Rapamycin worsens renal function and intratubular cast formation in protein overload nephropathy

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Rapamycin worsens renal function and intratubular cast formation in protein overload nephropathy.

Background. Rapamycin (sirolimus) is associated with functional nephrotoxicity in some patients with nephrotic glomerular diseases but the pathophysiologic mechanisms are not known. This study investigated the effects of rapamycin on renal function and structure in protein overload nephropathy.

Methods. Rats with protein overload nephropathy [induced by bovine serum albumin (BSA), 2.1 g by daily intraperitoneal injection, day 0 to day 3] received daily intraperitoneal injections of either vehicle [dimethyl sulfoxide (DMSO)], rapamycin (0.2 mg/kg, an inhibitor of mammalian target of rapamycin), or roscovitine (3.5 mg/kg, a small molecule cyclin-dependent kinase inhibitor) \((N = 9\) each) from day \(-3\) to day 3.

Results. In protein overload nephropathy, rapamycin caused severe acute renal failure and mild hypercholesterolemia (both \(P < 0.05\)). Rapamycin dramatically increased intratubular cast formation, and proximal tubular epithelial cells were swollen and engorged with increased cytoplasmic protein droplets. The number of 5-bromo-2′-deoxyuridine (BrdU)-positive tubular epithelial cells increased by more than 20-fold on day 3 in protein overload nephropathy, and this was attenuated by 65% with rapamycin \((P < 0.05)\), whereas roscovitine was ineffective. Rapamycin increased the protein expression of p27\(^{kip1}\) in tubular epithelial cells, but did not alter D-type cyclin expression or apoptosis.

Conclusion. Rapamycin caused a specific pattern of acute renal injury characterized by increased intratubular cast formation in protein overload nephropathy. This could be due to disruption of a potentially important compensatory mechanism in nephrotic glomerular diseases involving tubular epithelial cell protein endocytosis and proliferation.

Rapamycin (sirolimus) is a new immunosuppressant with potent antitumor and antiproliferative properties [1, 2]. Preclinical and clinical studies have now firmly established rapamycin as an excellent agent in calcineurin inhibitor–free immunosuppressant regimens to prevent long-term allograft rejection in solid organ transplantation, with the potential to reduce chronic allograft nephropathy [3, 4]. Indeed, because of these attributes, rapamycin also holds promise in the treatment of nephrotic glomerular diseases [5], such as membranous nephropathy and focal segmental glomerulosclerosis (FSGS), where calcineurin inhibitor–free regimens are also desperately needed [6, 7]. However, a recent preliminary study reported that six out of 11 patients with nephrotic glomerular diseases treated with rapamycin developed functional nephrotoxicity for unclear reasons [8].

Rapamycin binds to a specific cytosolic protein, FK binding protein-12, and this complex inhibits the activation of the mammalian target of rapamycin (mTOR), a kinase required for cell cycle progression [1, 2]. However, the antiproliferative actions are not restricted to T cells, and rapamycin also has these effects in other cell types, including tubular epithelial cells [9]. In nephrotic glomerular diseases, tubular epithelial cell proliferation is increased [10, 11], and this might be a compensatory mechanism to process the excess plasma proteins present in the lumen.

The aim of this study was to investigate the functional and pathologic effects of rapamycin in a model of glomerular disease characterized by heavy proteinuria and tubular epithelial cell proliferation. Protein overload nephropathy is induced in rats by the repeated injections of bovine serum albumin (BSA) and (by mechanisms that are not entirely clear) this causes nonimmune-initiated glomerular injury with an almost immediate onset of nephrotic range proteinuria [12]. During the first week, protein overload nephropathy is characterized by marked tubular epithelial cell proliferation and mild interstitial inflammation [12], making it an excellent in vivo model to investigate the antiproliferative actions of rapamycin on tubular epithelial cells in proteinuric conditions.
METHODS

Animals

Studies were performed in female Wistar rats (8 weeks old, derived from the Wistar rat colony at Westmead Hospital). Animals were fed standard rat pellets and allowed tap water ad libitum. All protocols were approved by the Animal Ethics Committee, Westmead Hospital, University of Sydney, and animals were handled according to the guidelines of the National Health and Medical Research Council of Australia.

Experimental design

In the first study, animals were divided into four groups according to body weight: (1) control group [saline + dimethyl sulfoxide (DMSO)] ($N=4$); (2) protein overload nephropathy + DMSO ($N=9$); (3) protein over- load nephropathy + rapamycin (0.2 mg/kg) (Sigma-Aldrich, Sydney, Australia) ($N=9$); and (4) protein overload nephropathy + roscovitine (3.5 mg/kg) (Sigma-Aldrich) ($N=9$). Protein overload nephropathy was induced by daily intraperitoneal injections of BSA (2.1 g) (A4503) (Sigma-Aldrich) under halothane anesthesia on days 0, 1, 2, and 3, as previously described [12]. Endotoxin was assessed in the injected BSA solution and found to be significantly below levels that increase glomerular permeability or cause tubular injury (0.03 μg/mL) (Kinetic Chromogenic Test) (assessed independently by AMS Laboratories Pty. Ltd., Sydney, Australia) [13]. The experimental drugs were administered daily by intraperitoneal injection 3 days prior to and then on each day of the BSA injections (that is, from day −3 through to day +3). Roscovitine is a small molecule cyclin-dependent kinase inhibitor [15] and is a potent suppressor of mesangial cell proliferation in the Thy1 model of glomerulonephritis [14]. It was included in the study to investigate whether it could inhibit tubular epithelial cell proliferation, and if so, whether this caused the same pathologic effects as rapamycin. For the control group, BSA and the experimental drugs were substituted with normal sterile saline and DMSO (the vehicle), respectively. The dose, route, and frequency of rapamycin and roscovitine administration were determined according to previous studies in rats [9, 14]. Doses of roscovitine higher than 3.5 mg/kg were not used as they cause toxic side effects in rats [14].

On day 2, nonfasting animals were placed in metabolic cages for 14 hours to determine urinary protein excretion. On day 3, approximately 3 hours after the final BSA injection, animals received a single intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU) (50 mg/kg). Three hours later, animals were anesthetized with ketamine:xylazine. A midline laparotomy was performed, blood was collected by cardiac puncture, the inferior vena and aorta were transected below the renal arteries, and nephrectomys were performed. Day 3 was chosen as the end of the study as preliminary experiments in our laboratory showed that tubular epithelial cell proliferation peaks between days 3 and 4 in this model.

To determine the effect of rapamycin on kidney structure and function in normal rats, in a second study, groups received either vehicle (DMSO) or rapamycin (0.2 mg/kg) (Sigma-Aldrich) ($N=9$) for the same duration as in experiment 1 (that is, 6 days). Blood, urine, and kidney tissue were collected as described in the first experiment. In addition, trough levels of rapamycin were assessed in whole blood, collected 6 hours after the last injection of rapamycin, by a high-performance liquid chromatography (HPLC) assay (Department of Clinical Pharmacology, Westmead Hospital).

Renal function

The serum creatinine, albumin, cholesterol, urinary protein, and urinary creatinine were assessed by an auto-analysier, as previously described [16].

Histology and immunohistochemistry

For histologic analysis midcoronal sections of kidney were fixed in either neutral buffered formalin or methyl carnoy and embedded in paraffin. Sections 3 μm in thickness were stained by the periodic acid-Schiff (PAS) method. For immunohistochemistry, sections were processed according to standard protocols and incubated with primary antibody (16 hours at 4°C) as previously described [16]. The primary antibodies used were anti-BrdU (Amersham Biosciences, Sydney, Australia), anti-rat monocye/macrophage (ED1) (Serotec, UK), anti-cyclin D1 (Neomarkers, Lab Vision, Fremont, CA, USA), anti-cyclin D3 (Neomarkers, Lab Vision), and anti-p27kip1 (Neomarkers, Lab Vision). Immunostaining for cyclin D1 and cyclin D3 required antigen unmasking in formalin-fixed sections with microwave heating for 5 minutes in citrate buffer (Antigen Decloaker) (Biocorta, San Diego, CA, USA), prior to application of the primary antibody. For p27kip1, antigen unmasking in formalin-fixed sections consisted of microwave heating for eight rounds in distilled water, before application of the primary antibody. A negative control (primary antibody omitted) was included in all experiments.

Assessment of tubular epithelial cell apoptosis

Tubular epithelial cell apoptosis was evaluated by the in situ cell death detection terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end-labeling (TUNEL) method using a commercial kit (Roche Diagnostics, Sydney, Australia) on formalin-fixed slides according to the manufacturer’s instruction. Permeabilization was achieved with proteinase K (20 μg/mL) treatment. TUNEL-positive cells
were visualized with diaminobenzidine (DAB). Positive and negative controls were prepared as recommended by the manufacturer. TUNEL-positive cells were defined, according to strict criteria, as DAB-positive cells with morphologic features of apoptosis.

**Quantification of immunohistology**

Random selection methods were used to determine the microscopic fields for evaluation. To assess cortical tubulointerstitial injury the average of a semiquantitative score in five cortical fields (×200) where 0 was no tubulointerstitial injury (tubular dilatation, tubular injury, and interstitial volume expansion); 1 was 1% to 25% of the field contained tubulointerstitial injury; 2 was 26% to 50% of the field contained tubulointerstitial injury; 3 was 51% to 75% of the field contained tubulointerstitial injury; and 4 was 76% to 100% of the field contained tubulointerstitial injury. To independently assess intratubular protein cast formation a semiquantitative score was measured in five cortical fields (×200) where 0 was no casts present in cortical tubules; 1 was 25% of cortical tubules contained casts; 2 was 50% of cortical tubules contained casts; 3 was 75% of cortical tubules contained casts; and 4 was 100% of cortical tubules contained casts. For BrdU, TUNEL, cyclin D1, and cyclin D3, the number of DAB-positive tubular epithelial cells/nuclei were counted in ten overlapping cortical fields (×200). For ED1, the number of DAB-positive interstitial cells were counted in ten overlapping cortical fields (×200). To assess the cortical expression of p27kip1, quantitative image analysis was performed (Optimas 6.51) (Media Cybernetics, Silver Spring, MD, USA). The percentage area positive for DAB was calculated from the mean of ten cortical fields (×400) for each animal.

**Statistics**

Data were analyzed using the JMP software package (SAS Institute, Cary, NC, USA). Multiple parametric and nonparametric comparisons were performed using one-way analysis of variance (ANOVA) with two-sample t test and the Kruskal-Wallis test. A P value less than 0.05 indicated significance. Unless otherwise stated, data were expressed as mean ± SEM.

**RESULTS**

**Rapamycin causes acute renal failure in protein overload nephropathy**

The repeated administration of rapamycin or roscovitine did not affect body weight in rats with protein overload nephropathy (DMSO 225 ± 7; rapamycin 230 ± 7; and roscovitine 235 ± 4 g on day 3). However, one rat in the rapamycin-treated group was euthanized on day 2 due to signs of physical distress, and another was anuric at the time of urine collection. Biochemical and proteinuria data are shown in Table 1. Rapamycin caused severe acute renal failure (as determined by the serum creatinine and creatinine clearance) and mild hypercholesterolemia, but did not significantly alter proteinuria (as

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Table 1. Biochemical data and kidney weight in the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Serum creatinine μmol/L</th>
<th>Serum albumin g/L</th>
<th>Serum cholesterol mmol/L</th>
<th>Proteinuria mg/day</th>
<th>Urine protein:creatinine ratio mg/mmol</th>
<th>Kidney weight g</th>
<th>Kidney weight/body weight ratio g/100 g</th>
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<tbody>
<tr>
<td>Saline + DMSO</td>
<td>4</td>
<td>26 ± 1</td>
<td>35 ± 1</td>
<td>2.3 ± 0.1</td>
<td>11 ± 0.1</td>
<td>0.21 ± 0.06</td>
<td>0.84 ± 0.07</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Protein overload nephropathy + DMSO</td>
<td>9</td>
<td>117 ± 27a</td>
<td>76 ± 4a</td>
<td>1.5 ± 0.1a</td>
<td>1074 ± 188b</td>
<td>20.8 ± 2.1a</td>
<td>1.50 ± 0.34a</td>
<td>0.68 ± 0.07a</td>
</tr>
<tr>
<td>Protein overload nephropathy + rapamycin</td>
<td>9</td>
<td>353 ± 54b</td>
<td>89 ± 5a</td>
<td>2.7 ± 0.4b</td>
<td>836 ± 194a</td>
<td>15.1 ± 2.5a</td>
<td>1.42 ± 0.14a</td>
<td>0.62 ± 0.05a</td>
</tr>
<tr>
<td>Protein overload nephropathy + roscovitine</td>
<td>9</td>
<td>73 ± 11a</td>
<td>70 ± 1a</td>
<td>1.7 ± 0.1a</td>
<td>1449 ± 187a</td>
<td>20.8 ± 2.7a</td>
<td>0.74 ± 0.07b, 0.53 ± 0.02a</td>
<td></td>
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</tbody>
</table>

DMSO = dimethyl sulfoxide. Data are expressed as mean ± SEM.

*P < 0.05 versus control + vehicle; †P < 0.05 versus protein overload nephropathy + vehicle; ‡In the protein overload nephropathy + rapamycin group, the data for proteinuria and urine protein:creatinine ratio are for seven animals only as one animal was euthanized on day 2 before urine could be obtained and another was anuric on the day of urine collection.
determined either by the timed collection or the urinary protein:creatinine ratio (Fig. 1) (Table 1). In contrast, roscovitine attenuated renal dysfunction as assessed by creatinine clearance ($P < 0.05$) and there was a trend for a decrease in the serum creatinine.

Rapamycin increases intratubular cast formation in protein overload nephropathy

In PAS-stained sections, protein overload nephropathy was characterised by tubular dilatation, interstitial edema and inflammation with cast formation, compared to the control group (saline + DMSO) (Fig. 2). Rapamycin treatment worsened renal cortical tubulointerstitial injury (Fig. 2) (mean tubulointerstitial injury score: protein overload nephropathy + DMSO 2.6 ± 0.2; protein overload nephropathy + rapamycin 3.4 ± 0.3) ($P < 0.05$). This was characterized by a dramatic increase in the number of intratubular proteinaceous casts, consisting of homogenous eosinophilic material (protein overload nephropathy + DMSO 1.7 ± 0.3; protein overload nephropathy + rapamycin 2.9 ± 1.3) ($P = 0.02$). In five of the nine protein overload nephropathy animals treated with rapamycin, the cast formation was severe and diffuse throughout the cortex and medulla. In some areas, Bowman’s space of glomeruli was also filled with proteinaceous material (Fig. 2). In contrast to rapamycin, there was a trend for an improvement in the roscovitine-treated group ($2.1 ± 0.2$) ($P = 0.07$).

Rapamycin reduces cortical tubular epithelial cell proliferation but does not affect apoptosis in protein overload nephropathy

Kidneys from rats with protein overload nephropathy had a granular surface and increased in weight by almost twofold (Table 1). The proliferation of cortical tubular epithelial cells (assessed by the number of BrdU-positive nuclei) was increased by more than 20-fold in the vehicle-treated protein overload nephropathy group, and reduced by almost 65% with rapamycin treatment (Fig. 3). There was a nonsignificant trend for roscovitine to reduce tubular epithelial cell proliferation ($P = 0.22$). The number of TUNEL-positive tubular epithelial cells was increased in protein overload nephropathy (saline + DMSO 2.1 ± 0.4 cells per field and protein overload nephropathy + DMSO 3.7 ± 0.5 cells per field) ($P < 0.05$), as shown previously [12], but this not altered by rapamycin (4.0 ± 0.4) or roscovitine (3.2 ± 0.8).

Rapamycin does not alter D-type cyclin expression in tubular epithelial cells

To investigate the molecular mechanisms of rapamycin-mediated suppression of tubular epithelial cell proliferation in protein overload nephropathy, the expression of D-type cyclins (which promotes the G1/S phase transition of the cell cycle) was examined.

Rapamycin increases cytoplasmic protein absorption droplets in tubular epithelial cells

Proximal tubular epithelial cells from rats treated with rapamycin were swollen and engorged with increased intracytoplasmic protein droplets (Fig. 2). Very occasionally, tubular epithelial cells were detached from the basement membrane and present in the lumen in the rapamycin-treated animals, but no areas of necrosis were seen.
Fig. 3. Photomicrographs and quantitation of the renal cortex. (A) Photomicrographs show 5-bromo-2′-deoxyuridine (BrdU)-positive tubular epithelial cells (TECs) (×200). Abbreviations are: PON, protein overload nephropathy; DMSO, dimethyl sulfoxide. (B) Quantitation of the number of BrdU-positive cortical tubular epithelial cells (mean ± SD). *P < 0.05 compared to protein overload nephropathy + DMSO [diaminobenzidine ((DAB) with methyl green counterstain, magnification ×200].

Fig. 4. Photomicrographs and quantitation of the renal cortex. (A) Photomicrographs show cyclin D1 (upper panels) and cyclin D3 (lower panels) immunostaining in the experimental groups [diaminobenzidine (DAB) with methyl green counterstain, magnification ×200]. Both cyclin D1 and D3 were detected in the nuclei of multiple tubular epithelial cells (arrows). Abbreviations are: PON, protein overload nephropathy; DMSO, dimethyl sulfoxide. (B) Quantitation of the number of cyclin D1- or cyclin D3-positive tubular epithelial cells (mean ± SE). Abbreviations are: Rapa, rapamycin; Rosco, roscovitine. *P < 0.05 compared to the control group (saline + DMSO).

By immunohistochemistry, cyclin D1 and cyclin D3 protein were constitutively expressed in the nuclei of tubular epithelial cells in control animals (Fig. 4A). In protein overload nephropathy, the number of cyclin D1-positive, but not cyclin D3-positive tubular epithelial cells was reduced in protein overload nephropathy on day 3 compared to the control group (P = 0.03). The expression of cyclin D-type cyclins in tubular epithelial
cells in protein overload nephropathy, however, were not altered by rapamycin or roscovitine treatment (Fig. 4).

**Rapamycin increases p27kip1 in tubular epithelial cells in protein overload nephropathy**

In some cell types, rapamycin up-regulates the G1/S transition cyclin-dependent kinase inhibitor, p27kip1. To test this hypothesis in protein overload nephropathy, immunostaining for p27kip1 was performed. In control animals, nuclear staining for p27kip1 was constitutively present in the nuclei of cortical tubular epithelial cells (Fig. 5). There was a trend for tubular epithelial cell p27kip1 to be reduced in protein overload nephropathy animals treated with the vehicle. Rapamycin treatment, however, increased p27kip1 protein expression in tubular epithelial cells ($P < 0.05$). Under these circumstances, p27kip1 was present not only in the nucleus but also in the cytosol of tubular epithelial cells (Fig. 5A). Roscovitine did not significantly alter p27kip1 expression in tubular epithelial cells.

**Rapamycin and roscovitine reduce interstitial monocyte accumulation in protein overload nephropathy**

Interstitial monocyte accumulation was increased almost twofold in protein overload nephropathy, as assessed by ED1 immunohistochemistry (saline + DMSO $25.6 \pm 7.5$ cells/mm$^2$ and protein overload nephropathy + DMSO $49.5 \pm 5.0$ cells/mm$^2$) ($P < 0.05$). Both rapamycin and roscovitine reduced interstitial monocyte accumulation in protein overload nephropathy (protein overload nephropathy + rapamycin $16.2 \pm 5.0$ cells/mm$^2$ and protein overload nephropathy + roscovitine $30.8 \pm 5.0$ cells/mm$^2$) ($P < 0.05$ compared to protein overload nephropathy + DMSO).

**Rapamycin does not cause acute renal failure or cast nephropathy in normal rats**

In normal rats, treatment with rapamycin increased the serum cholesterol (normal + DMSO $1.5 \pm 0.1$ mmol/L and normal + rapamycin $2.1 \pm 0.1$ mmol/L) ($P < 0.01$) but did not alter serum creatinine (normal + DMSO $33 \pm$
5 µmol/L and normal + rapamycin 30 ± 3 µmol/L), proteinuria (normal + DMSO 6 ± 1 mg/day and normal + rapamycin 7 ± 6 mg/day), or induce intratubular protein cast formation in normal rats (Fig. 6). The mean trough levels in whole blood achieved with this dose of rapamycin was 10.3 ± 1.2 ng/mL.

DISCUSSION

The main findings of the present study were (1) rapamycin caused severe acute renal failure in protein overload nephropathy; (2) rapamycin increased intratubular protein cast formation and appeared to disrupt the intracytoplasmic processing of proteins in tubular epithelial cells in protein overload nephropathy; (3) rapamycin inhibited proteinuria-induced tubular epithelial cell proliferation in vivo, through a mechanism that may involve the upregulation of p27kip1; and (4) the adverse pathologic renal effects of rapamycin were specific to protein overload nephropathy, as they did not occur in normal rats given identical and therapeutically relevant doses of rapamycin.

In previous preclinical studies, the effects of rapamycin on renal function and structure have been extensively examined in normal rats [1]. However, these studies did not reveal whether rapamycin had any unique effects on kidney pathophysiology in proteinuric conditions. Fervenza et al [8] recently reported that rapamycin caused functional nephrotoxicity in humans with glomerular disease. In addition, another study also recently reported that rapamycin use was associated with the continuing deterioration of renal function, in kidney transplant patients with chronic allograft nephropathy whose baseline proteinuria was greater than 1.5 g per day [17]. In contrast, in patients with proteinuria lower than this, rapamycin improved graft function at 12 months [17]. However, the question as to whether rapamycin had any specific pathologic effects in the kidney in proteinuric conditions had not been addressed. The results of the current study provide the first experimental evidence to suggest that rapamycin nephrotoxicity in proteinuric renal diseases could, in some circumstances, be due to a unique pattern of pathologic injury involving the induction of protein cast nephropathy.

It is well known that rapamycin exacerbates renal function in experimental models of acute renal ischemia, through mechanisms that involve impaired tubular epithelial cell regeneration and increased apoptosis [9]. However, the pathogenesis of functional nephrotoxicity in protein overload nephropathy appears to be different. Consistent with previous observations in rats with acute renal ischemia [9], rapamycin reduced tubular epithelial cell proliferation but, in contrast, did not increase tubular epithelial cell apoptosis in protein overload nephropathy [9]. Rather, increased intratubular protein cast formation was the most dramatic histologic feature of rapamycin treatment in protein overload nephropathy. Cast formation was quite diffuse and widespread in more than 50% of the animals. In some areas, Bowman's space was filled with proteinaceous material. The latter supports the possibility that the acute renal failure was the consequence of intratubular obstruction secondary to cast nephropathy. The nephrotoxicity of rapamycin in protein overload nephropathy seems to be pathologically analogous to other types of acute renal failure that are mediated by protein-induced intratubular obstruction, such as rhabdomyolysis, acute tumor cell lysis, and paraprotein-associated kidney disease in humans [18, 19].

The mechanisms by which rapamycin promotes protein cast formation in protein overload nephropathy require further investigation. Interestingly, increased intratubular cast formation was also present in kidney biopsies from renal transplant patients with delayed graft function who received rapamycin [20]. In the latter study, it was hypothesised that denuded tubular epithelial cells might act as a nidus for cast formation in the lumen. However, in our study in protein overload nephropathy, detached tubular epithelial cells in association with luminal casts (which were very eosinophilic and homogenous) were rarely present. Instead, another striking morphologic feature in the rapamycin-treated protein overload nephropathy group was that some proximal tubular epithelial cells were swollen and engorged with increased cytoplasmic protein droplets. These morphologic observations suggest that rapamycin (through effects on mTOR) disrupted the intracellular processing of endocytosed proteins in tubular epithelial cells, as suspected from in vitro studies by Dixon and Brunskill [21]. Alternatively, reduced tubular epithelial cell proliferation may also have decreased the overall capacity of the nephron to process the increased concentration of albumin in tubular lumen. Whatever the exact pathogenesis, we postulate that the end result was an increase in the amount of luminal protein reaching the distal tubule, thereby promoting protein aggregation and cast formation. Indeed, rapamycin has been reported to increase proteinuria in some transplant recipients [17, 22], and our data raise the hypothesis that this might, at least in part, be mediated by a tubular mechanism.

To investigate the molecular mechanisms of the antiproliferative effect of rapamycin in tubular epithelial cells, the expression of D-type cyclins and p27kip1 in protein overload nephropathy was determined. D-type cyclins promote the G1/S phase transition of the cell cycle. In some cell types D-type cyclin expression can be suppressed by rapamycin [23–25], but this was not the mechanism in protein overload nephropathy. Instead, our data clearly show that rapamycin increased the protein expression of p27kip1 in cortical tubular epithelial cells. This observation is consistent with previously known effects of
Rapamycin [26]. Interestingly, rapamycin altered the subcellular localization of p27\(^{kip1}\) and caused a significant increase in expression in the cytosol of tubular epithelial cells.

In contrast to rapamycin, roscovitine failed to reduce tubular epithelial cell proliferation in protein overload nephropathy, despite being used at doses appropriate to suppress mesangial cell proliferation in rats [14]. Yet, roscovitine conferred partial functional and histologic protection in protein overload nephropathy, most likely due to the anti-inflammatory effects of this compound. On the other hand, rapamycin also reduced monocytic accumulation. Therefore, the divergent effects of rapamycin and roscovitine in protein overload nephropathy, again emphasize that tubular epithelial cell proliferation is an important compensatory response during the early phase of this model.

In contrast to the results of the current study, rapamycin was renoprotective in the passive Heymann nephritis rat model of membranous nephropathy [27], and in the Han:Sprrd model of polycystic kidney disease [28]. Moreover, unpublished data from our laboratory also show that chronic rapamycin treatment for up to 6 weeks reduces renal hypertrophy and does not induce cast nephropathy in adriamycin nephropathy (a toxin-induced model of FSGS). These data suggest that rapamycin-mediated nephrotoxicity in glomerular disease is complex and could be determined by the rapidity and severity of the proteinuria (which is very high in protein overload nephropathy compared to Heymann nephritis and adriamycin nephropathy), the composition of the proteinuria as well as other disease-specific factors. These intriguing observations warrant further investigation in future studies, as it may help define the risk factors for developing rapamycin-induced nephrotoxicity in human glomerular disease [29].

CONCLUSION

Rapamycin causes acute renal failure in protein overload nephropathy due to a specific pattern of renal injury characterized by intratubular obstruction secondary to increased protein cast formation. The latter could be due to disruption of a potentially important compensatory mechanism in nephrotic glomerular disease involving tubular epithelial cell protein endocytosis and proliferation. Moreover, in resembling recent clinical reports [8, 17, 20, 22, 29], these data raise further caution in the use of rapamycin in glomerular diseases characterized by nephrotic range proteinuria, until further information is available [29].

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