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Irreversible Tubulointerstitial Damage Associated with Chronic Aminonucleoside Nephrosis

Amelioration by Angiotensin I Converting Enzyme Inhibition

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Chronic aminonucleoside nephrosis is variably associated with tubulointerstitial damage, depending on the route and frequency of drug administration. Recently, different groups have shown this injurious tubulointerstitial process to be reversible, coinciding with the resolution of heavy proteinuria to normal values. The authors have previously shown that a single jugular intravenous administration of puromycin aminonucleoside (PA) to male Munich-Wistar rats produces a triphasic pattern of glomerular injury and proteinuria, which culminates in focal glomerulosclerosis 70 weeks after drug administration. The authors now report the later progression of the tubulointerstitial morphologic abnormalities associated with acute nephrosis (phase I), despite spontaneous resolution of glomerular injury during the intermediate period (phase II) in this model. Although treatment of rats with the angiotensin I converting enzyme inhibitor enalapril (50 mg/l drinking water) over the 70-week period did not affect the magnitude of proteinuria during the acute nephrotic phase, enalapril prevented the recurrence of proteinuria (phase III), as well as significantly reducing the severity of interstitial fibrosis, extent of tubular dilatation, and number of intratubular casts on semiquantitative scoring at the conclusion of the study. In addition, enalapril-treated rats had less low-molecular-weight protein excretion during the recurrent phase of proteinuria, suggesting a preservation of tubular functional capacity to reabsorb these proteins. In vitro ***cytotoxicity studies showed only the glomerular visceral epithelial cell to be sensitive to PA, in contrast with rat tubular epithelium and other cellular controls. Although the exact pathogenetic mechanism responsible for the development of the tubulointerstitial damage remains unknown, PA*** in vitro ***does not adversely affect rat tubular epithelium; there is however a clear correlation between the magnitude of recurrent proteinuria and the severity of tubulointerstitial morphologic abnormalities, as suggested by the beneficial effect of converting enzyme inhibition on both of these untoward processes. (Am J Pathol 1990, 137:1323–1332)***

Aminonucleoside nephrosis is an experimental model of nephrotic syndrome that, depending on the dosage schedule and the route of administration employed, progresses to focal and segmental glomerulosclerosis (FSGS).[1–6] Eddy and Michael[7] have recently shown that one intraperitoneal injection of puromycin aminonucleoside (PA) produces a transient, reversible acute nephrotic syndrome that is accompanied by tubulointerstitial cellular infiltrates, which abate as the nephrotic syndrome resolves. These authors suggested that these tubulointerstitial abnormalities may be a consequence of proteinuria during the nephrotic state. This is consistent with earlier thoughts that the observed tubular cell swelling and dilatation of the tubules in this glomerulopathy were secondary to the proteinuria.[8] Micropuncture experiments in

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aminonucleoside nephrosis have demonstrated that the proteinuria is of glomerular and not tubular origin.[9] Thus there is enhanced delivery of protein to the proximal tubular epithelium, which is reflected by the large numbers of protein reabsorption droplets seen in these cells by immunofluorescence.[10] Despite evidence for abnormalities in proximal tubule protein uptake and catabolism in acute aminonucleoside nephrosis, direct measures of proximal tubular function and parameters of cellular injury that correlate the increased protein delivery with tubulointerstitial damage are lacking.

Earlier investigators, using a chronic aminonucleoside model, have suggested that glomerulosclerosis is a consequence of the amount of urinary protein excretion.[11] It has also been noted that many progressive glomerulopathies are accompanied by a variable degree of tubulointerstitial damage.[1,12] In another toxic glomerulopathy, adriamycin nephrosis, Bertani et al[13] have alternatively suggested that the observed tubulointerstitial abnormalities were a consequence of long-lasting proteinuria that induced intratubular cast formation and a subsequent interstitial inflammatory reaction.

Recently we have shown that a single jugular intravenous injection of PA produces a triphasic glomerular disorder characterized by massive proteinuria and an acute nephrotic syndrome lasting 2 to 3 weeks (phase I); a period of spontaneous clinical recovery during which the nephrotic syndrome abates and protein excretion falls to near-normal levels (phase II); and then recurrent proteinuria (phase III), which progressively worsens, culminating in glomerulosclerosis.[14,15] Converting-enzyme inhibition (CEI) ameliorated the recurrent proteinuria and glomerular injury in this model.[15] Because of the apparent strain differences between the Sprague-Dawley and the Munich-Wistar rat, in that the latter develops much more severe tubulointerstitial injury accompanying the chronic glomerular lesion,[14,15] we were interested in assessing whether the prevention of only the late, recurrent phase of proteinuria (phase III) with the converting enzyme inhibitor, enalapril, altered the expression of tubulointerstitial disease at the conclusion of the study, 70 weeks after the single jugular intravenous injection of PA in the Munich-Wistar rat. The present investigation represents new studies that were conducted to explain histologic observations noted in our earlier report.[15] Specifically this present study attempts to relate the histologic amelioration in the tubulointerstitial areas in enalapril-treated PA rats, 70 weeks after PA, to the prevention of recurrent proteinuria by the CEI. To examine this, we evaluated patterns of protein excretion at 8, 40, and 70 weeks after PA. In addition, we also assessed *in vitro* whether PA has toxic effects on renal cell lines, other than its principal target, the glomerular visceral epithelial cell.

Methods

Three groups of adult male Munich-Wistar rats, weighing 220 to 260 g, were studied. Under Brevital anesthesia (50 mg/kg intraperitoneally), the right internal jugular vein was cannulated and injected with either 3 ml of saline (sham) or PA (Sigma Chemical Co., St. Louis, MO), 50 mg/kg dissolved in 3 ml of saline, given as a single injection over 3 minutes with a constant infusion pump. All rats were maintained on a standard diet (Rodent Laboratory Chow No. 5001; Ralston Purina Co., St. Louis, MO). One cohort of puromycin-injected rats (PA) received no therapy and served as nephrotic controls, whereas another group (designated PA/CEI) was treated with the angiotensin I converting-enzyme inhibitor (CEI), enalapril (Merck, Sharp & Dohme, West Point, PA) at a dose of 50 mg/liter in the drinking water starting 1 week before injection. An additional group of rats received a single intraperitoneal injection of PA (150 mg/kg) to assess variability in the magnitude of proteinuria during phase I as determined by the route of administration. Urine was collected over a 24-hour period in metabolic cages at 2, 8, 40, and 70 weeks after PA administration, at which time the study was terminated. No animals died between phases I and III. Total urine protein was quantitated by the sulfosalicylic acid (SSA) method.[16] Daily low-molecular-weight (LMW) protein excretion was quantitated at 8, 40, and 70 weeks after PA administration by filtering a sample of urine from each of the animals through a PM-10 membrane in an Amicon ultrafiltration cell (Amicon Corp., Danvers, MA) to permit the passage of proteins with molecular weight (MW) less than 10,000. For each urine sample, the fraction of the total urinary protein that was less than 10,000 MW was measured by the Lowry method.[17] Using this fraction and the total daily urinary protein excretion, as determined by the SSA method, the amount of daily LMW protein excreted was determined. Twenty-four–hour urinary albumin excretion rates were determined by the single radial immunodiffusion technique.[18]

Renal Function Studies

Measurements of glomerular filtration rate (GFR) were performed before killing the animals in sham, PA, and PA/CEI rats at 2 (n = 10 in each group), 8 (n = 11 in each group), and 70 weeks (n = 8 for PA; n = 9 for Sham; and n = 9 for PA/CEI) after PA injection.[15] Rats were anesthetized with Inactin (100 mg/kg intraperitoneally) and placed on a temperature-regulated table. The left femoral artery was catheterized, and a baseline collection of 0.28 ml of blood was obtained for measurement of hematocrit (Hct) and inulin 'blanks'. This arterial catheter was used for subsequent periodic blood sampling. After tracheos-

tomy, venous catheters were inserted for infusions of inulin and plasma. Intravenous infusions of rat plasma and 4% to 10% inulin solution in 0.9% NaCl were started at rates of 6.0 and 1.2 ml/hour, respectively. The left ureter was catheterized for urine collection.

Because the plasma volume of rats prepared for clearance studies is reduced by approximately 20%,[19] euvolemia was maintained using the following protocol: Isoncotic rat plasma was infused at 0.1 ml/minute in a total amount equal to 1% of the body weight, followed by a reduction in infusion rate to 0.6 ml/kg/hour, to maintain the Hct constant.

For calculation of GFR, 15- to 20-minute urine collections were obtained for determination of inulin concentration. These measurements permitted calculation of GFR (inulin clearance) by standard formulas.

Preparation for Light Microscopy

At 70 weeks after PA administration, animals were anesthetized with Inactin, and the kidneys were perfused, *in vivo*, *via* an infrarenal aortic cannula for 2 to 3 minutes. The perfusate solution consisted of a 0.1 mol/l 〈molar) cacodylate buffer containing 5% sucrose (pH 7.4) followed by the fixative, 2% glutaraldehyde in cacodylate buffer. Both kidneys were removed, sectioned coronally, and immersed in 2% glutaraldehyde in cacodylate buffer for an additional 2 hours. After fixation, these coronal sections were rinsed for an additional 2 hours in 0.1 mol/l cacodylate buffer with 5% sucrose (pH 7.4), embedded in paraffin, and sectioned at approximately 2- to 3-μ thickness for staining with periodic acid-Schiff reagent (PAS), phosphotungstic acid-hematoxylin (PTAH), and hematoxylin and eosin (H&E). The PAS stain was used to demonstrate intratubular casts as well as to show segmental areas of glomerulosclerosis/hyalinosis and fibrosis in the surrounding tubulointerstitial areas. The PTAH stain was used to evaluate the degree of interstitial fibrosis, as has previously been reported.[20] The H&E stain was used to evaluate the nature of the cellular infiltrate within tubulointerstitial areas. In addition, smaller randomly selected sections of renal cortex were postfixed in 2% osmium tetroxide for 1 hour; dehydrated in acetone; and embedded in epoxy resin (Epon, Bioscience Products, New York). One-micron thin sections were stained with 1% toluidine blue. All tissue was viewed and photographed with a Leitz-Wetzlar photomicroscope.

Scoring of Tubulointerstitial Disease at Seventy Weeks

Interstitial fibrosis was scored by evaluating 25 randomly selected cortical areas in a stained hemicoronal section using the 10× objective for the presence of PTAH-positive connective tissue foci as follows: 0 absent; 1+ (1 focus per field); 2+ (2 to 3 foci per field); 3+ (more than 3 foci per field). The number of PAS-positive intratubular casts seen at 10× magnification in 25 randomly selected areas at the corticomedullary junction were graded as follows: 0 absent; 1+ (1 to 2 per field); 2+ (2 to 5 per field); 3+ (more than 5 per field). The extent of tubular dilatation was assessed from the largest external diameters of 25 randomly selected tubules that were deemed to be circular at the corticomedullary junction, using the 40× objective as follows: 0 (less than 10 μ); 1+ (less than 20 μ); 2+ (less than 20 to 30 μ); 3+ (more than 50 μ). To avoid distortion in measuring the largest external diameters of tubules at the corticomedullary junction, the 1-μ toluidine blue-stained sections were used.

Cytotoxicity Assay

To assess whether there is any differential susceptibility to cellular injury by PA and also to gauge whether this known glomerular visceral epithelial cell toxin[21] also damages cortical tubular epithelium, we performed cytotoxicity assays on rat glomerular visceral epithelial cells (GEC); rat glomerular mesangial cells (MC); rat cortical tubular epithelial cells (CTEC); and Madin-Darby Canine Kidney cells (MDCK), as a nonrodent tubular epithelial cell control, from established cell lines,[21] which were grown in their respective media in 24-multiwell plates until confluent. At that time, 0.5 ml of a ^{51}sodium chromate (^{51}Cr, 0.01 mC/ml medium) solution was added to the confluent monolayers for 2 hours at 37°C. The media then was removed and cells were washed twice with 0.5 ml of sterile Hank's medium buffered with HEPES (H-H). Then, varying concentrations of PA in H-H (0, 40, 100, 250, 500 μg/mL) were added to the monolayers for 5 hours at 37°C. After both the 5-hour incubation with PA and the lysis of the monolayer with a 12-hour incubation with 0.5 ml of 1-N NH_4OH, the media were counted after the addition of liquid scintillation fluid (New England Nuclear, Boston, MA) in a Beckman liquid scintillation counter. To establish absolute chromium release, different wells of cells were treated with 0.05% Triton X for 5 hours, followed by 1-N NH_4OH lysis overnight, and counted similarly. The percentage of chromium released (percentage ^{51}Cr released) was calculated as the ratio of I and II divided by the ratio of III and IV multiplied by a factor of 100, where I represents the counts per minute (cpm) of ^{51}Cr released after PA incubation; II represents the cpm of ^{51}Cr released after NH_4OH incubation for monolayer lysis; III represents the cpm of ^{51}Cr after incubation with Triton X; and IV is the cpm of ^{51}Cr released after NH_4OH incubation after treatment with Triton X.

Statistics

All values are expressed as the means ± standard error of the mean (SEM). Statistical analysis was performed by unpaired *t*-test or one-way analysis of variance, followed by computation of modified *t* values and multiple pairwise comparisons, according to the method of Bonferroni,[22] as appropriate.

Results

During acute nephrosis, 2 weeks after PA delivery, both the PA and PA/CEI groups manifested severe tubulointerstitial abnormalities, which included interstitial edema, tubular atrophy and dilatation, and intratubular casts with intraluminal cellular debris (Figure 1). No interstitial fibrosis was noted with either PAS or PTAH stains. The degree of severity of these lesions was not qualitatively different between the two nephrotic groups. At this time, 24-hour proteinuria values were comparable in the PA and PA/CEI groups (139 ± 13 mg/day and 117 ± 18 mg/day, respectively), while the sham group excreted 5 ± 1 mg/day ($P < 0.005$ *versus* PA and PA/CEI). Another group of weight-matched Munich-Wistar rats injected with a single intraperitoneal dose of PA (150 mg/kg body weight) excreted 102 ± 15 mg/day of protein 2 weeks after injection, which was not different from the two nephrotic groups above at that time. Thus, early proteinuria is equivalent with the two dosage regimens, and unaffected by concurrent converting enzyme inhibition administration.

Eight weeks after PA administration, which represents the phase of spontaneous recovery from the acute nephrotic syndrome, there were virtually no glomerular abnormalities present in either the PA or PA/CEI groups,[15] yet the above-mentioned tubulointerstitial abnormalities persisted and were comparable among the two groups. In addition, both the PA and PA/CEI groups manifested foci of early interstitial fibrosis, as evidenced by a sparse distribution of both PAS and PTAH-positive material surrounding the tubules. Figure 2 demonstrates the patterns of protein excretion in the three groups during this phase. The low-molecular-weight (LMW < 10,000) protein excretion rates, in the PA and PA/CEI groups, were not different at 5.0 ± 0.7 mg/day and 4.5 ± 0.9 mg/day, respectively. The sham animals excreted 3.2 ± 0.3 mg/day, which was significantly lower than the PA group ($P < 0.02$). Likewise, the total daily protein excretion rates in the PA and PA/CEI groups were not different at 13 ± 1 mg/day and 10 ± 1 mg/day, respectively. The sham group, excreting 8 ± 1 mg/day, again was significantly lower than only the PA group ($P < 0.05$). The daily urinary albumin excretion rate in the PA group was significantly higher than in the PA/CEI group (22.8 ± 3.9 *versus* 8.4 ± 1.4 mg/day, $P < 0.05$). The sham group excreted 4.6 ± 1.0 mg/day, which was not different from the PA/CEI group, but significantly lower than the PA group ($P < 0.05$). The greater absolute values for albuminuria in all groups are due to the much greater sensitivity of the single radial immunodiffusion method as compared with the SSA assay. Also, at 8 weeks after PA administration, whole-kidney glomerular filtration rates (GFR), as assessed by inulin clearance, were similar in the three groups (each n = 8 to 9), ranging from 1.25 ± 0.09 ml/minute in the PA/CEI group to 1.34 ± 0.06 ml/minute in the sham group. The value for the PA group was 1.28 ± 0.06 ml/minute.[15]

At 40 weeks after PA administration, during the phase of recurrent proteinuria, LMW protein, total daily protein, and daily urinary albumin excretion rates were all significantly greater in the PA group as compared with the PA/CEI and sham cohorts (Figure 3). At the termination of the study (70 weeks after PA administration), the pattern observed at 40 weeks was still present. Total daily protein

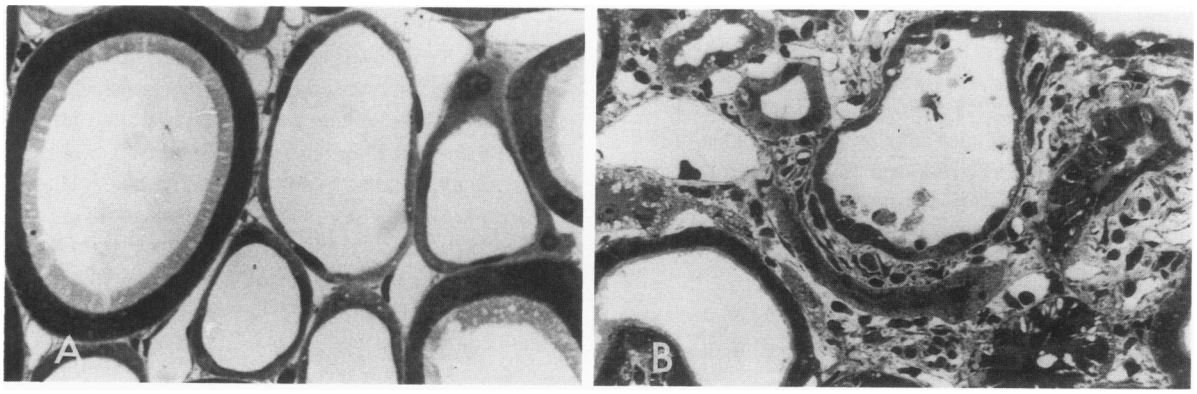

Figure 1. *Representative photomicrographs of rat tubulointerstitial areas 2 weeks after puromycin aminonucleoside (PA) delivery.* **A** *is from an animal infused with saline and demonstrates normal tubulointerstitial areas, while* **B** *is from a nephrotic (PA) rat and exhibits tubulointerstitial injury features including tubular atrophy and collapse, tubular dilatation, intraluminal cellular debris, and interstitial edema (Toluidine blue, ×300).*

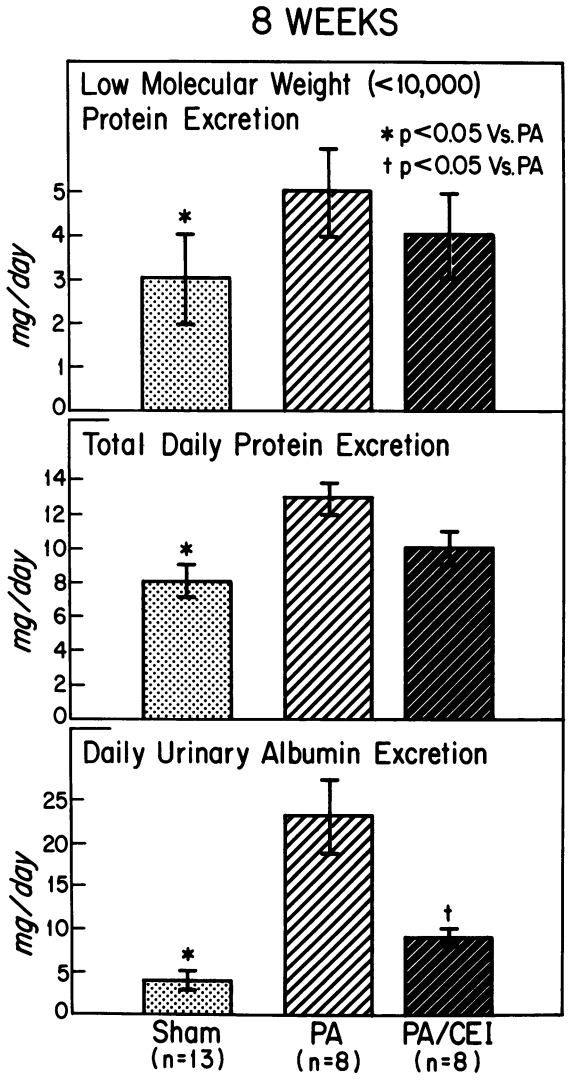

Figure 2. *Patterns of urinary protein excretion 8 weeks after PA administration. During this phase of spontaneous recovery from the nephrotic syndrome, note that although the total daily protein excretion is not different between the PA and PA/CEI groups, the daily urinary albumin excretion rate is significantly lower in the PA/CEI group as contrasted to the PA one.* * P < 0.05, *sham* vs. *PA*; ‡ P < 0.05, *PA* vs. *PA/CEI. Values are means* ± *SEM.*

and albumin excretion rates were significantly higher in the PA group, and the LMW protein excretion rate was numerically greater in the PA group as compared with both the PA/CEI and sham rats (Figure 4). With regard to inulin clearance values at 70 weeks, unlike the values for GFR at 8 weeks after PA, there were now obvious differences between the PA group (1.14 ± 0.13 ml/minute) and the PA/CEI (1.43 ± 0.09 ml/minute, $P < 0.05$), as well as the Sham group (1.51 ± 0.12 ml/minute, $P < 0.05$).[15] This pattern of depressed GFR in the PA group is indicative of early renal insufficiency, while values in PA/CEI rats suggest preservation of renal function at levels comparable to those in Sham rats.

In concert with the worsening urinary protein excretion patterns and declining GFR, the tubulointerstitial abnormalities also were more severe in the PA group, as depicted in Table 1. Semiquantitative scores for interstitial fibrosis, intratubular casts, and tubular dilatation were all significantly worse in the PA group as contrasted with the other two groups. Figure 5 demonstrates the above lesions. In the upper panel, tubulointerstitium from a Sham rat is shown and serves as a normal control. Figure 5b through d exhibit the types of interstitial injury seen in PA rats 70 weeks after PA administration. As is evident, there were extensive areas of tubular atrophy, tubular dilatation with PAS-positive intratubular cast material, and an intense fibrotic response, as evidenced by the interstitial PAS and PTAH positivity. Figure 6 shows these lesions at higher magnification and, in addition, the photomicrograph in

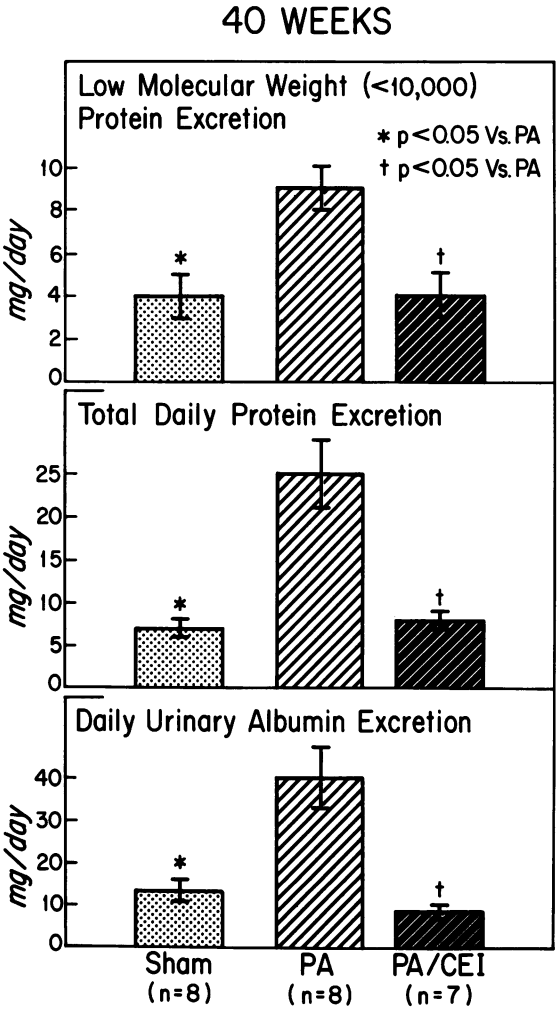

Figure 3. *Patterns of urinary protein excretion 40 weeks after PA administration. During this phase of recurrent proteinuria, there are now significant differences in daily low molecular weight, total protein, and urinary albumin excretion in Sham* vs. *PA and PA/CEI* vs. *PA.* * P < 0.05, *sham* vs. *PA*; ‡ P < 0.05, *PA* vs. *PA/CEI. Values are expressed as means* ± *SEM.*

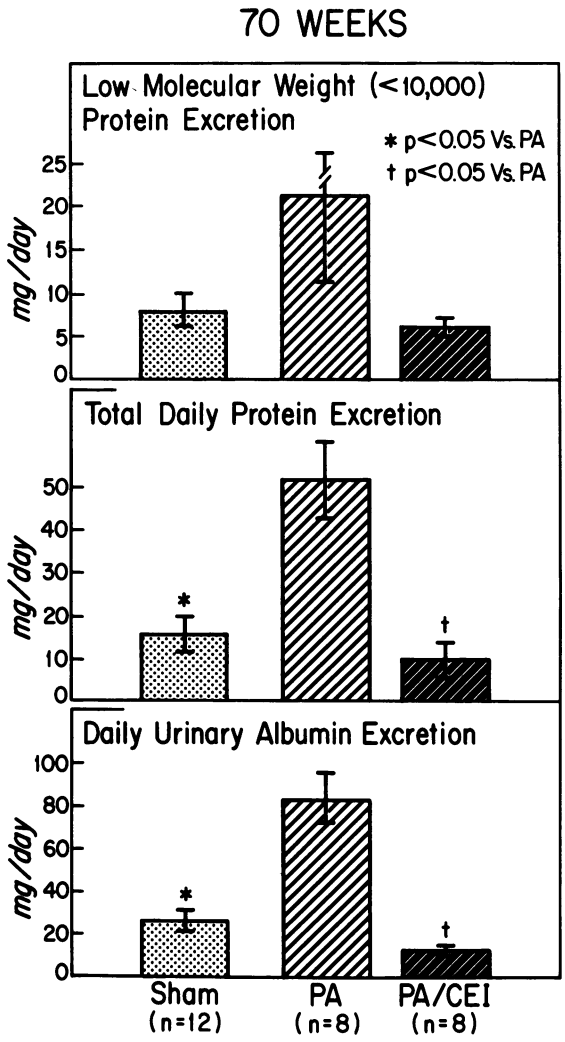

Figure 4. *Patterns of urinary protein excretion 70 weeks after PA administration, at the conclusion of the study. Note that there are still statistically significant differences in daily total protein and urinary albumin excretion in Sham* vs. *PA and PA/CEI* vs. *PA. Although low molecular weight protein excretion was numerically higher in PA rats as compared to PA/CEI and Sham rats, these differences did not reach statistical significance.* * P < *0.05, sham* vs. *PA;* ‡ P < *0.05, PA* vs. *PA/CEI. Values are means* ± *SEM.*

Figure 6c shows that several of the abnormal interstitial areas in PA rats had a dense mononuclear cell infiltrate surrounding both glomeruli and tubules.

With regard to whether PA produces a direct injurious effect on tubular epithelium, the results of cytotoxicity assays on different cell lines appear in Figure 7. At all concentrations of PA, over a range of 0 to 500 μg/ml in the tissue culture medium for 5 hours' incubation, the glomerular visceral epithelial cell released significantly greater percentages of the labeled chromium as contrasted to the cortical tubular epithelial cell line, the MDCK cell line, and the glomerular mesangial cell, all of which failed to release the intracellular label above baseline. Thus, PA was cytotoxic to GEC cells, and did not injure the other epithelial and mesangial cell lines, suggesting a specific effect.

Discussion

The present study describes the tubulointerstitial morphologic abnormalities that develop in chronic aminonucleoside nephrosis as it evolves over 70 weeks in the Munich-Wistar rat. This model lends itself well to such an investigation, as its triphasic pattern of proteinuria, which includes an intermediate period of spontaneous functional and morphologic recovery, provides a means to assess the effects of protein-induced tubulointerstitial injury at different times. Specifically, it allows modulation of the magnitude of glomerular injury during only the late, recurrent phase of proteinuria with concurrent CEI administration[15] and assessment of the impact of this pharmacologic maneuver on tubulointerstitial injury. Although CEI administration had no discernible ameliorative effect on the severity of either the glomerular or tubulointerstitial injury induced by PA during phase I, as evidenced by proteinuria, GFR studies, and qualitative assessment of morphologic abnormalities, respectively, the prevention of proteinuria and albuminuria during phase III with enalapril was associated with a significant reduction in the severity of tubulointerstitial histologic injury, assessed semiquantitatively.

Our findings differ with those reported by Eddy and Michael[7] and Olbricht et al,[23] in that the observed tubulointerstitial morphologic abnormalities in PA rats reported here were irreversible, being present 8 weeks after PA administration, when spontaneous recovery from the nephrotic syndrome has already occurred. Olbricht et al[23] noted both lethal and sublethal cell injury with evidence of early regeneration in many proximal tubules, while many distal convoluted tubules contained either homogeneous-appearing casts or cellular debris. However, at 28 days after PA injection, the histopathologic picture resembled that observed in the control animals.[23]

Because it is possible that the manner in which PA is delivered to the animal may determine the severity of the glomerular lesion, perhaps because of varying transient

Table 1. *Tubulointerstitial Abnormalities in Chronic Aminonucleoside Nephrosis 70 Weeks After PA Administration*

	Intratubular casts	Interstitial fibrosis	Tubular dilatation
PA (n = 8)	2.81 ± 0.13	2.88 ± 0.12	2.38 ± 0.31
PA/CEI (n = 7)	1.43 ± 0.41‡	0.57 ± 0.07†	1.00 ± 0.15†
SHAM (n = 11)	0.73 ± 0.12*	0.82 ± 0.15*	0.77 ± 0.17*

* *P* < 0.001 *vs.* PA.
† *P* < 0.001 *vs.* PA.
‡ *P* < 0.01 *vs.* PA.

intrarenal concentrations of the drug, we sought to assess whether the magnitude of initial proteinuria is determined, in part, by the route of PA administration (ie, intravenous *versus* intraperitoneal). Whether this difference in dosage regimen explains the persistence of tubulointerstitial abnormalities throughout the course of our model was addressed by injecting another cohort of Munich-Wistar rats with a single dose of PA (150 mg/kg body weight) intraperitoneally. There were no significant differences observed between peak proteinuria values at 2 weeks in PA and PA/CEI rats injected intravenously and in PA rats injected intraperitoneally, suggesting that the different route of administration of PA cannot account for the previously reported reversible nature of the tubulointerstitial injury[7] by altering the magnitude of initial proteinuria.

Prevention of the recurrence of proteinuria and albuminuria at 40 and 70 weeks (phase III) with the converting enzyme inhibitor enalapril was accompanied by a significant reduction in the severity of interstitial fibrosis, tubular dilatation, and intratubular cast formation at the conclusion of this study, suggesting that an injurious relationship exists between proteinuria, and more specifically albuminuria, and tubulointerstitial damage. Low–molecular-weight protein excretion probably does not play a major role in the pathogenesis of tubulointerstitial injury, but rather the lower values in the PA/CEI rats serve as physiologic correlates to the less severe tubulointerstitial morphologic features noted in this group. Decreased urinary excretion of LMW protein suggests preservation of the tubular functional capacity to reabsorb these proteins.[24] Under physiologic conditions, absorption of many LMW proteins is nearly complete and the principal site for this process is the proximal tubule, a segment of the nephron with a prominent and well-developed endocytotic apparatus.[25]

Although the exact pathogenetic mechanism(s) responsible for the development of these tubulointerstitial morphologic abnormalities remains unknown, there has been speculation that this process may be related to the proteinuria resulting from the underlying glomerular lesion[7] and not from a direct effect of PA on the tubular epithelium analogous to what has been described for the glomerular visceral epithelium.[12] In fact, our cytotoxicity assay data shows that the glomerular visceral epithelial cell is uniquely sensitive to PA, and both rat and canine tubular epithelial cell lines do not manifest cytotoxic effects in response to a dose range of PA. Eddy and Michael,[7] in their acute aminonucleoside nephrosis model, have demonstrated that there was a significant correlation between the quantitative albuminuria and the number of interstitial mononuclear cells present. This observation is analogous to our findings that significant reductions in daily albumin excretion rates at 8 and 40 weeks were accompanied by significantly less severe tubulointerstitial morphologic abnormalities at 70 weeks after PA administration. One hypothetical mechanism by which increased delivery of filtered protein to the tubular epithelium is noxious is based on the idea that an excessive concentration of absorbed proteins appearing within the lysosomes of proximal tubular epithelial cells during proteinuria may lead to cellular damage, possibly due to the leakage of lysosomal enzymes into the cytosol.[26] Paigen and Peterson[27] have stated that, because of increased protein uptake, the ly-

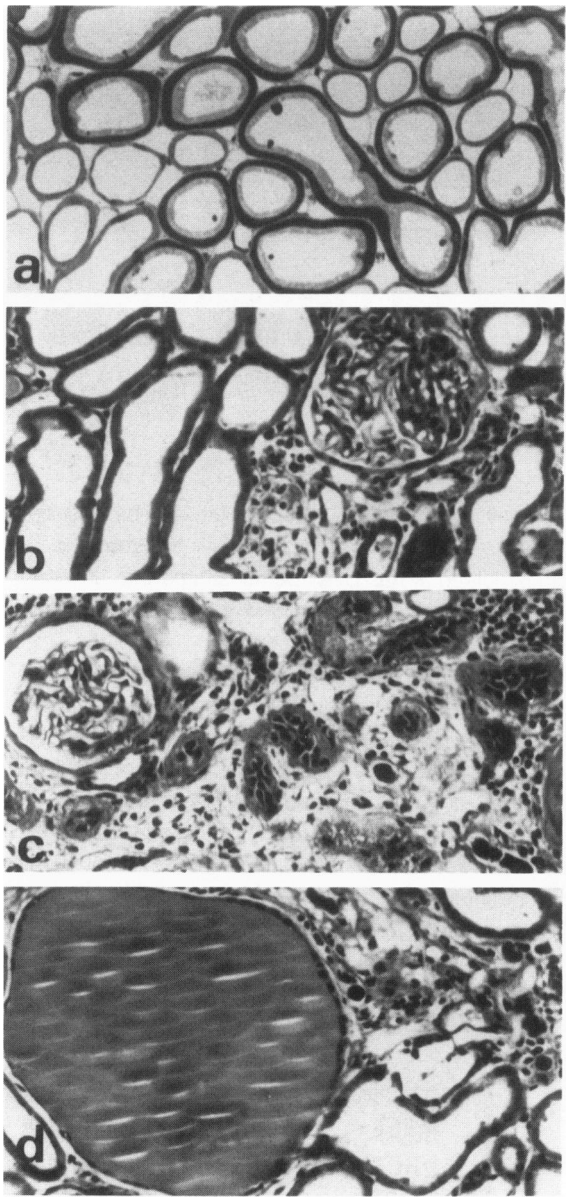

Figure 5. *Representative photomicrographs from Sham and PA rats 70 weeks after PA administration.* **a** *is from a Sham animal and demonstrates normal tubulointerstitial architecture.* **b** *through* **d** *demonstrate the spectrum of morphologic abnormalities observed in PA rats, including tubular atrophy and dilatation, interstitial edema, intratubular cast formation, and interstitial fibrosis. A microscopic cyst also appears in* **d** *(Toluidine blue, ×200).*

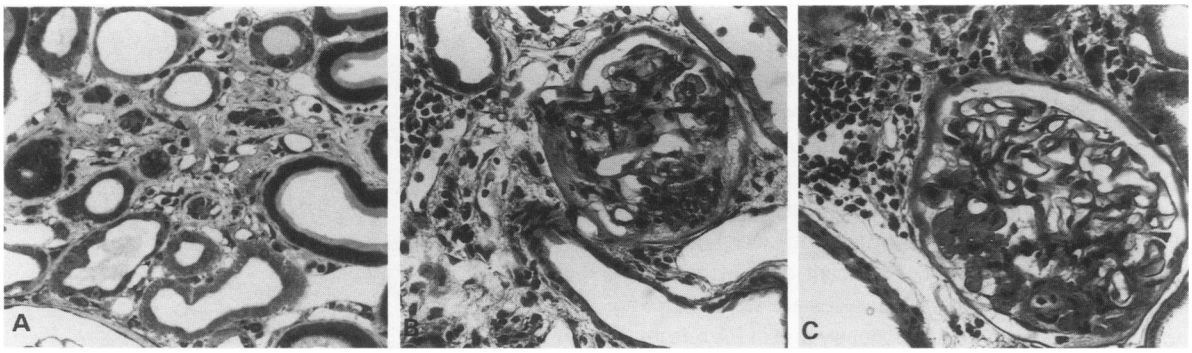

Figure 6. *Representative photomicrographs from PA rats 70 weeks after PA administration. Panels* **A** *through* **C** *reveal marked tubular atrophy, interstitial fibrosis, and mononuclear cell infiltrate surrounding glomeruli manifesting segmental glomerulosclerosis (Toluidine blue, ×480).*

sosomes in proximal tubular epithelium may be 'overfed,' thereby exhausting and extruding their enzymes into the tubular lumen. It has been demonstrated that aminonucleoside nephrosis is associated with increased urinary excretion of lysosomal enzymes.[28]

Bertani et al[13] have noted in a chronic adriamycin nephrosis model that distal tubule cast formation occurs in association with the heavy proteinuria. This cast formation was thought to precede the development of interstitial damage[13] and was similar to our observation that the presence of intratubular casts 2 weeks after PA administration antedates the demonstration of PAS- or PTAH-positive interstitial staining, which is indicative of fibrosis. Those authors further proposed that distal tubular casts may then progressively increase in size and cause intratubular obstruction with rupture of the tubular basement membrane (TBM), leading to leakage of THP into the interstitium, ultimately eliciting an interstitial inflammatory reaction.[13] Hoyer and Seiler[29] have noted that, although the major pathogenetic contribution of intratubular THP appears to be tubular obstruction by urinary casts, disruption of tubular integrity and urinary extravasation of THP may have a pathogenetic role in the interstitial nephritis of a number of human renal disorders. It has been demonstrated in a rat model that an immunologic response develops to this protein and includes an immune complex tubulointerstitial nephritis mediated by autoantibodies to THP.[30,31] Increased THP excretion has been noted in the nephrotic syndrome.[32] Hoyer and Seiler[29] have suggested that an increase in THP excretion might be related to increased sodium chloride delivery to the distal tubule secondary to the nephrotic syndrome or to proximal tubular dysfunction. The efficacy of converting enzyme inhibition in preventing recurrent proteinuria and in ameliorating the

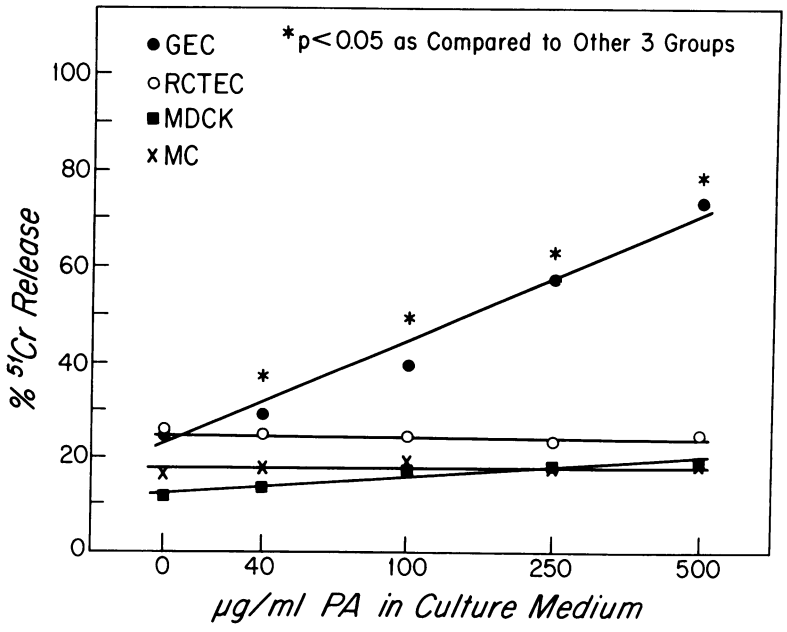

Figure 7. *Cytotoxicity of PA, as assessed by percentage of ^{51}Chromium release (% ^{51}Cr released) on four kidney cell lines: rat glomerular visceral epithelial cell (GEC); rat cortical tubular epithelial cell (RCTEC); Madin Darby canine kidney cell (MDCK, a nonrodent tubular epithelial cell control); and rat glomerular mesangial cell (MC). At all doses of PA added to culture medium, the GEC released significantly greater amounts of labeled chromium as compared to the three other cell lines.*

severity of the subsequent tubulointerstitial abnormalities in this model has been attributed to a reduction in the glomerular capillary hydraulic pressure (P_{GC}).[15] Alternatively Alfrey and Tomford[33] have suggested that protective maneuvers (such as protein restriction and phosphate depletion) may be efficacious in preventing tubulointerstitial nephritis in other experimental models of progressive glomerulopathy, by decreasing energy requirements and oxygen consumption by the tubules. Protein restriction, by reducing GFR, theoretically can decrease sodium reabsorption and sodium-potassium adenosine triphosphatase (ATPase) activity in the tubules.[33] It seems likely that the maintenance of a normal GFR in rats with chronic aminonucleoside nephrosis treated with enalapril could be operating similarly, in that the significantly lower GFR values in PA rats at 70 weeks (and, therefore, presumed single-nephron hyperfiltration) may indicate increased nephron work with potentially deleterious consequences with regard to tubular injury. Interestingly Agus et al[34] have demonstrated an inhibitory effect of dietary protein restriction on the development of immune-mediated interstitial nephritis, which was in part related to the selective abrogation of effector T-cell immunity.

In summary, although the exact pathogenetic mechanisms responsible for the tubulointerstitial damage remains unknown, PA *in vitro* does not adversely affect rat tubular epithelium; however there is a clear correlation between the magnitude of recurrent proteinuria and the severity of the tubulointerstitial morphologic abnormalities, as suggested by the beneficial effect of converting enzyme inhibition on both of these untoward processes.

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