology similar to normal rats.

Table 1. Glomerular hemodynamics in normal, oxonic acid, and oxonic acid + tempol rats

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>PGC, mmHg</th>
<th>SNGFR, nl/min</th>
<th>QA, nl/min</th>
<th>AR, dyn·s·cm⁻⁵</th>
<th>ER, dyn·s·cm⁻⁵</th>
<th>Kf, nl·s⁻¹·mmHg⁻¹</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>123±1.4</td>
<td>48±1.2</td>
<td>28±1.5</td>
<td>95±6.5</td>
<td>3.2±0.2</td>
<td>1.8±0.2</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td>Oxonic acid</td>
<td>149±2.4</td>
<td>57±1.5</td>
<td>18±1.7</td>
<td>69±6.7</td>
<td>5.77±0.7</td>
<td>3.11±0.3</td>
<td>0.01±0.002</td>
</tr>
<tr>
<td>OA + tempol</td>
<td>136±4.7</td>
<td>48±1.8</td>
<td>28±1.8</td>
<td>126±10.7</td>
<td>2.97±0.2</td>
<td>1.28±0.1</td>
<td>0.03±0.005</td>
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<tr>
<td>N vs. OA</td>
<td>0.001</td>
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<td>0.01</td>
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<td>N vs. OA + tempol</td>
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<td>OA vs. OA + tempol</td>
<td>0.05</td>
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Applicable values are means ± SE. MAP, mean arterial pressure; QA, glomerular plasma flow; PGC, glomerular capillary hydrostatic pressure; SNGFR, single-nephron glomerular filtration rate; AR, afferent resistance; ER, efferent resistance; Kf, ultrafiltration coefficient; OA, oxonic acid; N, normal.
Peroxynitrite can produce novel products such as 3-NT that serve as an important biological marker in vivo (32). Hyperuricemic rats showed an increased immunostaining of 3-NT in tubulointerstitial areas of 2.7 times relative to normal rats. Tempol treatment partially reduced 3-NT immunostaining.

4-HNE is a biologically active aldehyde produced during membrane lipid peroxidation in inflammation and oxidative stress (8). We studied intrarenal lipid peroxide using an anti-4-HNE antibody. Tubulointerstitial staining of 4-HNE was significantly enhanced in OA-treated rats; an increase of 3.9 times relative to normal rats was observed. Scavenging of superoxide radical in hyperuricemic rats improved lipid peroxidation.

Because NOX-4 subunit functions as the catalytic moiety of the NADPH-oxidase enzyme, changes in the expression of this subunit is of utmost importance during physiopathological conditions (26). We selected to evaluate NOX 4 because this isoform is highly expressed in the proximal convoluted tubule cells of the renal cortex (11) and was previously shown that increased levels of UA upregulated the expression of this isoform in adipocytes (39). We observed that NOX-4 immunostaining was increased 2.76 times in hyperuricemic rats relative to normal rats; simultaneous treatment with tempol reduced significantly NOX-4 overexpression.

Previous studies have shown that the intrarenal AII content correlates closely with quantification of renal AII immunostaining by computer image analysis (9). On the other hand, it has been previously shown that OA administration induced the overexpression of renin in juxtaglomerular cells (24), a finding that suggests the possibility that AII synthesis might be increased in hyperuricemic rats. Consistent with this hypothesis, renal AII was significantly increased in hyperuricemic rats; moreover, when we analyzed both normal and OA-dosed rats together, a strong linear correlation between UA and AII was observed ($r = 0.89, P < 0.0001$). In addition, as was previously reported in cyclosporin A (CsA)-treated rats (27), tempol treatment prevented renal AII overexpression in hyperuricemic rats.

Finally, the following correlations were observed: UA vs. 3-NT ($r = 0.56, P = 0.03$), urinary excretion of NO$_2$/NO$_3$ vs. 3-NT ($r = -0.66, P = 0.02$), NOX-4 vs. 3-NT ($r = 0.85, P < 0.0001$), AII vs. NOX-4 ($r = 0.49, P = 0.03$) vs. 4-HNE ($r = -0.78, P = 0.002$), single nephron GFR vs. 3-NT ($r = -0.69, P = 0.005$), PG C and 3-NT vs. 4-HNE ($r = 0.92, P < 0.0001$).

**DISCUSSION**

In the present study, we tested the hypothesis that ROS might contribute to the endothelial dysfunction and systemic and renal hemodynamic changes associated with experimentally induced hyperuricemia. This hypothesis was based on cell-culture studies that UA may function as a prooxidant under certain circumstances (6, 19, 39) and the possibility that the reduction in NO could be mediated by an increase in oxidative stress (5).

The principal finding was that OA-induced hyperuricemia triggered intrarenal oxidative stress revealed by significant increments of NOX-4 protein expression, protein nitration, and lipid peroxidation; moreover, these alterations occurred in concert with AII renal overexpression. These findings suggest that the excessive synthesis of ROS contributed to the decreased bioavailability of NO, as was indicated by the suppression of the excretion of NO metabolites, as well as the development of systemic hypertension, renal cortical vasoconstriction, and intrarenal microvascular damage. Chronic scavenging of superoxide with tempol was able to prevent the systemic and renal alterations induced by hyperuricemia despite plasma UA remaining elevated. More-
over, because tempol treatment prevented the development of preglomerular damage and decreased blood pressure, glomerular pressure was maintained at normal values as well.

An imbalance between prooxidants and antioxidants results in oxidative stress, which is the pathogenic consequence of increased oxidant synthesis that overcomes the cellular antioxidant capacity. Superoxide anion can be generated in the

Fig. 3. Representative micrographs of afferent arteriole morphology, tubulointerstitial markers of oxidative stress, 3-NT and 4-HNE, and NOX-4 and AII expressions in normal, oxonic acid and oxonic acid + tempol-treated rats (all 400×, except NOX-4, 200×).
kidney by xanthine oxidoreductase (XO) or NADPH oxidase (56). However, both enzymes apparently are regulated differently; for example, in AII-infused rats (54) and during chronic renal disease (51), NADPH oxidase is upregulated, at least in renal cortex; in contrast, XO remain unchanged or is downregulated (51, 54). NADPH oxidase is also upregulated in the kidney of animals with diabetes (31). On the contrary, in the genetic model of essential hypertension [spontaneously hypertensive rats (SHR)], both XO and NADPH oxidase are upregulated (2, 23). Recently we reported that UA was able to stimulate NADPH oxidase with oxidant generation, reduction in NO levels, and the formation of peroxynitrite in cultured adipocytes (39). Moreover MCP-1 upregulation and NF-κB activation induced by UA have been shown to be inhibited by antioxidants in vascular muscle cells (19) and proximal tubular cells, respectively (15). A new finding resulting from the present studies was that mild hyperuricemia induced a significant increase of NOX-4 protein expression in kidney, as was previously observed in adipocytes (39), suggesting that increased production of superoxide radical derived from this enzyme may have a role in the renal damage induced by hyperuricemia. However, we cannot rule out the participation of different sources of oxygen radicals in this pathology.

One potential mechanism by which UA might induce oxidative stress could be by stimulation of the renin angiotensin system (RAS). Corry et al. (6) also recently demonstrated that UA stimulated proliferation, AII production, and oxidative stress and decreased NO bioavailability in vascular smooth muscle cells; these effects were prevented by the administration of losartan or captopril, suggesting that they are mediated by the activation of RAS. We have also reported that hyperuricemia activates the intrarenal RAS in rodents (24); in this model, the administration of enalapril improved systemic hypertension, renal structural damage, and plegmerular arteriolopathy in hyperuricemic rats (24, 25). Toma et al. (48) have also reported that UA causes immediate renin release, utilizing an elegant afferent arteriole-isolated glomerular preparation. Furthermore, hyperuricemia is closely linked to elevated plasma renin activity in humans (30). In turn, AII is a strong activator of renal and vascular NADPH oxidase (33); this fact suggests that superoxide-mediated oxidative stress during hyperuricemia likely resulted from the direct and/or indirect activation of intrarenal NADPH oxidase. In effect, in the present study, we found that hyperuricemia induced a significant increment of intrarenal AII expression; furthermore, a strong linear correlation between UA and AII was noted when the analysis included both normal and OA-dosed groups (r = 0.89, P < 0.0001). Interestingly, it has been reported that tempol increased the expression of renin in SHR (55); in contrast it prevented the increment in the expression of renal AII in CsA-treated rats through an unknown mechanism (27). In the present studies, we confirmed that tempol prevented the intrarenal overexpression of AII in OA-dosed rats. In addition, we found a mild but positive correlation between AII and NOX-4 (r = 0.49, P = 0.03). These findings suggest that AII is partially mediating the increment in the expression of NOX-4; however, it is likely that other mechanisms are participating as well.

Several studies suggest that the increased production of superoxide is implicated in the decreased bioavailability of NO in several experimental models of renal damage and hypertension as well as in human hypertension (10, 40, 47, 54). A reliable approach to detect the interaction between NO and superoxide is the formation of peroxynitrite, which can be evaluated by documenting the presence of 3-NT residues (32). In our studies, increased immunostaining of 3-NT in hyperuricemic rats suggests that endothelial dysfunction is related to the excessive production of superoxide; moreover, we found a correlation between UA and 3-NT (r = 0.56, P = 0.03) and negative correlations between urinary excretion of NO_2/NO_3 with 3-NT (r = −0.66, P = 0.02) and with 4-HNE (r = −0.78, P = 0.002).

In the present report as well as in a previous study (34), we reported an unusually suppressed urinary excretion of NO metabolites in OA-treated rats. A possible explanation for this outcome is that NO_2/NO_3 metabolites are extensively reabsorbed in the proximal tubule (45) but with the maintenance of a linear relationship between plasma NO_2/NO_3 levels and its urinary excretion rate (13). Interestingly, plasma concentrations of sodium nitrate in the lowest range were associated with the lowest fractional excretion and the highest tubular reabsorption rates (13). Since it was previously shown that OA-dosed rats have decreased plasma concentrations of NO metabolites in plasma (21), its tempting to speculate that increased tubular reabsorption rates of nitrates occurred in hyperuricemic animals, resulting in an almost suppressed excretion of this metabolite in this condition.

The reaction of NO with superoxide has been shown to limit the relaxing effect of the former on afferent arterioles (54). In the present study, scavenging of superoxide with tempol prevented the development of renal vasconstriction in hyperuricemic rats; in addition, a negative correlation between 3-NT and single nephron GFR was observed (r = −0.69, P = 0.005). We previously showed that supplementation of L-arginine (NO synthase substrate) to hyperuricemic rats augmented the synthesis of NO (34); that effect likely overcame the increased production of superoxide, improving renal vasodistraction.

Another marker of increased oxidative stress is lipid peroxidation (8). Hyperuricemic rats showed increased immunostaining of 4-HNE. In this regard, there are reports indicating that UA is able to amplify the oxidation of lipids (1, 29); moreover, in cultured adipocytes, UA was associated with the oxidation of membrane lipids secondary to the activation of NADPH oxidase (39). On the other hand, peroxynitrite is a potent oxidation species that has been found to cause lipid peroxidation per se (32). In the present study, treatment with tempol to hyperuricemic rats significantly reduced the immunostaining of 4-HNE; furthermore, we found a strong positive correlation between the renal immunostaining of 3-NT and 4-HNE (r = 0.92, P < 0.0001).

We have reported that one of the main mechanisms associated with the renal damage induced by hyperuricemia is the alteration of autoregulatory capacity in plegmerular vessels that allows the transmission of systemic hypertension to glomerular capillaries (34–37); this defect is linked to the microvascular lesion induced by UA in the afferent arteriole (25). Glomerular pressure is regulated by the relative contribution of AR and ER as well as by arterial pressure. Thus glomerular hypertension may result from a proportional increment of ER relative to AR, or alternatively due to an inadequate autoregulatory response of AR to an increment in systemic pressure. In
the present study, we observed that both normal and OA-dosed animals had the same ER/AR ratio (normal 0.55 ± 0.03, OA 0.56 ± 0.03), indicating a similar level of ER relative to AR in both groups. In contrast, we observed a mild but significant linear relationship between both systolic and MAP vs. glomerular pressure (SBP vs. PGC r = 0.45, P = 0.04; MAP vs. PGC r = 0.48, P = 0.02). This finding suggests the transmission of systemic hypertension to glomerular capillaries, as was previously reported by us in hyperuricemic rats and by others in fawn-hooded rats (44). Thus, in OA-treated rats, we have the unusual situation of renal vasoconstriction and apparent arteriopathy coexisting with glomerular hypertension. In the present study, antioxidant therapy to hyperuricemic rats prevented the rise of glomerular pressure; this beneficial effect was likely the consequence of the preservation of the integrity of preglomerular vessels and a lower systemic pressure.

In conclusion, mild hyperuricemia is associated with intrarenal oxidative stress, which contributes to the development of the systemic hypertension and the renal abnormalities induced by increased UA. Moreover, scavenging of superoxide anion attenuated the adverse effects induced by increased UA. Further studies will be necessary to determine the relevance of these findings in the setting of hyperuricemia-associated cardiovascular and renal diseases in humans.

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