Rapamycin Ameliorates Proteinuria-Associated Tubulointerstitial Inflammation and Fibrosis in Experimental Membranous Nephropathy

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Proteinuria is a risk factor for progression of chronic renal failure. A model of proteinuria-associated tubulointerstitial injury was developed and was used to examine the therapeutic effect of rapamycin. Two studies were performed. In study A, proteinuric rats were given sheep anti-Fx1A to induce experimental membranous nephropathy; control rats received normal sheep serum. Four weeks later, groups were subdivided and underwent laparotomy alone (two kidneys), nephrectomy alone (one kidney), or nephrectomy with polectomy (0.6 kidney). Renal function and morphology were evaluated 4 wk later. Whereas control rats never developed proteinuria, anti-Fx1A induced severe proteinuria. Proteinuria was unaffected by renal mass reduction. Proteinuric rats developed tubulointerstitial disease that was most severe in rats with 0.6 kidneys. Renal function (GFR) was reduced by loss of renal mass and was reduced further in proteinuric rats with 0.6 kidneys. In study B, the effect of rapamycin on the expression of candidate proinflammatory and profibrotic genes and the progression of proteinuria-associated renal disease were examined. All rats received an injection of anti-Fx1A and were nephrectomized and then divided into groups to receive rapamycin or vehicle. Gene expression, renal morphology, and GFR were evaluated after 4, 8, and 12 wk. Rapamycin reduced expression of the proinflammatory and profibrotic genes (monocyte chemotactic protein-1, vascular endothelial growth factor, PDGF, TGF-β1, and type 1 collagen). Tubulointerstitial inflammation and progression of interstitial fibrosis that were present in vehicle-treated rats were ameliorated by rapamycin. Rapamycin also completely inhibited compensatory renal hypertrophy. In summary, rapamycin ameliorates the tubulointerstitial disease associated with chronic proteinuria and loss of renal mass.

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Chronom proteinuria is an important and independent risk factor for progressive loss of renal function that occurs in many patients with chronic renal failure. Therapeutic interventions aimed at reducing protein excretion (e.g., angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) have been shown to slow progression of renal disease and have become part of standard medical practice for the treatment of proteinuria (1,2). However, these strategies seldom completely normalize proteinuria and rarely halt the progressive loss of renal function. Therefore, the development of additional and/or alternative interventions that slow or prevent proteinuria-associated tubulointerstitial injury remains an important therapeutic goal.

It has been suggested that proteinuria contributes to progression of renal disease by exacerbating tubulointerstitial disease (3,4). Studies in experimental models suggest that proteinuria induces a proinflammatory and profibrotic milieu within the renal parenchyma (5,6) by stimulating the production of proinflammatory and profibrotic cytokines, chemokines, and growth factors such as IL-8, RANTES, monocyte chemotactic protein 1 (MCP-1), and TGF-β (7–13). However, the mechanisms of proteinuria-associated tubulointerstitial disease have proved difficult to study.

The first objective of this study was to characterize a model of progressive interstitial disease and renal dysfunction that could be attributable primarily to proteinuria. We used an accelerated model of experimental membranous nephropathy (eMN) in rats with passive Heymann nephritis (PHN) for this purpose. PHN is induced in rats by administration of sheep antiserum raised against a proximal tubular cell brush border fraction (Fx1A) (14). This results in a noninflammatory form of glomerular disease that resembles human membranous nephropathy with immune complexes deposited in an exclusively subepithelial location (15). Proteinuria is due to complement-mediated podocyte injury (15). Reduction in renal mass in PHN accelerates the development of tubulointerstitial disease (16).

In the second part of this study, we tested the hypothesis that rapamycin would ameliorate proteinuria-associated interstitial disease. Rapamycin is used clinically to suppress rejection of transplanted organs (17). The immunosuppressive effect of rapamycin is due to inhibition of the mammalian target of

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rapamycin (mTOR), a serine/threonine kinase that is necessary for proliferation and clonal expansion of activated T cells (18). Inhibition of mTOR has been documented at rapamycin doses as low as 0.1 mg/kg per day in the rat. In addition to its immunosuppressive actions, rapamycin inhibits growth factor–mediated proliferation and survival of many nonimmune cells, including endothelial cells, renal tubular cells, and fibroblasts (19–22). In this study, we demonstrate that a relatively low dose of rapamycin markedly ameliorates the development of the intrarenal inflammation and interstitial fibrosis that is associated with proteinuria in eMN.

**Materials and Methods**

**Animal Protocols**

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were used for all experiments. The rats were housed in the Laboratory Animal Science Center at Boston University School of Medicine and allowed free access to water and chow (Purina 5001). Two studies were performed. The aim of the first study (study A) was to establish a model of proteinuria–associated chronic interstitial disease. The second study (study B) was designed to use the model characterized in study A to test the hypothesis that rapamycin would ameliorate interstitial injury associated with chronic proteinuria. All protocols were reviewed and approved by the Boston University Institute for Animal Care and Use Committee.

**Study A.** Rats that weighed 200 to 250 g were divided into two groups: proteinuric and control. Proteinuria was induced in one group (n = 48) by the administration of sheep anti-Fx1A (14). The control group (n = 36) received normal sheep serum. A cohort of the proteinuric rats (n = 12) were killed at weekly intervals in a pilot study to assess the time of appearance and disappearance of anti-Fx1A from the tubular brush border.

Four weeks after anti-Fx1A administration, all control rats and the remaining proteinuric animals (n = 36 in each group) were divided into three subgroups and subjected to right nephrectomy only (one kidney), right nephrectomy with left upper poleectomy (0.6 kidney), or laparotomy without removal of renal mass (two kidneys). After another 4 wk, all rats were anesthetized, GFR was measured by inulin clearance, and kidneys then were harvested for morphologic evaluation. At the end of the study, animals were killed while under anesthesia. Six rats (three controls and three proteinuric) died before completion of the study protocol, reducing the number of animals to 33 in each group.

**Study B.** In a subsequent experiment, the effect of rapamycin on progression of proteinuria–associated tubulointerstitial disease was studied. In this study, all rats (n = 52) were given anti-Fx1A to induce proteinuria then uninephrectomized 4 wk later as described in study A. One week after the nephrectomy, the rats were divided into two groups that received daily subcutaneous injections of either rapamycin (0.5 mg/kg per d) or the vehicle (carboxymethyl cellulose + poly-ethylene-sorbitan monoleate in ddH2O; Wyeth Pharmaceuticals, Madison, NJ). On the basis of a pilot study, we chose a dose of rapamycin that is below that usually used as monotherapy for rats after kidney transplantation (0.8 to 3.0 mg/kg per d) and that does not adversely affect renal function or hematopoiesis (23–25).

Weight gain, protein excretion rate, and BP were measured during the study period. After 1 (n = 2/group), 4, 8, and 12 wk (n = 8/group) of treatment, inulin clearance was measured in cohorts from each group. The animals then were killed, and renal tissues were prepared for histology and RNase protection assay.

**Functional Studies**

**Proteinuria.** In both studies A and B, protein excretion was measured every 2 wk after nephrectomy. Rats were placed in metabolic cages with free access to water, and urine was collected over a 16- to 24-h period. Urine volume and protein concentration were measured using the Bio-Rad method (Bio-Rad, Hercules, CA), and daily protein excretion rate was calculated.

**BP.** In both studies, tail-cuff BP was measured in unanesthetized rats using a NARCO polygraph (Narcotrace, Houston, TX) connected to a pressure transduction system (26). After rats were habituated to the procedure, systolic BP was measured five times on each occasion, and the average of the five measurements was calculated.

**Inulin Clearance.** GFR was assessed by inulin clearance. Rats were anesthetized with intraperitoneal pentobarbital sodium (55 mg/kg). Rats were tracheostomized and placed onto a thermostatically controlled operating table that maintained rectal temperature between 36 and 38°C. The jugular vein and bladder were catheterized to allow for intravenous infusion and urine collection. The femoral artery was catheterized for blood sampling and continuous measurement of mean arterial pressure using a pressure transducer. Inulin clearance was determined by continuous intravenous infusion of 1H inulin (0.06 μCi/min). Blood and urine samples were obtained during three consecutive 20-min clearance periods. Samples of plasma and urine (5 μl each) were added to separate vials and counted using a liquid scintillation counter (Tricarb 1600TR; Packard Instruments, Meriden, CT). Inulin clearance was calculated using the standard formula (27).

**Morphometry**

Formalin-fixed sections were stained with periodic acid-Schiff (PAS) and Masson’s trichrome. Sections were coded by one investigator (R.F.), then digitally photographed and analyzed by another blinded investigator (R.B.). Photographs of each section were taken using a stereomicroscope (Nikon Ophiphot, Melville, NY) fitted with a digital camera (Diagnostic Instruments, Sterling Heights, MI). Using two PAS- and two trichrome-stained sections from each animal, six random low-power (×100 magnification) photographs were taken of each section (representing approximately 10% of the cortex) and morphometrically analyzed using point counting. To this end, a standardized grid of 70 points was superimposed on each photograph. The number of points over glomeruli, tubular epithelia, or interstitial tissues was determined and used to assess the proportion of the cortex occupied by each structure. A similar technique was used to assess the proportion of the cortex that was infiltrated by inflammatory cells (PAS) or fibrous tissue (Masson’s trichrome). In addition, the proportion of points that fell in the tubular lumen was used as an indicator of tubular simplification and dilation.

Glomerular volume was calculated from the mean glomerular cross-sectional area as previously described (28). Briefly, mean glomerular tuft area (A C) was determined in each animal using computer-assisted pixel counting in the same photographs used for morphometry. The mean A C for each animal was calculated from all available glomerular profiles, and tuft volume (V C) was calculated as V C = (B/8k) × (A C)1.5, where B is the shape coefficient for an idealized spherical glomerulus (B = 1.38) and k is a size distribution coefficient (k = 1.10).

**Immunohistochemistry**

We detected the presence and distribution of sheep anti-Fx1A antibody in frozen sections of kidney tissue obtained from 12 rats that were killed at weekly intervals (3 rats/wk) after antibody injection. Sections were stained with FITC-conjugated anti-sheep IgG (F5137; Sigma, St. Louis, MO). For detection of CD45, frozen sections from four animals in
each of the study A subgroups were stained with anti-rat CD45 (550566; BD Biosciences, Pharmingen, San Diego, CA). Antibodies were detected using appropriate secondary antibodies in conjunction with the Vectastain ABC kit (AK-5002; Vector Laboratories, Burlingame, CA). Stained sections were coded, then photographed by a blinded investigator (R.B.) and analyzed by color-limited pixel counting (Adobe Photoshop 8.0; Adobe Systems Inc., San Jose, CA).

**Multiprobe RNase Protection Assay**

In study B, the effect of rapamycin treatment on the expression of proinflammatory, profibrotic, and matrix-modulating genes was examined using RNase protection assays.

**RNA Extraction.** RNA was extracted from 0.5 g of kidney cortex using the TRIzol (Invitrogen, Carlsbad, CA) one-step method (29). RNA was purified further by two rounds of phenol-chloroform (70/30% vol/vol) extraction then ethanol-precipitated and resuspended in DEPC-treated H2O. After quantification and quality control, samples were aliquotted and stored at −80°C.

**Probe Generation.** Custom-made multiprobe template sets (BD Biosciences) were chosen to represent renally expressed growth factors, cytokines, chemokines, and matrix modulators that have previously been shown to modulate renal fibrosis (9,30,31). Probe sets used in study B were #62 (vascular endothelial growth factor [VEGF], MCP-1, PDGF-A, TGF-β1, TGF-β2, TGF-β3, TGF-βRI, TGF-βRII, bone morphogenetic protein 7 [BMP-7], L32, and glyceraldehyde-3-phosphate dehydrogenase [GAPDH]), #84 (matrix metalloprotease-2 [MMP-2], MMP-9, TNF receptor p75 [TNF Rp75], TNF Rp55, macrophage inflammatory protein-2, plasminogen activator inhibitor 1, tissue inhibitor of metalloproteinase 1[TIMP1], TIMP2, L32, and GAPDH), and #85 (IL-10, IL-1, IL-12, type 1 collagen, type 2 collagen, TNF-α, L32, and GAPDH). Radiolabeled probes were generated from the templates using the T7 RNA polymerase (Promega, Madison, WI) according to the manufacturer’s instructions.

**Hybridization and RNase Digestion.** At the end of study B, 15 μg of total RNA from each sample (n = 4 per group at 4, 8, and 12 wk) was used for analysis. The RNA was re-precipitated with ethanol then resuspended in hybridization buffer. Radiolabeled probes (10⁶ cpm) were added to each sample, which then was denatured at 90°C for 5 min. Hybridization was performed overnight at 56°C. Unprotected RNA and probes were then digested using the RNase cocktail (100 μl of RNase buffer, 10 mM Tris [pH 7.5], 30 mM sodium acetate, and 5 mM EDTA with 75 U of RNase T1 and 24 ng of RNase A; BD Biosciences). After digestion, RNase was removed by proteinase K digestion followed by phenol-chloroform extraction. Protected hybrids (dsRNA) were precipitated with ethanol using 3 μg of RNA as a carrier, then resuspended in loading buffer.

**PAGE Gel and Quantification.** Samples were loaded and run using a preheated denaturing sequence gel (5% acrylamide, 8 M urea, TBE). Undigested probes served as size markers. The radioactivity of each band was quantified by phosphoimaging (Cyclone Phospho Screen; Packard, Doener’s Grove, IL). Readings for each gene were normalized to the GAPDH signal.

**Statistical Analyses**

Data are expressed as the mean ± SE. Groups were compared using ANOVA and the t test, with the Bonferroni correction for multiple comparisons. Differences in gene expression between the vehicle- and rapamycin-treated groups were compared at baseline and during treatment using the Wilcoxon rank sum test for nonparametric data. Results were considered statistically significant at P < 0.05.

**Results**

**Study A**

**Distribution of Heterologous Sheep IgG.** In proteinuric rats, intense granular staining of sheep IgG was present in glomerular capillary loops (the classical site of immune complexes in membranous nephropathy) 1 wk after the injection of anti-Fx1A. As has been previously described, staining was also present along the brush border of the proximal tubules (Figure 1A) (14). No antibody staining was evident in kidneys of control rats. Whereas heterologous antibody staining persisted within glomeruli, the tubular brush border staining was cleared and anti-Fx1A was undetectable 3 wk after injection (Figure 1A).

In contrast to previous studies (16,32), we delayed renal mass reduction in our studies for 4 wk after administration of anti-Fx1A, i.e., a period well after clearance of tubular-bound anti-Fx1A (Figure 1A), to avoid potentially confounding tubulotoxic effects of brush border–bound deposition of anti-Fx1A.

**Effects of Proteinuria and Renal Mass Reduction on Weight, BP, Proteinuria, and Renal Function.** Proteinuric animals gained less weight during the study period and at the end of the study were smaller than control animals (424 ± 33 g versus 492 ± 20 g, respectively). Weight was not altered by renal mass reduction. Control animals did not develop proteinuria (Figure 1B), whereas anti-Fx1A induced in all animals severe proteinuria that persisted for the duration of the study. Renal mass reduction did not alter the severity of proteinuria in animals that received anti-Fx1A (Figure 1B). Systolic BP was normal and comparable among groups (132 ± 5 versus 137 ± 4 mmHg in control and proteinuric groups at the end of the study, respectively) and was unaffected by renal mass reduction. GFR in proteinuric and control rats with two kidneys were comparable (Figure 1C). However, there was a trend toward a lower GFR in proteinuric compared with control rats with one kidney (P = 0.054), and GFR was significantly lower in 0.6 kidney proteinuric compared with 0.6 kidney control rats (P < 0.004; Figure 1C).

**Effect of Reduction in Renal Mass and Proteinuria on Glomerular Hypertrophy.** Glomerular hypertrophy occurred in response to the reduction in renal mass in control rats (Figure 1D). In addition, proteinuric rats had significantly larger glomeruli than control rats regardless of their renal mass (Figure 1D).

**Effects of Proteinuria and Renal Mass Reduction on Tubulointerstitial Disease.** There was no evidence of tubulointerstitial inflammation in any of the control rats (Figure 2A). However, in all proteinuric rats, a mononuclear infiltrate was present in the renal interstitium as evidenced by morphometric analysis of PAS-stained sections (Figure 2, A and C) and by CD45 staining (data not shown). The infiltrate was of comparable severity in proteinuric rats with one and two kidneys. However, the cortical inflammation was substantially worse in 0.6 kidney proteinuric rats (Figure 2, A and D). Similarly, tubular atrophy and dilation were greater in kidneys from all proteinuric compared with nonproteinuric rats, being most severe in the 0.6 kidney proteinuric animals (data not shown).
Interstitial fibrosis was assessed in sections of kidneys stained by the Masson's trichrome stain. There was minimal evidence of fibrosis in all nonproteinuric animals, whereas proteinuric rats with one or two kidneys had mild but significant amounts of fibrosis (Figure 2B). In contrast, a substantial amount of interstitial fibrosis was present in proteinuric rats with 0.6 kidneys (Figure 2, B and D).

Study B: Effect of Rapamycin on Proteinuria-Associated Tubulointerstitial Disease

Effects of Rapamycin on Body Weight, BP, GFR, and Proteinuria. In study B, severe proteinuria was induced in all rats (n = 52) by administration of anti-Fx1A (Figure 3). Rats were divided into two groups (n = 26 each), all of which received daily subcutaneous injections of either rapamycin (0.5 mg/kg per d) or the rapamycin vehicle. Rats from both groups tolerated therapy well and gained weight comparably during the 12-wk treatment period (P = 0.18 vehicle versus rapamycin). In this study, which was considerably longer than study A, proteinuric rats developed mild systolic hypertension. There was no difference between treatment groups (at study end, rapamycin and vehicle systolic BP were 153 ± 6 and 156 ± 7 mmHg, respectively). In addition, there was a trivial decline in GFR during the treatment period (ΔGFR between weeks 4 and 12 in rapamycin and vehicle groups was −0.17 ± 0.12 and −0.28 ± 0.31 ml/min per 450 g, respectively), which was not different between groups (P = 0.81).

Anti-Fx1A induced similar levels of proteinuria in rats from the two groups before treatment. As described previously in PHN (33), the rats had maximal protein excretion 4 to 6 wk after anti-Fx1A injection. In the vehicle group, this peak was followed by a gradual decline in protein excretion (Figure 3). Rapamycin decreased proteinuria by a further 28, 56, and 49% at 6, 8, and 12 wk, respectively (Figure 3).

Effects of Rapamycin on Progression of Histologic Lesions in Proteinuric Animals. A mild interstitial inflammatory infiltrate was seen at baseline in all nonproteinuric animals, whereas proteinuric rats with one or two kidneys had mild but significant amounts of fibrosis (Figure 2B). In contrast, a substantial amount of interstitial fibrosis was present in proteinuric rats with 0.6 kidneys (Figure 2, B and D).
As rapamycin was given in a therapeutic regimen in this study, mild interstitial fibrosis was evident in the baseline samples that were taken before initiation of treatment (Figure 6, A and C). Whereas vehicle-treated rats developed progressively worsening interstitial fibrosis, rapamycin-treated animals had no significant progression of disease (Figure 6, B and C). The decreased progression of fibrosis was associated with decreased expression of type 1 collagen and fibrosis (D) both were exacerbated by severe renal mass reduction (0.6 kidney). \*P < 0.05 proteinuric versus controls with the same renal mass; \*P < 0.05 versus two-kidney rats from the same group.

**Figure 2.** Effect of proteinuria and renal mass reduction on interstitial inflammation and fibrosis. (A) Inflammation of the renal interstitium. Representative periodic acid-Schiff (PAS)-stained sections of kidney cortex show that nonproteinuric (control) rats did not develop interstitial inflammation even when nephron mass was reduced. However, interstitial inflammation was present (filled arrows) in kidneys of proteinuric rats. (B) Interstitial fibrosis. Representative trichrome-stained section of kidney cortex from rats with proteinuria demonstrates blue-staining interstitial fibrosis (open arrows), which was most severe in rats with the combination of proteinuria and reduced renal mass. Tubular simplification and dilation (Φ) was prominent in animals with proteinuria and 0.6 kidneys. Morphometric analysis of tissue demonstrates that proteinuria-induced interstitial inflammation (C) and fibrosis (D) both were exacerbated by severe renal mass reduction (0.6 kidney). \*P < 0.05 proteinuric versus controls with the same renal mass; \*P < 0.05 versus two-kidney rats from the same group.

**Effect of Rapamycin on Renal and Glomerular Hypertrophy.** Glomerular tuft volume (Figure 7, A and B) and kidney size (Figure 7C) were markedly lower in rapamycin-treated rats. Thus, an unexpected finding of our study was that rapamycin completely inhibited the compensatory renal and glomerular hypertrophy associated with proteinuria and renal mass reduction.

**Discussion**

The initial objective of this study was to examine the effects of renal mass reduction in experimental membranous nephropathy, a model of proteinuria that is not associated with severe
glomerular inflammation or sclerosis (7,14,16). We show that animals with proteinuria and normal renal mass or uninephrectomy develop patchy interstitial inflammation (Figure 2A) and fibrosis (Figure 2B). Despite heavy proteinuria, these animals have normal renal function that remains stable for several months. These findings are consistent with previous chronic studies of experimental membranous nephropathy (7,16,32,33).

We demonstrate that experimental membranous nephropathy is associated with glomerular hypertrophy (Figure 1D), a phenomenon that is known to occur in response to both renal mass reduction and proteinuria (34,35). We also provide novel evidence that when renal mass reduction is severe (0.6 kidney), interstitial inflammation and fibrosis associated with experimental membranous nephropathy are markedly exacerbated and renal function deteriorates (Figure 1C). The deleterious effects of reduced renal mass on interstitial disease and renal function could be due to a number of factors, including an increase in single nephron protein flux (28) and/or glomerular hypertrophy, which is known to contribute to the progression of chronic renal disease (34–36).

The second objective of the study was to examine the effect of rapamycin in this model of proteinuria-associated progressive tubulointerstitial disease. Proteinuric states are thought to initiate an intrarenal inflammatory response by stimulating the production of proinflammatory cytokines and chemokines (11,37,38). Because rapamycin is known to inhibit responses induced by a number of cytokines (IL-2 [39], IL-4 [40], IL-7 [41], and IL-15 [42]), we examined the hypothesis that rapamycin...
inhibits proteinuria-associated renal disease and that these effects are mediated by inhibition of expression of cytokines and growth factors.

Rapamycin markedly attenuates both the interstitial inflammatory (Figure 4) and fibrotic (Figure 6) responses associated with proteinuria-induced renal disease. In addition, rapamycin reduces the expression of cytokines and profibrotic growth factors such as MCP-1, PDGF, and TGF-β1 (Figure 5) as well as the expression of type 1 collagen (Figure 6, D and E). These findings are also consistent with other available evidence that rapamycin inhibits the growth factor–stimulated proliferation of fibroblasts and is a potent inhibitor of processes that lead to progressive fibrosis (20,43).

An unexpected finding of the study was that the glomerular hypertrophy that occurred in response to nephrectomy and proteinuria was markedly inhibited by rapamycin therapy (Figure 7). This finding is consistent with the well-described importance of mTOR signaling in stimulating protein synthesis and cell growth (44). Because glomerular hypertrophy contributes to the progression of chronic renal disease (34–36), it is likely that the effects of rapamycin on glomerular hypertrophy contributed to some extent to the protective effects of rapamycin on tubulointerstitial disease.

We therefore have demonstrated that rapamycin ameliorates the progression of proteinuria-induced renal disease by at least three mechanisms: inhibition of interstitial inflammation, reduction of intrarenal fibrosis, and the attenuation of the hypertrophy of resident renal cells. Although the interstitial inflammation and fibrosis in this model were associated with tubular "dropout," tubular cell proliferation and apoptosis were not prominent features. This is in keeping with the slow progression of renal failure in this model.

Rapamycin treatment resulted in a substantial reduction in proteinuria in our model (Figure 3). However, the effects of rapamycin on tubulointerstitial disease cannot be explained by the reduction in proteinuria, which occurred relatively late and well after the interstitial inflammation and fibrosis had already occurred. However, the ability of rapamycin to reduce proteinuria could potentially have additional benefits in this model. These effects of rapamycin are clearly of potential clinical importance and suggest that rapamycin may represent a viable treatment option for the treatment of proteinuric renal diseases in humans.

It is noteworthy that although therapy with rapamycin markedly reduced tubulointerstitial disease, the decline in proteinuria, which occurred relatively late and well after the interstitial inflammation and fibrosis had already occurred. However, the ability of rapamycin to reduce proteinuria could potentially have additional benefits in this model. These effects of rapamycin are clearly of potential clinical importance and suggest that rapamycin may represent a viable treatment option for the treatment of proteinuric renal diseases in humans.

It is interesting that the available evidence suggests that rapamycin has the potential to be beneficial or deleterious, depending on the contribution of mTOR-dependent cellular proliferation and hypertrophy to disease pathogenesis. Our group previously reported that inhibition of tubular cell pro-

**Figure 5.** Rapamycin ameliorates proteinuria-associated proinflammatory and profibrotic gene expression. Rapamycin ameliorated the expression of proinflammatory chemokine monocyte chemotactic protein 1 (MCP-1) and several profibrotic candidate genes. (A) RNase protection assay of profibrotic growth factors vascular endothelial growth factor (VEGF), PDGF-A, TGF-β1, and TGF-β receptor 1 (TGF-βR1) and the chemokine MCP-1. Lane 1 contains undigested labeled probe that was used as a ladder. Rapamycin-treated animals are shown in lanes 2 through 9, and vehicle-treated animals are in lanes 10 through 17. Even-numbered lanes (2 through 16) were derived from the baseline nephrectomy and represent the level of expression in proteinuric animals before treatment, whereas odd-numbered lanes (3 through 17) represent the level of expression after 4 wk of rapamycin or vehicle therapy. (B through E) Quantitative analysis of the effects of rapamycin on MCP-1 (B), VEGF (C), PDGF-A (D), and TGF-β1 (E) gene expression over the course of the 12-wk study. *P < 0.05 vehicle versus rapamycin; †P < 0.05 versus baseline.
liferation by rapamycin delays recovery from acute renal failure (21). Others have reported that the same effect of rapamycin is beneficial in polycystic kidney disease, in which excessive tubular cell proliferation seems to play a pathogenic role (45). Similar to the beneficial effects that have been ascribed to rapamycin after renal transplant or coronary artery stenting (46), the data presented above suggest that rapamycin has beneficial effects in chronic proteinuric disease by inhibiting proteinuria-associated inflammation and fibrosis. These same effects of rapamycin (decreased inflammation and fibrosis) have been reported to impair wound healing after transplant (47).

In summary, we have demonstrated that experimental membranous nephropathy is associated with progressive injury of the kidney characterized by interstitial inflammation, fibrosis, and glomerular hypertrophy. Renal mass reduction in this model exacerbates the development of interstitial disease and glomerular hypertrophy. Because experimental membranous nephropathy is not associated with either glomerular inflammation or sclerosis, the renal disease observed in this model

Figure 6. Rapamycin slows the progression of interstitial fibrosis in proteinuric rats postnephrectomy. (A) A trichrome-stained kidney section showing early interstitial fibrosis (filled arrows) and proteinaceous tubular casts (arrowheads) that were present at baseline in both groups. (B) Trichrome-stained sections show that interstitial fibrosis (arrows) worsened over time in the vehicle-treated rats but did not progress in rats that were given rapamycin. Tubular dilation was also lessened by rapamycin. (C) Morphometric assessment (n = 8/group at each time point) confirmed that progressive worsening of interstitial fibrosis in the vehicle group was significantly ameliorated by rapamycin. (D and E) RNase protection assay confirmed that mRNA for type 1 collagen (Col1A1), which was expressed equally before treatment, was decreased by rapamycin at all time points during treatment. *P < 0.05 vehicle versus rapamycin; †P < 0.05 versus baseline. Magnification, ×100 in A.
Figure 7. Rapamycin inhibits compensatory renal hypertrophy. (A) PAS-stained kidney sections photographed at the same magnification from vehicle- and rapamycin-treated rats after 8 wk of treatment. Sections shown here were chosen for their large number of representative glomeruli. Circles denote the size of glomeruli in the vehicle group. Note that the glomeruli from rapamycin-treated rats appear smaller. No glomerulosclerosis was noted at this time. (B) Morphometric analysis of glomerular tuft size confirmed that glomerular hypertrophy developed in the vehicle group after nephrectomy and that rapamycin completely inhibited this hypertrophy. (C) Similarly, the increase in kidney weight that occurred in the vehicle group after nephrectomy was not seen in the rapamycin group. These changes were not associated with significant differences in body weight between the groups. \(^*P < 0.05\) vehicle versus rapamycin; \(^{1}P < 0.05\) versus baseline. Magnification, \(\times 200\) in A.

likely is due to the proteinuria per se. We provide novel evidence that rapamycin, in relatively low doses (compared with those used for monotherapy after transplantation), substantially ameliorates proteinuria-induced interstitial inflammation and fibrosis as well as compensatory glomerular hypertrophy. Additional studies will be necessary to elucidate further the mechanisms that are responsible for the protective effects of rapamycin on proteinuria-associated progressive renal disease and to determine whether rapamycin has similar effects in humans with proteinuric states.

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