Focal and Segmental Glomerulosclerosis Following a Single Intravenous Dose of Puromycin Aminonucleoside

JONATHAN R. DIAMOND, MD, and
MORRIS J. KARNOVSKY, MB, BCh

From the Department of Pathology, Harvard Medical School, Boston, Massachusetts

Focal and segmental glomerulosclerosis (FSGS) represents a final pathologic pattern of a number of human renal disorders. Among laboratory models, repeated intraperitoneal injections of the aminonucleoside of puromycin (PA) produces a histologic pattern not unlike the human process. A single intravenous dose of this drug usually results in glomerular morphologic changes in rats resembling those in human nephrotic syndrome with minimal changes. This report describes acute and chronic glomerular injury that begins as early as 8 days after a single central administration of PA and progresses to FSGS within an 18-week period. It seems likely that minimal change disease and FSGS are two pathologic processes in the same continuum of disease. In this model, the severity and persistence of the glomerular lesion may represent irreversible glomerular epithelial cell (GEC) injury secondary to the toxic effects of PA. (Am J Pathol 1986, 122:481–487)

IN MAN, focal and segmental glomerulosclerosis (FSGS) is believed to represent a final pathologic pattern of a number of clinical renal disorders.[1–3] Laboratory models also exist with glomerular abnormalities that are very similar to the human pathologic process. In the rat, FSGS occurs spontaneously, as either a congenital lesion or with aging,[4–7] follows renal ablation,[8,9] and develops after the administration of adriamycin[10,11] or after repeated doses of aminonucleoside of puromycin (PA).[12–14]

Recently, our laboratory produced a pathologic process that had many similarities to FSGS, 8 days after a single central intravenous injection of the aminonucleoside of puromycin.[15] The glomerular abnormalities, with light microscopy, included mesangial cell proliferation and matrix expansion, obliteration of capillaries, and development of synechiae; whereas with transmission electron microscopy, glomerular epithelial cell (GEC) foot processes were markedly spread.[15] In order to assess whether this acute glomerular injury became progressively worse in a protracted period of time, we studied animals for 18 weeks after a single intravenous injection of the drug. This is in contrast to an earlier report by Lannigan,[16] where the glomerular morphologic findings were normal after a solitary intravenous injection of PA up until 35 weeks after drug administration. At that time, only an increase in PAS-positive material, which was presumably mesangial matrix, was noted in axial zones of some glomeruli. Lannigan[16] also reported that more advanced degrees of glomerular damage, which resemble what are now recognized as histologic features consistent with FSGS, did not become evident until after 35 weeks following PA delivery.

Materials and Methods

Male Sprague–Dawley rats (Charles River, Wilmington, Mass), weighing 180–250 g, were studied. After the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight), 7 rats received a single intravenous injection, over 5 minutes, of PA (5 mg/100 g body weight, Sigma Chemical Company, St. Louis, Mo) via a tapered polyethylene catheter (PE 50) placed in the right internal jugular vein. The drug was dissolved in 3 ml of 0.9 g/100

Supported by NIH Grant AM 13132 and NIADDK Training Program in Academic Nephrology (2T322AM07241-07).

Dr. Diamond is a recipient of a Clinician-Scientist Award from the American Heart Association.

Accepted for publication October 11, 1985.

Address reprint requests to Jonathan R. Diamond, MD, Department of Pathology, Harvard Medical School, Building D-2, Room 347, 25 Shattuck Street, Boston, MA 02115.

ml sodium chloride. Another 5 rats received an equal volume of 0.9 g/100 ml sodium chloride. No apparent acute toxic effects of drug infusion were noted. The cannula was removed, and the vein was ligated at the end of the infusion. The animals were allowed free access to standard Purina Rat Chow and water. At 10, 63, 84, and 126 days after the intravenous injection, the rats were placed into metabolic cages, and a 24-hour urine collection was obtained for measurement of protein and creatinine. Total protein was quantitated by the Lowry method.[17] Serum for creatinine was obtained at the end of each 24-hour collection. Random animals from each group were sacrificed at 10, 42, and 84 days after PA administration for histologic study, with the remainder continuing for the duration of the study.

Preparation for Light Microscopy

Animals were anesthetized with ether, and the kidneys were perfused, *in vivo*, via an infrarenal aortic cannula. The perfusate solution consisted of a 0.1 M cacodylate buffer containing 5% sucrose (pH 7.4) for 2–3 minutes, followed by 2% glutaraldehyde in 0.1 M cacodylate buffer for 1–2 minutes. Both kidneys were removed, and the cortices were separated from the remainder of the kidneys and immersed in 2% glutaraldehyde in cacodylate buffer for an additional 2 hours. After fixation 1–2-mm cortical sections were rinsed for 2 hours in 0.1 M cacodylate buffer with 5% sucrose (pH 7.4) and prepared for light microscopy. Thick sections approximately 1μ were stained with either 1% toluidine blue or periodic acid–Schiff (PAS) reagent and viewed with a Leitz photomicroscope.

Statistical significance between groups was determined by using the Student *t* test.

Results

Values for body weight, 24-hour urinary volume and protein excretion, and creatinine clearance, after either PA or saline infusion, appear in Table 1. Weight gain was significantly lower in the PA group, at all time intervals after treatment. Daily urinary volumes between the two groups were not different. Ten days after PA administration, the mean value for 24-hour urinary protein excretion rose to 190.3 ± 136.2 mg/day as compared with the saline control animals ($P < 0.05$). Thereafter, urine protein excretion rate, in the PA group, declined, but persisted above normal control levels (Table 1). The creatinine clearance in PA-treated animals was significantly lower than in the saline control group at 10, 63, 84, and 126 days after administration of the drug ($P < 0.025$, < 0.025, < 0.05, and 0.025, respectively).

Light Microscopy

Review of whole kidney sections revealed no difference in morphologic alterations between juxtamedullary and outer cortical glomeruli. Figure 1 demonstrates histologic changes 10 days after PA administration. Evident abnormalities include segmental mesangial proliferation with matrix expansion, obliteration of glomerular capillary lumens, and adhesions between the glomerular tuft and Bowman's capsule. Changes in the GECs consisted of cell swelling, bleb formation, and protein reabsorption droplets. Forty-two days after PA delivery (Figure 2), the process is more global, with an increase in mesangial matrix and expansion of the mesangium as confirmed by PAS stain. In addition, it is more difficult to discern the glomerular capillary loops. In Figure 3, 84 days after PA, there is shrinkage of the glomerular tuft, with mesangial cell and matrix

Table 1—Serum and Urinary Measurements in Experimental Animals

Parameter measured	Treatment	Baseline value	Time interval after treatment			
			Day 10	Day 63	Day 84	Day 126
Body weight (kg)	PA	198 ± 7*	264 ± 46†	432 ± 26‡	497 ± 5†	585 ± 7†
	Saline	200	334 ± 13	514 ± 5	552 ± 24	645 ± 7
Urinary volume (ml/day)	PA	7.2 ± 0.9	6.3 ± 3.9	3.7 ± 3.1	8.1 ± 6.9	3.7 ± 1.1
	Saline	7.7 ± 0.8	7.9 ± 1.3	5.7 ± 1.7	4.5 ± 1.1	9.0 ± 0.7
Urinary protein (mg/day)	PA	14.5 ± 1.4	190.3 ± 136.2§	41.8 ± 19.4	81.6 ± 26.2	105.0 ± 60.2
	Saline	15.6 ± 2.8	31.2 ± 7.0	36.7 ± 5.3	62.7 ± 8.0	58.0 ± 2.8
Creatinine clearance (ml/min)	PA	1.17 ± 0.30	0.89 ± 0.42‖	0.65 ± 0.17‖	0.82 ± 0.06§	0.78 ± 0.06‖
	Saline	1.17 ± 0.45	1.80 ± 0.45	1.11 ± 0.26	1.11 ± 0.20	1.49 ± 0.41

* Values are expressed as the mean ± 1 SD.
† PA versus saline groups, $P < 0.010$.
‡ PA versus saline groups, $P < 0.001$.
§ PA versus saline groups, $P < 0.05$.
‖ PA versus saline groups; $P < 0.025$.
Statistical significance determined by the Student *t* test.

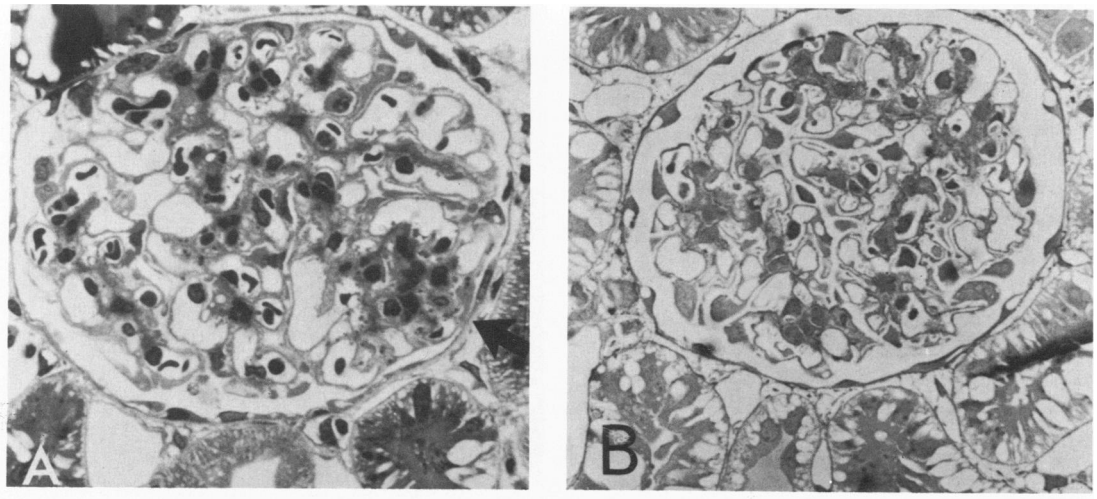

Figure 1—Light micrographs of glomeruli from PA-treated rats 10 days after drug administration. **A**—Glomerulus demonstrating segmental mesangial cell proliferation, mesangial matrix expansion, collapse of capillary lumens, and a small adhesion between Bowman's capsule and segment of glomerular tuft (*arrow*). (Toluidine blue, ×645) **B**—Glomerulus showing increased mesangial matrix with mesangial expansion. (PAS, ×645)

proliferation and marked collapse of capillary lumens. In addition, the epithelium lining Bowman's capsule has now become cuboidal, with large nuclei and prominent nucleoli. Also evident at 84 days after PA were scattered early hyalinosis lesions, which appeared as PAS-positive, crescent-shaped collections located subendothelially or within segments of expanded mesangium (Figure 4). At 126 days after PA, 42% ± 5.1% and 13.5% ± 5.1% of glomeruli examined showed segmental areas of mesangial proliferation or glomerulosclerosis/hyalinosis (Figure 5), respectively, in both outer cortical and juxtamedullary areas. However, in contrast to the renal ablation model, no areas of extensive hyalinosis or global glomerulosclerosis[9] were observed at the end of the 18-week period. There was no histologic evidence of hypertensive damage in the renal vasculature at any of the time periods after PA administration.

Discussion

Glomerular morphologic changes in rats with aminonucleoside nephrosis resembling those in human nephrotic syndrome with minimal changes have been demonstrated.[18] In addition, spontaneous resolution of the GEC lesion, on electron microscopy, accompanied by normalization of protein excretion occurred approximately 4 weeks after drug administration.[18] More re-

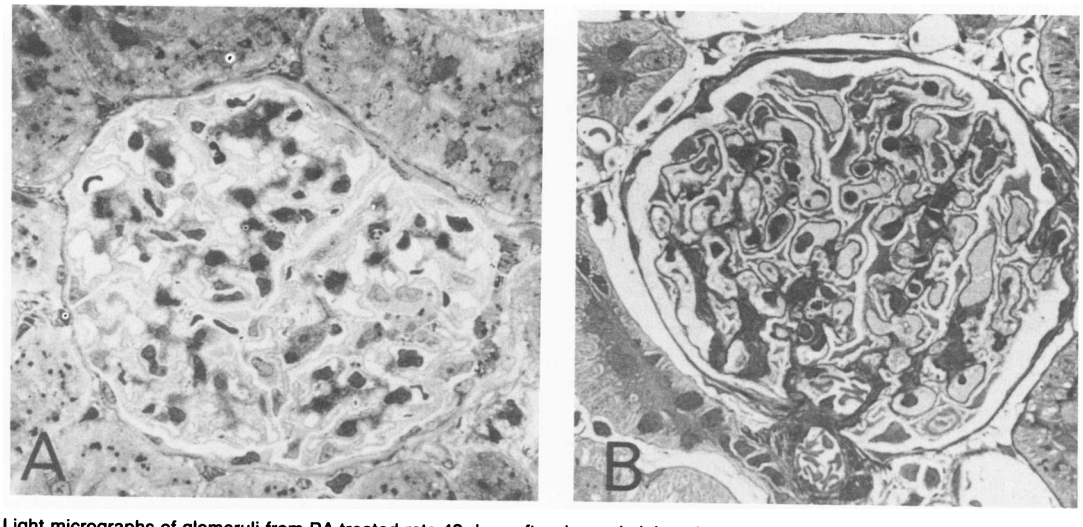

Figure 2—Light micrographs of glomeruli from PA-treated rats 42 days after drug administration. **A**—Glomerulus demonstrating more global morphologic abnormalities with increase in mesangial matrix and mesangial expansion. (Toluidine blue, ×645) **B**—PAS stain of glomerulus confirming increased deposition of glycosaminoglycans substance within the expanded mesangium. (×645)

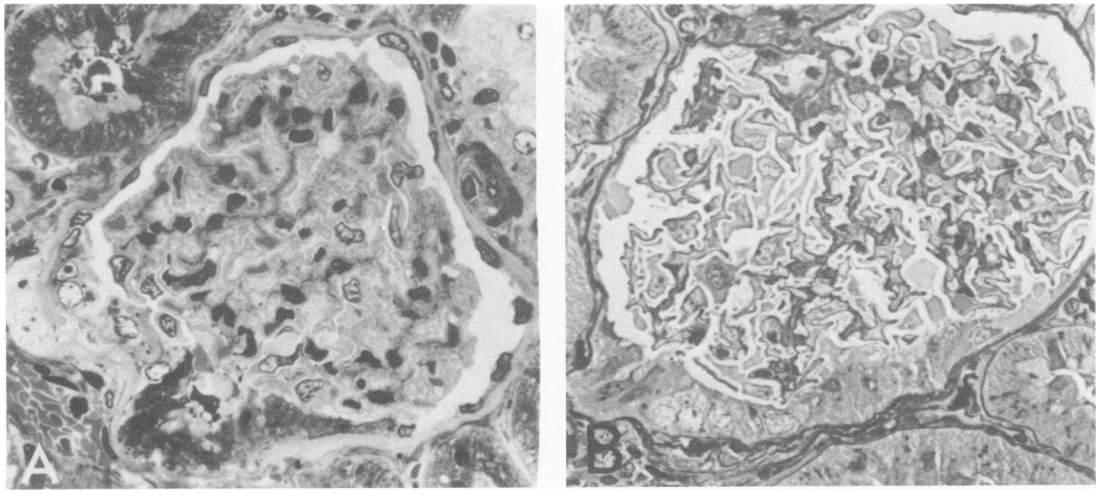

Figure 3—Light micrographs of glomeruli from PA-treated rats 84 days after drug administration. **A**—Shrunken glomerular tuft with marked global obliteration of capillary loops, mesangial cell, and matrix proliferation. Note also the presence of cuboidal epithelial cells lining Bowman's capsule with large nuclei and prominent nucleoli. (Toluidine blue, ×645) **B**—Glomerular tuft which is shrunken with capillary lumens that are difficult to discern. Again, the epithelial lining of Bowman's capsule has an altered histologic appearance. (PAS, ×645)

cent studies[12-14] have revealed that repeated intraperitoneal injections of PA can result in persistent proteinuria accompanied by morphologic abnormalities consistent with FSGS. In addition, Glasser et al[12] demonstrated that unilateral nephrectomy augmented proteinuria and markedly accelerated development of FSGS in rats given PA. Our data show that one-third of the usual dosage of PA employed, when injected as a single intravenous bolus, results in acute and chronic glomerular injury consistent with FSGS.

In an attempt to explain the differences in the course of the histologic progression of disease in rats receiving a single central intravenous dose of PA, as compared with other studies where the drug was administered via tail vein or intraperitoneally, we regard minimal change disease and FSGS as two pathologic processes in the same continuum of disease. According to Hoyer,[19] the morphologic features of the PA model vary depending on the magnitude of the initial injury, time after exposure to PA, and whether ongoing injury is occurring; either minimal change disease or FSGS may be seen despite a common primary cause. With the urinary protein excretion being significantly higher in the PA group at only Day 10 following drug delivery, our data, likewise, suggest that the magnitude of the initial glomerular injury may predict whether FSGS will occur. Although the precise mechanism for the development of FSGS following a single intravenous dose of PA remains obscure, one may speculate that the GECs suffered an irreversible insult secondary to PA. In our model, PA was administered directly into the central venous circulation by rapid bolus injection. Theoretically, the drug may have more reliably achieved higher concentrations within the kidney, as compared with previous studies in which the drug was administered as either single or repeated doses via the tail vein or intraperitoneally. In fact, Derr et al[20] have shown that there is an extremely rapid rate of urinary excretion of the drug when given to clinically normal animals. Thus, greatly elevated acute and transient levels of PA within the glomerulus may have produced such profound GEC injury that FSGS developed. Because our animals were normal prior to PA infusion, there is no reason to suspect that there was another ongoing renal insult that would have retarded the excretion of the drug.

With regard to the pathogenesis of FSGS in models utilizing PA, a few hypotheses have been proposed. First,

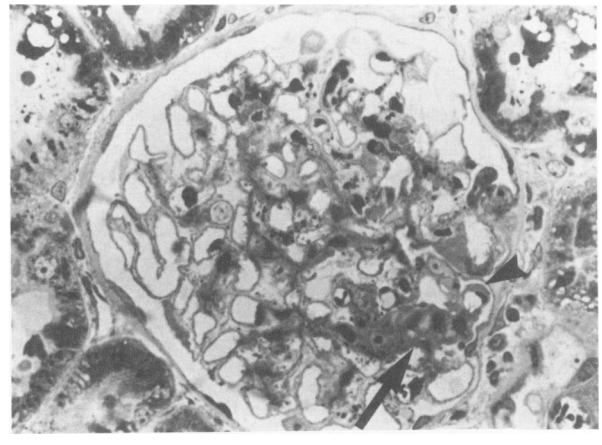

Figure 4—Light micrograph from a PA-treated rat 84 days after drug administration showing an early hyalinosis lesion (*arrowhead*) and segmental expansion of mesangium by hyalinosislike material and other materials. (Toluidine blue, original magnification ×645)

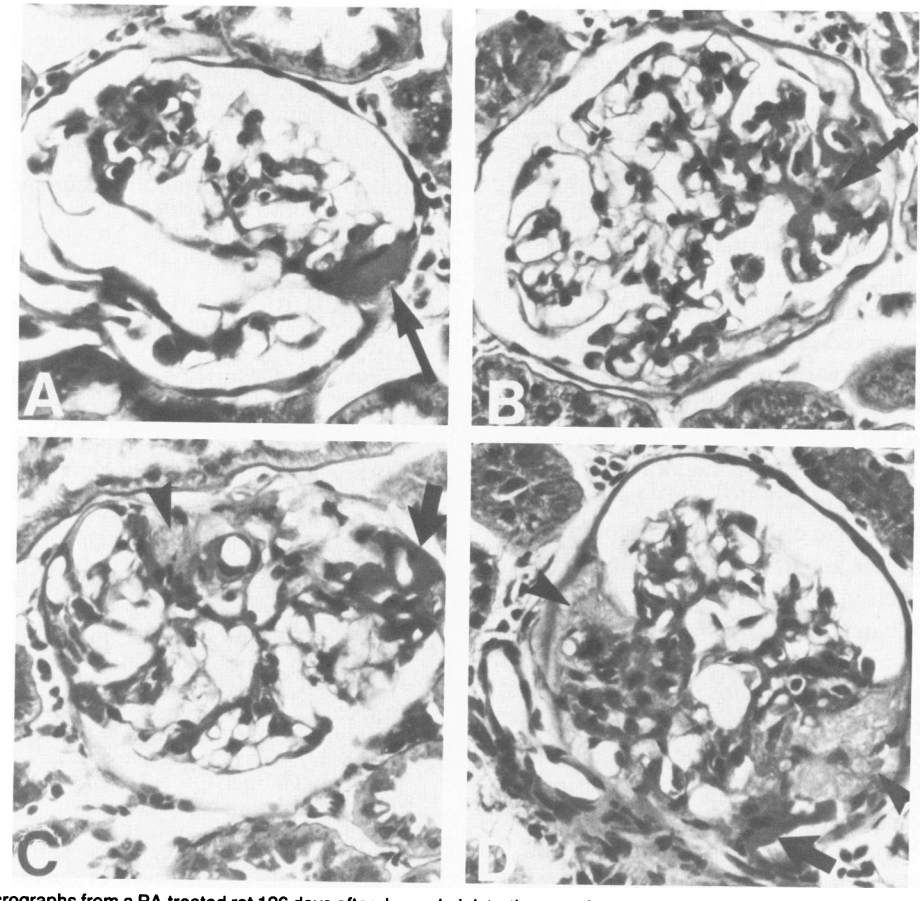

Figure 5—Light micrographs from a PA-treated rat 126 days after drug administration. **A** and **B**—Representative glomeruli demonstrating segmental areas of hyalinosis in peripheral capillary loops (*arrows*) with adhesions to Bowman's capsule. **C** and **D**—Representative glomeruli showing segmental necrotic foci in the glomerular capillary tuft (*arrowheads*) in addition to hyalinosis lesions (*arrows*). (PAS, ×600)

it has been demonstrated that there is enhanced mesangial accumulation of macromolecules in aminonucleoside nephrosis, which could cause "mesangial overloading."[21,22] This could then produce mesangial cell injury, mesangial cell proliferation, and matrix overproduction and ultimately glomerular sclerosis.[2] Available data suggest that the increased mesangial flux of macromolecules is probably not on a hyperfiltration or hyperperfusion basis, because direct micropuncture measurements have revealed significant reductions in single nephron glomerular filtration rate[23,24] and glomerular plasma flow rate[24] at 2 weeks after PA administration. Thus, there must be permselectivity changes within the glomerular basement membrane (GBM) and/or direct cell injury, independent of glomerular hemodynamic perturbations, in aminonucleoside nephrosis that ultimately leads to FSGS. The absence of glomerular hyperfiltration and/or hyperperfusion may also explain the lack of extensive hyalinosis in our animals, as contrasted with the renal ablation model.[9] Olson et al[25] have also recently suggested that early endothelial damage is caused by hyperfiltration, with the consequent accumulation of plasma constituents in the subendothelial space forming the hyalinosis lesions.

A second possibility is based on a correlation observed by Velosa et al.[13] In that study there was a direct relationship between the percentage of glomeruli with FSGS, polyanion loss, and total protein excreted, as well as a demonstration that areas of glomerular sclerosis corresponded to areas devoid of polyanion.[13] In summarizing the available data, Cotran and Rennke[26] suggested that in human diseases, such as congenital nephrosis or diabetes mellitus, as well as in experimental models, such as aminonucleoside nephrosis, there may be an abnormality in the turnover of proteoglycan or other anionic moieties by glomerular cells.

A third potential mechanism, which is not mutually exclusive with loss of charge selectivity, involves PA-mediated GEC injury that is independent of glomerular hyperfiltration and/or hyperperfusion and produces an abnormality in mesangial cell (MC) growth regulation. Castellot et al[27] have demonstrated, *in vitro*, that exogenous heparin, when added to culture medium, inhibited proliferation of both exponentially growing

MCs and MCs released from growth arrest. In addition, these authors noted that conditioned medium from GEC cultures possesses an inhibitor of MC growth and that this substance has heparinlike properties. Fishman and Karnovsky[28] have shown, *in vitro*, that the GEC is both morphologically and functionally injured by PA. Although the data remain conflicting, Caulfield and Farquhar[29] have shown that there is progressive loss of fixed negative charges from both the epithelial cell coat and the GBM. Since then, Mynderse et al[30] have found, *in vivo*, a marked loss of heparan sulfate proteoglycan in animals treated with PA; whereas Kerjaschki et al[31] have recently demonstrated that there is defective glycosylation of podocalyxin, a sialoglycoprotein, in addition to alterations in heparan proteoglycan. However, Kanwar and Kakubowski[32] have recently shown, using quantitative electron-microscopic autoradiography, that there are no significant alterations in the anionic sites rich in heparan sulfate proteoglycan in aminonucleoside nephrosis. Indeed, it would be interesting if the PA-mediated GEC injury initially produced a reduction in heparan biosynthesis, and this, in turn, secondarily resulted in an abnormality in MC growth regulation that could ultimately lead to the mesangial changes seen in FSGS. In fact, investigators have utilized different exogenous heparin species in the renal ablation,[33,34] the spontaneously hypertensive rat,[35] and the habu snake venom[36] models and have demonstrated protective effects in these disease states where MC proliferation play an integral role.

Clearly, additional investigation is needed to more properly discern the precise pathogenetic mechanism of FSGS following PA administration. This modified method of drug delivery, though, provides an easy and reliable technique for producing the chronic glomerular lesion. Because micropuncture studies, early in the course of the lesion, do not support glomerular hyperfiltration or hyperperfusion,[23,24] this model offers another approach for examining the natural progression of glomerular diseases to FSGS where augmented glomerular hemodyamics perturbations may not be involved.

References

1. Hostetter TH, Rennke HG, Brenner BM: Compensatory renal hemodynamic injury: A final common pathway of residual nephron destruction. Am J Kidney Dis 1982, 1:310–314
2. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 1982, 307:652–659
3. Baldwin DS: Chronic glomerulonephritis: Nonimmunologic mechanisms of progressive glomerular damage. Kidney Int 1982, 21:109–114
4. Kreisberg JI, Karnovsky MJ: Focal and segmental glomerular hyalinosis and sclerosis in the fawn-hooded rat. Am J Pathol 1978, 92:637–646
5. Abramowsky CR, Aikawa M, Swinehart GL, Snajdar RM: Spontaneous nephrotic syndrome in a genetic rat model. Am J Pathol 1984, 117:400–408
6. Couser WG, Stilmant MM: Mesangial lesions and focal glomerular sclerosis in the aging rat. Lab Invest 1975, 33:491–501
7. Elema JD, Arends A: Focal and segmental glomerular hyalinosis and sclerosis in the rat. Lab Invest 1975, 33:554–561
8. Shimamura T, Morrison AB: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. Am J Pathol 1975, 79:95–101
9. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. Am J Physiol 1981, 241:F85–F93
10. Bertani T, Rocchi G, Mecca G, Sacchi G, Remuzzi G: Adriamycin-induced chronic proteinuria: A new model of glomerular focal sclerosis (Abst) Kidney Int 1983, 23:192A
11. Fajardo LF, Eltringham JR, Stewart JR, Klauber MR: Adriamycin nephrotoxicity. Lab Invest 1980, 43:242–253.
12. Glasser RJ, Velosa JA, Michael AF: Experimental model of focal sclerosis. I. Relationship to protein excretion in aminonucleoside nephrosis. Lab Invest 1977, 36:519–526
13. Velosa JA, Glasser RJ, Nevins TE, Michael AF: Experimental model of focal sclerosis. II. Correlation with immunopathologic changes, macromolecular kinetics, and polyanion loss. Lab Invest 1977, 36:527–534
14. Grond J, Weening JJ, Elema JD: Glomerular sclerosis in nephrotic rats. Comparison of the long term effects of adriamycin and aminonucleoside. Lab Invest 1984, 51:277–285
15. Diamond JR, Bonventre JV, Karnovsky MJ: The role of oxygen free radicals in aminonucleoside nephrosis. Kidney Int 1986, 29:478–483
16. Lannigan R: The production of chronic renal disease in rats by a single intravenous injection of aminonucleoside of puromycin and the effect of low dosage continuous hydrocortisone. Br J Exp Pathol 1963, 44:326–333
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin method. J Biol Chem 1951, 193:265–275
18. Ryan GB, Karnovsky MJ: An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. Kidney Int 1975, 8:219–232
19. Hoyer JR: Focal segmental glomerulosclerosis. Semin Nephrol 1982, 2:253–263
20. Derr RF, Alexander CS, Nagasawa HT: Metabolism of puromycin aminonucleoside in the normal, pre-nephrotic, and nephrotic rat. Proc Soc Exp Biol Med 1967, 125:248–252
21. Seiler MW, Hoyer JR, Krueger TE: Altered localization of protamine-heparin complexes in aminonucleoside nephrosis. Lab Invest 1980, 43:9–17
22. Grond J, Koudstaal J, Elema JD: Mesangial function and glomerular sclerosis in rats with aminonucleoside nephrosis. Kidney Int 1985, 27:405–10
23. Ichikawa I, Rennke HG, Hoyer JR, Badr KF, Schor N, Troy JL, Lechene CP, Brenner BM: Role of intrarenal mechanisms in the impaired salt excretion of experimental nephrotic syndrome. J Clin Invest 1983, 71:91–103.
24. Bohrer MP, Baylis C, Robertson CR, Brenner BM: Mechanisms of the puromycin-induced defects in the trans-

glomerular passage of water and macromolecules. J Clin Invest 1977, 60:152–161
25. Olson JL, De Urdaneta AG, Heptinstall RH: Glomerular hyalinosis and its relation to hyperfiltration. Lab Invest 1985, 52:387–398
26. Cotran RS, Rennke HG: Anionic sites and the mechanisms of proteinuria. N Engl J Med 1983, 309:1050–1051
27. Castellot JJ, Hoover RL, Harper PA, Karnovsky MJ: Heparin and glomerular epithelial cell-secreted heparin-like species inhibit mesangial cell proliferation. Am J Pathol 1985, 120:427–435
28. Fishman JA, Karnovsky MJ: Effects of the aminonucleoside of puromycin on glomerular epithelial cells *in vitro*. Am J Pathol 1985, 118:398–407
29. Caulfield JP, Farquhar MG: Loss of anionic sites from the glomerular basement membrane in aminonucleoside nephrosis. Lab Invest 1978, 39:505–512
30. Mynderse LA, Hassell JR, Kleinman HK, Martin GR, Martinez-Hernandez A: Loss of heparan sulfate proteoglycan from glomerular basement membrane of nephrotic rats. Lab Invest 1983, 48:292–302
31. Kerjaschki D, Vernillo AT, Farquhar MG: Reduced sialylation of podocalyxin, the major sialoprotein of the rat kidney glomerulus in aminonucleoside nephrosis. Am J Pathol 1985, 118:343–349
32. Kanwar YS, Jakubowski ML: Unaltered aninoic sites of glomerular basement membrane in aminonucleoside nephrosis. Kidney Int 1984, 24:613–618
33. Olson JL: Role of heparin as a protective agent following reduction of renal mass. Kidney Int 1984, 25:376–382
34. Purkerson ML, Hoffsten PE, Klahr S: Pathogenesis of the glomerulopathy associated with renal infarction in rats. Kidney Int 1976, 9:407–417
35. Purkerson ML, Joist JH, Greenberg JM, Kay D, Hoffsten PE, Klahr S: Inhibition by anticoagulant drugs of the progressive hypertension and uremia associated with renal infarction in rats. Thromb Res 1982, 26:227–240
36. Coffey A, Karnovsky MJ: Heparin inhibits mesangial cell proliferation *in vivo* induced by habu snake venom. Am J Pathol 1985, 120:248–255