Effect of elevated serum uric acid on cisplatin-induced acute renal failure

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Effect of elevated serum uric acid on cisplatin-induced acute renal failure. Am J Physiol Renal Physiol 292: F116–F122, 2007; doi:10.1152/ajprenal.00160.2006.—Marked hyperuricemia is known to cause acute renal failure via intrarenal crystal deposition. However, recent studies suggest mild hyperuricemia may have vasoactive and proinflammatory effects independent of crystal formation. We therefore tested the hypothesis that mild hyperuricemia might exacerbate renal injury and dysfunction in a model of cisplatin-induced acute renal failure in the rat. Cisplatin was administered to normouricemic and hyperuricemic rats (the latter generated by administering the urate oxidase inhibitor, oxonic acid). Recombinant urate oxidase (rasburicase) was administered in a third group to assess the effect of lowering uric acid on outcomes. Other control groups include normal rats and hyperuricemic rats without cisplatin-induced injury. Cisplatin induced injury of the pars recta (S3) segment of the proximal tubule in association with a mild monocyte infiltration. Hyperuricemic rats showed significantly greater tubular injury and proliferation with significantly greater macrophage infiltration and increased expression of monocyte chemoattractant protein-1. However, renal function was not different between normouricemic and hyperuricemic rats with cisplatin injury. Treatment with rasburicase reversed the inflammatory changes and lessened tubular injury with an improvement in renal function (relative to the hyperuricemic group). No intrarenal crystals were observed in any groups. These data provide the first experimental evidence that uric acid, at concentrations that do not cause intrarenal crystal formation, may exacerbate renal injury in a model of acute renal failure. The mechanism may relate to a proinflammatory pathway involving chemokine expression with leukocyte infiltration.

urate oxidase; rasburicase

ACUTE RENAL FAILURE (ARF) is a common complication associated with the treatment of cancers and is associated with a marked increased risk for mortality (19). While there are numerous etiologies, one of the more common causes is the tumor lysis syndrome (20). While classically observed with hematological malignancies, tumors lysis may also occur with the treatment of solid cancers (5, 8) and can also rarely develop spontaneously in the absence of any treatment (1).

The mechanism by which tumor lysis syndrome causes ARF is thought to be multifactorial (20). The rapid destruction of tumor cells leads to a release of their intracellular contents into the circulation with a marked rise in serum potassium and phosphate. The increased nucleotide release and turnover result in increased synthesis of uric acid, which may rise to levels of 12 mg/dl or more (20). While renal biopsy is not typically performed, renal failure is thought to result primarily from the precipitation of uric acid, xanthine, and calcium phosphate in the renal tubules (20). Support for a key role for uric acid in causing the ARF is provided by animal models in which experimentally induced hyperuricemia has been shown to lead to marked uricosuria with intratubular crystal deposition, tubular obstruction, and a marked local inflammatory response (17, 18). Prevention of ARF in subjects undergoing chemotherapy is largely aimed at maintaining adequate hydration, reducing serum uric acid levels, and alkalinization of the urine (which promotes urate solubilization) (20). Recently, the use of recombinant urate oxidase, rasburicase, has been shown to be markedly effective at lowering uric acid and appears to be superior to allopurinol in promoting diuresis and improving renal function in these patients (32).

As discussed above, the mechanism by which hyperuricemia may predispose subjects to ARF is thought to be due exclusively to the precipitation of urate crystals within the renal tubules, resulting in a type of obstructive uropathy (20). However, there has been the recent recognition that experimental hyperuricemia may have numerous proinflammatory and vasoconstrictive effects independent of intrarenal crystal deposition (21, 33, 34, 39). Soluble uric acid has been shown to inhibit endothelial cell proliferation and migration and to inhibit endothelial nitric oxide bioavailability (14, 16, 26). Experimental hyperuricemia also causes profound renal vasoconstriction (18, 34) and over time both causes and exacerbates chronic renal injury (15, 27). Furthermore, uric acid can activate inflammatory cells (2), stimulate monocyte chemoattractants (13), and induce pro-oxidative effects in vascular cells (13). Uric acid may also have a key role in the dendritic cell activation to injured cells (36). Interestingly, under certain conditions uric acid may also function as an antioxidant with potential cytoprotective qualities (2).

We therefore tested the hypothesis that an elevation of uric acid, at concentrations that do not promote intrarenal crystal deposition, might exacerbate renal injury and dysfunction in a model of ARF. The model of ARF we selected was that induced by cisplatin (CP) in which proximal tubular injury is thought to be due to a combination of direct cytotoxicity, intrarenal vasoconstriction, and oxidative stress (9, 23). Interestingly, pretreatment uric acid levels predict the risk for azotemia in CP-treated patients (29). Furthermore, serum uric acid also rises following CP treatment although to levels not associated with intrarenal crystal deposition (from 6.1 ± 1.0 to 8.3 ± 1.3 mg/dl in one study) (30). However, uric acid levels

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in this range are known to be associated with significant systemic endothelial dysfunction which can be improved by lowering serum uric acid (22).

**MATERIALS AND METHODS**

**Experimental design.** Adult male Sprague-Dawley rats (200–250 g, Charles River Laboratories, Wilmington, MA) were housed in individual metabolic cages and fed a standard diet (Harlan Teklad, Madison, WI) with water ad libitum. After 1 wk, rats were randomized into five groups. Group I (CP group) consisted of six rats that received a single dose of CP (5 mg/kg body wt; Sigma, St. Louis, MO) in normal (0.9%) saline. Group II (CP-OA group) consisted of six rats that received CP but also were made acutely hyperuricemic by the daily gavage of the urate oxidase inhibitor, oxonic acid (OA; Sigma). The OA was administered at a dose of 750 mg/kg body wt in 0.5 ml of 0.25% methyl cellulose solution for 5 consecutive days beginning immediately after injection of the CP. Group III (CP-OA/raasburicase) consisted of six rats with CP-OA and also received intraperitoneal injection of human recombinant urate oxidase (25 mg/kg rasburicase, Sanofi-Aventis, Paris, France) in vehicle for 5 consecutive days.

**Group IV** (OA, n = 6) received daily gavage with methylcellulose without OA; similarly, group I also received daily vehicle by gavage. All animal procedures were approved by the Animal Care Committee at the University of Florida, Gainesville, Florida.

**Renal function.** Urine was collected between days 4 and 5 by housing rats in metabolic cages with free access to water, but not to food, for 16 h. At day 5 rats were killed under isoflurane anesthesia (Webster Veterinary Supply, Bessemer, AL) via cardiac exsanguination and the serum was separated for the measurement of renal function. Blood urea nitrogen (BUN), serum creatinine and sodium, urine creatinine, protein and sodium excretion were determined using a VetACE automated biochemistry machine (Alfa Wassermann, West Caldwell, NJ). Creatinine clearance rates (CrCl) and uric acid clearance rates (CUA) were calculated as \( C_{\text{Cr}} \times V/\text{Plcr} \), where \( U \) and \( P \) denote urinary and serum creatinine concentrations and \( V \) represents volume of urine in millimeters per minute, respectively. Serum and urine uric acid were measured by a modified carbonate-phosphotungstate method (12).

**Renal histology and quantification of morphology.** Methyl Carnoy’s fixed tissue was processed and paraffin embedded, and 3-μm sections of renal tissues were stained with periodic acid-Schiff (PAS) reagent and hematoxylin-eosin. Alcohol-fixed tissue was processed and paraffin embedded and 7-μm sections were stained for uric acid crystals using De Galantha stain. As a positive control, we utilized kidney tissue from a rat with severe hyperuricemia and acute urate nephropathy induced with combined OA and uric acid administration (17, 18, 21).

All histological analyses were performed blinded by a renal pathologist (B. Croker). For each kidney biopsy specimen, a specific analysis was made of coronal sections of specific tubular segments, including the proximal convoluted tubule in the cortex, the proximal straight tubule (pars recta or S3 segment) in the outer stripe of the outer medulla (OSOM), the medullary thick ascending limb (mTAL) in the inner stripe of the outer medulla (ISOM), and the collecting duct from the cortex to the inner medulla. For each tubular segment, a tubular injury score was determined based on the presence of tubular cell swelling, brush-border loss, nuclear condensation, and karyolysis. In addition, the peritubular capillaries and interstitium were evaluated for an interstitial inflammatory score based on the presence of interstitial cell infiltration and capillaritis. Each score was graded from 0 to 5, with 0 = no changes present; 1 < 10%; 2 = 10 to 25%; 3 = 25 to 50%; 4 = 50 to 75%; and 5 = >75% changes in the specific region.

**Immunohistochemistry.** Monococyte macrophage infiltration was identified by indirect immunoperoxidase using the ED-1 monoclonal antibody (BD Pharmingen, San Diego, CA) and proliferating cells were identified using anti-PCNA antibody (Sigma). Bound primary antibodies were detected with appropriate secondary antibodies (Rockland Immunocchemicals, Gilbertsville, PA), followed by mouse peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16). Negative controls consisted of omission of the primary antibody with the appropriate peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16). Negative controls consisted of omission of the primary antibody with the appropriate peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16). Negative controls consisted of omission of the primary antibody with the appropriate peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16). Negative controls consisted of omission of the primary antibody with the appropriate peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16). Negative controls consisted of omission of the primary antibody with the appropriate peroxidase anti-peroxidase (DAKO, Carpinteria, CA) and diamino-benzidine substrate to generate a brown color (16).

For quantification of immunohistochemistry staining, stained sections were imaged using an Axioplan 2 imaging microscope (Carl Zeiss, Munich, Germany) and analyzed using Zeiss AutoMeasure software (Axiovision 4.1, Carl Zeiss). Single-image frames (700 × 550 μm) were captured at ×100 magnification, and the entire outer medullary region was measured. The mean percentage of each scanned area with positive staining was recorded. All analyses were performed by a blinded observer.

**RNA isolation, reverse transcription, and real-time PCR.** Kidney tissues were frozen in liquid nitrogen for RNA and protein 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. Reverse transcription reactions were performed in a one-step protocol using a iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California). Reverse transcription yields were confirmed by PCR using specific primers for each target gene.

**Materials.** All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

**Table 1. Uric acid and renal function**

<table>
<thead>
<tr>
<th>Group</th>
<th>UA, mg/dl</th>
<th>BUN, mg/dl</th>
<th>Cr, mm/dl</th>
<th>Ccr, mg/min·1.73 m–2</th>
<th>UUA, mg/24 h</th>
<th>CUA, mg/min·1.73 m–2</th>
<th>UAB, mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.0±0.4</td>
<td>14.0±0.9</td>
<td>0.3±0.04</td>
<td>3.3±0.6</td>
<td>2.2±1.3</td>
<td>0.08±0.02</td>
<td>11.5±7.9</td>
</tr>
<tr>
<td>OA</td>
<td>2.9±0.8</td>
<td>14.0±0.6</td>
<td>0.3±0.1</td>
<td>3.7±1.4</td>
<td>3.5±1.4</td>
<td>0.09±0.03</td>
<td>10.2±7.1</td>
</tr>
<tr>
<td>CP</td>
<td>2.6±0.6</td>
<td>65.0±18.6</td>
<td>1.6±0.4</td>
<td>0.6±0.3</td>
<td>1.1±1.2</td>
<td>0.03±0.02</td>
<td>18.0±7.7</td>
</tr>
<tr>
<td>OA/CP</td>
<td>5.7±2.0</td>
<td>89.8±38.7</td>
<td>2.0±0.6</td>
<td>0.4±0.2</td>
<td>4.0±1.4</td>
<td>0.05±0.03</td>
<td>19.3±6.3</td>
</tr>
<tr>
<td>OA/CP/raasburicase</td>
<td>2.9±1.4</td>
<td>52.6±7.4</td>
<td>1.1±0.1</td>
<td>0.9±0.3</td>
<td>4.4±1.4</td>
<td>0.12±0.02</td>
<td>19.7±7.0</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 in each group. OA, rats fed oxonic acid; CP, rats injected with cisplatin; OA/CP, rats fed oxonic acid, injected with cisplatin; OA/CP/uricase, rats fed oxonic acid, injected with uricase; UUA, serum uric acid; BUN, serum urea nitrogen; Ccr, serum creatinine; CUA, uric acid clearance; UAB, uric acid; UUA, serum uric acid; BUN, serum urea nitrogen; Ccr, serum creatinine; CUA, uric acid clearance; UAB, serum albumin excretion; NS, not significant. Note: To convert serum creatinine in mg/dl to mmol/l, multiply by 88.4; urea nitrogen in mg/dl to mmol/l, multiply by 0.357; uric acid in mg/dl to mmol/l, multiply by 59.48; albumin in mg/dl to g/l, multiply by 10.
Histological changes in rat kidney

Fig. 1. Proliferating (PCNA+) cells in cisplatin (CP)-induced acute renal failure shown in a histogram of the relative numbers of proliferating cells in the outer medulla of rats with CP-induced renal injury and controls. Significantly greater PCNA-positive cells were observed in hyperuricemic rats with CP-induced renal injury compared with normouricemic and uricase-treated rats. The majority of these cells were tubular. (Measured by comparing relative % area positive staining using computer image analysis; control is set at 100.) OA, oxonic acid.

CA) according to the manufacturer’s protocol. Reactions were incubated at 25°C for 5 min, 42°C for 30 min, 85°C for 5 min, and cooled at 4°C in a Thermocycler (Eppendorf, Hamburg, Germany). Primers of monocyte chemotactic protein-1 (MCP-1) were designed by Genetool software (BioTools, Alberta, Canada).

Real-time PCR analyses were performed using an Opticon PCR machine (MJ Research, Waltham, MA). The SYBR Green master mix kit (Bio-Rad Laboratories) was used for all reactions with real-time PCR. Briefly, PCR was performed at 94°C for 2 min followed by 40 cycles of denaturation, annealing, and extension at 94°C for 30 s, 55°C for 30 s, 72°C for 60 s, respectively, and final extension at 72°C for 10 min. GAPDH primers were used as a housekeeping gene to allow quantification. All PCR reactions for each sample were performed in duplicate. Ratios for each of MCP-1/GAPDH mRNA were calculated for each sample and are expressed as means ± SD.

Measurement of renal MCP-1 protein by ELISA. Renal MCP-1 levels were measured by a commercially available ELISA kit on kidney tissue samples (BD Pharmingen). Briefly, whole kidney was snap-frozen in liquid nitrogen at death and ground into powder with a pestle on dry ice. Kidney tissue powder (BD Pharmingen) was sonicated in 500 μl of lysis buffer (Cell Signaling Technology, Danvers, MA) and centrifuged at 10,000 g for 30 min at 4°C. Protein in the supernatant was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories). The well was coated with capture antibody for MCP-1 in carbonate bicarbonate buffer (pH 9.6) overnight at 4°C. The wells were washed three times with PBS containing 0.5% Tween 20 (PBST) and blocked with PBS containing 5% bovine serum albumin for 1 h at room temperature (RT). One hundred microliters of standards or rat whole kidney homogenates were added into wells and incubated for 2 h at RT. After being washed, 100 μl of detection antibody and enzyme mixture were removed by seven consecutive washes with PBST. Finally, 100 μl of substrate solution were added to each well and the plate was incubated in the dark at RT for 30 min. The reaction was stopped by addition of 50 μl of stop solution and the absorbance was measured at 405 nm with a microplate scanning spectrophotometer (Powerwave 200; BIO-TEK Instruments, Winooski, VT).

Statistical analysis. All data are presented as means ± SD. Differences in the various parameters in more than two groups were evaluated by a one-way ANOVA. Continuous variables between two groups were analyzed using Student’s t-test. Significance between groups was defined as P < 0.05.

RESULTS

The study was aimed at testing the hypothesis that mild hyperuricemia, at concentrations that do not lead to intrarenal crystal deposition, might exacerbate renal injury and dysfunction in a model of CP-induced ARF in the rat. To test this hypothesis, we induced mild hyperuricemia in rats with OA, which is a urate oxidase inhibitor. As shown in Table 1, normal rats in this study had serum uric acid levels of 2 mg/dl, whereas serum uric acid levels were ~2.9 mg/dl (P < 0.05) with OA treatment in association with an increase in urinary uric acid excretion. Serum uric acid increased spontaneously in rats with CP-induced ARF (serum uric acid 2.6 ± 0.6 mg/dl) likely due to a reduction in urinary uric acid excretion. Serum uric acid levels were highest in CP-treated rats that also received OA (serum uric acid 5.7 ± 2.0 mg/dl) but this was reduced by 50% in those animals that received rasburicase (P < 0.05 vs. CP-OA). While urinary uric acid excretion increased in all rats receiving OA, the mean increase was only

Table 2. Histological changes in rat kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular Injury</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT (S3)</td>
<td>CD</td>
</tr>
<tr>
<td>Control</td>
<td>0.0±0</td>
<td>0.0±0</td>
</tr>
<tr>
<td>OA</td>
<td>0.0±0</td>
<td>0.0±0</td>
</tr>
<tr>
<td>CP</td>
<td>2.05±1.5</td>
<td>0.167±0.4</td>
</tr>
<tr>
<td>OA/CP</td>
<td>4.3±1.9</td>
<td>0.67±0.5</td>
</tr>
<tr>
<td>OA/CP/rasburicase</td>
<td>2.33±1.6</td>
<td>0.0±0</td>
</tr>
</tbody>
</table>

P Values

Control vs. OA    NS    NS    NS    NS    NS
OA vs. CP       P<0.05    NS    NS    P<0.01    NS
CP vs. OA/CP       P<0.05    P<0.05    P<0.01    NS    P<0.05
OA/CP vs. OA/CP/rasburicase  P<0.05    P<0.05    P<0.01    NS    P<0.05
CP vs. OA/CP/rasburicase    NS    NS    NS    NS    NS

Values are means ± SD; n = 6 in each group. CP, cisplatin; ISOM, inner stripe of outer medulla; OSOM, outer stripe of outer medulla; CD, collecting duct; PT, proximal tubule; OA, oxonic acid; NS, not significant.
about twofold or less, and in no cases were intrarenal crystals detected using De Galantha stain.

Effect of hyperuricemia on renal injury. The administration of CP resulted in injury that almost exclusively involved the S3 segment of the proximal tubule (pars recta). The renal injury was characterized by loss of the brush border with karyolysis. Mild tubular proliferation, as noted by PCNA staining, was observed in the outer medulla (Fig. 1). No injury was observed in the convoluted proximal tubules in the cortex, in the mTAL, or in the collecting ducts. However, a low-grade inflammatory response was observed that was localized to the OSOM.

Injury to the proximal tubule (S3 segment) was also marked in the hyperuricemic rats that received CP (Table 2). While brush-border loss was similar in both groups, hyperuricemic rats had more tubular swelling and nuclear condensation resulting in significantly greater tubular injury scores for both the S3 proximal tubular cells and for collecting duct cells. Tubular proliferation (PCNA staining) was also significantly greater in the outer medulla (Fig. 1). However, the most striking difference between the two groups related to the interstitial inflammation and peritubular capillaritis, which was much more marked in the hyperuricemic rats, and which extended into the cortex as well as the ISOM (Table 2).

A third group consisted of hyperuricemic rats with CP-induced renal injury in which the increase in serum uric acid was largely prevented by the administration of recombinant urate oxidase inhibitor (rasburicase). These rats also showed injury of the S3 segment of the proximal tubules, but the injury was very similar to rats administered CP alone, and tubular swelling and karyolysis were minimal. Indeed, tubular proliferation was also similar to that observed with CP alone (Fig. 1). Furthermore, the peritubular capillaritis was mild and confined to the OSOM (Table 2).

Hyperuricemia increases interstitial inflammation and renal MCP-1 expression. The observation of a marked interstitial inflammatory response in the CP-OA rats led us to stain the tissue for monocyte macrophages with the ED-1 antibody. As shown in Fig. 3, CP-induced ARF is associated with an infiltration of ED-1+ macrophages into the OSOM. Hyperuricemic rats with CP injury displayed significantly more macrophages in the cortex and inner stripe (Table 2 and Fig. 2). Importantly, lowering uric acid with recombinant uricase resulted in a significant reduction in the monocyte macrophage infiltration (Fig. 2).

MCP-1 is considered one of the most important chemokines that drives the interstitial inflammatory response in models of kidney disease (38), and we previously reported that uric acid can stimulate MCP-1 expression in rat vascular smooth muscle cells (19). We therefore measured both MCP-1 mRNA (by quantitative PCR) and MCP-1 (by ELISA) in whole kidney tissues of rats from the various groups. As shown in Fig. 4, MCP-1 mRNA was increased threefold in hyperuricemic rats
with CP-induced injury compared with controls, although this did not reach statistical significance. However, MCP-1 protein was increased in the hyperuricemic rats with CP-induced renal injury (266.1 ± 68 vs. 390.4 ± 104 pg/mg kidney protein in CP vs. OA/CP groups, \( P < 0.05 \)). Furthermore, lowering uric acid levels with rasburicase prevented this increase (Fig. 3).

**Effect of hyperuricemia on renal function.** Mild hyperuricemia in the absence of CP did not affect renal function (assessed by serum BUN and creatinine levels) or cause proteinuria (Table 1). The administration of CP to normouricemic rats did result in modest renal dysfunction at day 5. Renal function tended to be worse in hyperuricemic rats with CP compared with CP alone, but this did not reach significance (serum creatinine 2.0 ± 0.6 vs. 1.6 ± 0.4, \( P = \) not significant, respectively; Table 1). However, the administration of rasburicase to the hyperuricemic CP rats did result in significantly improved renal function compared with CP alone (1.1 ± 0.1 vs. 2.0 ± 0.6, \( P < 0.01 \), respectively).

**DISCUSSION**

It is well known that severe hyperuricemia may precipitate ARF. The mechanism has been attributed to the consequence of marked uricosuria with the formation of uric acid crystals that obstruct the tubular lumina and incite an inflammatory response (“acute urate nephropathy”). While classically observed with tumor lysis associated with the treatment of hematological malignancies, this syndrome may also occur with other conditions associated with marked cell turnover, such as rhabdomyolysis (25).

Recently, there have been a number of studies that have suggested that hyperuricemia, at concentrations that do not cause intrarenal crystal deposition, may have marked hemodynamic and proinflammatory effects. In cell culture studies, soluble uric acid has been found to inhibit endothelial cell proliferation, stimulate vascular smooth muscle growth, and stimulate monocyte chemotaxis (2, 14, 16, 39). Uric acid rapidly inhibits endothelial nitric oxide release, likely via a mechanism that involves scavenging of nitric oxide (13, 14, 16, 28). Uric acid also stimulates the release of proinflammatory mediators from vascular cells, including the release of MCP-1 and CRP (13, 14). In animals hyperuricemia can cause renal vasoconstriction, as well as raise blood pressure, induce features of the metabolic syndrome, and cause chronic renal disease (15, 21, 26, 28).

The observation that mild hyperuricemia may have vasoconstrictive, antiangiogenic, and proinflammatory actions led us to hypothesize that mild hyperuricemia may accelerate ARF. Indeed, recent studies documented that ARF is not simply mediated by tubular cell injury, but rather is frequently accompanied by renal vasoconstriction, microvascular injury, and a local inflammatory response (4, 6, 7, 10, 24, 37, 42).

To test this hypothesis, we induced mild hyperuricemia in rats by administering the urate oxidase inhibitor, OA. This was necessary as rats have the enzyme which degrades uric acid to allantoin, and hence the normal uric acid levels in the rat vary between 1 and 2 mg/dl. However, urate oxidase was mutated during hominoid evolution, and as a consequence humans have higher levels that are also less regulatable.

The induction of mild hyperuricemia was not associated with any acute renal injury by itself, but the rise in uric acid levels was also relatively small (~40–50%). However, when OA was administered to rats with CP-induced ARF, the rise in uric acid was accentuated. Renal function was not significantly different from rats treated with CP alone; however, hyperuricemic rats developed worse renal injury. In control rats with CP-induced injury, there was marked injury to the vulnerable S3 segment of the proximal tubule within the medullary rays and the OSOM, similar to what has been observed by others (41). Tubular injury was significantly greater in hyperuricemic rats with CP-induced injury, and tubular proliferation was also greater. However, the major finding was the presence of a much greater local inflammatory response, with increased
The data thus support the hypothesis that mild or moderate hyperuricemia may have a role in accelerating ARF. These data are consistent with epidemiological studies suggesting that an elevated uric acid may increase the risk of patients to CP-induced ARF (29). There have been a number of studies that have reported the use of the uric acid-lowering drug, allopurinol, in models of ARF. Allopurinol is a xanthine oxidase inhibitor that blocks the formation of uric acid. However, the inhibition of xanthine oxidase also results in blocking superoxide and hydrogen peroxide generation, and hence this agent was previously used as a means to reduce oxidants. Indeed, there is strong evidence that oxidants are involved in toxic ARF (3), including in CP-induced renal injury (35). There is also evidence for oxidant involvement in ischemic ARF. However, the role of allopurinol in protection in ischemic renal failure has been controversial, as there are reports that it is both beneficial (31, 40) as well as ineffective (11). Importantly, in none of these studies was uric acid raised using a urate oxidase inhibitor to mimic the human condition, nor were uric acid levels followed.

The design of our study allowed us to separate the effects of uric acid from that of coproduced oxidants generated during the xanthine oxidase reaction. Thus, by using uricase we were able to lower uric acid directly. Interestingly, urate oxidase actually generates oxidants during the degradation of uric acid; in contrast, OA blocks oxidant generation via the same pathway. These studies thus emphasize that it is likely the uric acid per se was accelerating the renal injury. However, it remains possible that the rasburicase is providing protection via some other unknown mechanism.

These studies do not rule out the involvement of oxidants in the mechanism by which uric acid mediates its effects. Thus, uric acid itself may have either prooxidative or antioxidant effects. Indeed, we previously reported that the stimulation of MCP-1 in vascular smooth muscle cells by uric acid can be blocked by antioxidants (13). Thus it remains possible that uric acid may be mediating prooxidant effects on various intrarenal cell populations through a direct mechanism, or possibly secondarily as a consequence of stimulating the leukocyte infiltrate.

In conclusion, mild hyperuricemia was shown to significantly increase renal tubular injury and inflammation in a model of CP-induced ARF in the rat. The primary mechanism appeared to be the stimulation of monocyte chemokines with an enhanced infiltration of infiltrating leukocytes. Importantly, this effect was not mediated by intrarenal crystal deposition. The data thus support the hypothesis that mild or moderate hyperuricemia may be a contributing factor in the renal injury accompanying CP-induced ARF.

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DISCLOSURES

R. J. Johnson has a consultancy with TAP Pharmaceuticals, Nephromics, and Scios.

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