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Acute rapamycin nephrotoxicity in native kidneys of patients with chronic glomerulopathies

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Abstract

Background. Based on its success as a transplant immunosuppressor, there is intense interest in using rapamycin in the treatment of progressive glomerulopathies involving native kidneys. However, we call attention to the potential toxicity associated with the use of rapamycin in this setting.

Methods. We conducted a study to examine the efficacy and safety of rapamycin in patients with progressive chronic renal failure. Eleven patients with either focal segmental glomerulosclerosis, immunoglobulin A nephropathy, membranous nephropathy or membrano-proliferative glomerulonephritis and progressive renal failure (defined as an increase in $>25\%$ of baseline serum creatinine over the last year or loss of glomerular filtration rate ≥ 5 ml/min/year as determined by the Cockcroft–Gault formula), proteinuria ≥ 1.0 g/24 h and with a creatinine clearance of ≥ 20 ml/min/1.73 m^2 were entered into a 12 month study. Patients were treated with rapamycin, starting at 5 mg/day, orally, aiming for target blood levels of 7–10 ng/dl. All patients were on treatment with an angiotensin-converting enzyme inhibitor and/or an angiotensin receptor blocker, aiming to control blood pressure $\leq 145/90$ mmHg.

Results. Six patients developed acute renal failure, defined as an increase in serum creatinine ≥ 0.5 mg/dl (baseline: 3.2 ± 0.9 mg/dl; peak: 5.6 ± 1.6 mg/dl; $P < 0.01$, paired t-test). In four patients, discontinuation of the drug resulted in improvement of renal function close to baseline levels. One patient required haemodialysis and had no subsequent recovery of renal function. In another patient, renal function recovered after discontinuation of the drug and then rapamycin was resumed at a lower dose when creatinine returned to baseline. This resulted in a second acute increase in serum creatinine that failed to return to baseline when the medication was discontinued. Four other patients had the following adverse events: skin rash, severe hypertriglyceridaemia, diarrhoea and hyperkalaemia. In none of the subjects were rapamycin levels >15 ng/dl.

Conclusions. Rapamycin can cause nephrotoxicity in some patients with chronic glomerulopathies. Whether the toxicity is solely related to rapamycin, due to the combination of proteinuria and rapamycin, or other unknown factor use is presently undetermined.

Keywords: acute renal failure; glomerulonephritis; glomerulopathies; nephrotoxicity; rapamycin

Introduction

Despite evidence that mechanical factors such as glomerular hypertension and hypertrophy are likely contributors, at least initially, in some forms of progressive renal disease, the exact molecular mechanisms underlying the progression of chronic renal disease are largely unknown. However, numerous observations suggest those cellular events, including cellular proliferation macrophage activation, cytokine release and the generation of local inflammatory mediators, play an important role in this process.

Rapamycin is a new immunosuppressive medication that prolongs allograft survival [1]. Such therapeutic effects of rapamycin are derived from the inhibition of proliferation of T lymphocytes. Rapamycin also inhibits proliferation of fibroblasts, endothelial, mesangial and smooth muscle cells. Since proliferation of these cells also contributes to the disease progression in chronic glomerulopathies, we considered whether the use of rapamycin could retard progression and improve renal survival in these patients.

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Subjects and methods

Eleven patients aged >18 years old with a biopsy-proven diagnosis of focal segmental glomerulosclerosis (FSGS), immunoglobulin A nephropathy (IgAN), membranous nephropathy (MN) or membrano-proliferative glomerulonephritis (MPGN) and progressive renal failure were entered into a 12 month study. Progressive renal failure was defined as an increase in >25% of baseline serum creatinine over the last year or loss of glomerular filtration rate (GFR) $\geq$5 ml/min/year as determined by the Cockcroft–Gault formula. All patients had proteinuria $\geq$1.0 g/24 h and a creatinine clearance of $\geq$20 ml/min/1.73 m^2 prior to the start of rapamycin. All patients had to be on an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker, aiming to maintain blood pressure $\leq$145/90 mmHg. Patients with diabetes mellitus, positive hepatitis B surface antigen or hepatitis C antibody and patients who had used corticosteroids, cytotoxic drugs, mycophenolate mofetil (MMF) or cyclosporin A therapy in the last 3 months prior to enrolment were excluded. In addition, patients with history of a disease which could be associated with a secondary glomerulopathy, e.g. malignancy, were also excluded. Patients were started on 5 mg rapamycin, orally, every day, aiming to achieve target drug levels between 7 and 10 ng/dl. Serum creatinine, full blood count and rapamycin levels were performed bi-weekly during the first month, once for the second and third months, and every 3 months after that. Dose adjustments were done according to drug-level determinations. The study was reviewed and approved by the Institutional Research Board and all patients signed an informed consent.

Results

Between February and November 2002, 11 patients, two with FSGS, five with IgAN, three with MN and one with MPGN, entered the study. At the start of treatment, sitting and standing blood pressure readings were 143/81 $\pm$ 20/13 and 141/79 $\pm$ 31/14 mmHg, respectively. Six patients developed acute renal failure, defined as an increase in serum creatinine $\geq$0.5 mg/dl over baseline, which occurred within the first 6 weeks of starting rapamycin (baseline: 3.2 $\pm$ 0.9 mg/dl *vs* peak creatinine: 5.6 $\pm$ 1.6 mg/dl; $P < 0.01$, paired *t*-test) (Table 1). In none of the subjects were rapamycin levels >15 ng/dl (mean: 10 $\pm$ 2.8 ng/dl). In five patients, discontinuation of the drug resulted in improvement of renal function, although in only three did creatinine return to baseline. One patient required haemodialysis and had no subsequent recovery of renal function. In another patient, renal function recovered after discontinuation of the drug and then rapamycin was resumed at a lower dose (3 mg/day) when creatinine returned to baseline. This resulted in a second acute increase in serum creatinine that failed to return to baseline when the medication was discontinued (Figure 1).

Four patients had early adverse events: hypertriglyceridaemia (1245 mg/dl) that failed to be controlled with the use of lipid lowering medication, acute diarrhoea secondary to *Clostridium difficile* in the stools, generalized skin rash and hyperkalaemia. All these side effects occurred within the first 2 weeks of starting rapamycin and the patients discontinued the study. Only one patient was able to complete the 12 month study period.

Table 1. Serum creatinine values (mg/dl) in patients who developed acute renal failure

Patient	At baseline	At peak	After discontinuing rapamycin
1	3.9	7.5	3.6
2	2.2	5.9	2.1
3	3.7	7.3	6.8[a]
4	2.1	3.4	2.4
5	4.3	4.9	4.4
6	2.8	4.5	3.5
Mean $\pm$ SD	3.2 $\pm$ 0.9	5.6 $\pm$ 1.6	3.8 $\pm$ 1.7[b]

[a]Haemodialysis started. [b]P = not significant (baseline *vs* discontinuing drug), paired *t*-test.

Discussion

In a select group of patients with native kidneys and chronic glomerulopathies, the use of rapamycin was complicated by an unusual number of side effects. Of particular relevance were the six cases of acute renal failure associated with the use of rapamycin. None of the patients underwent renal biopsy at the time of their acute renal failure, which is a drawback of the study. However, the timing of the onset of acute renal failure within 6 weeks after starting rapamycin and the recovery of renal function after stopping the medication is consistent with the hypothesis that the use of rapamycin had an acute detrimental effect on renal function. Rapamycin has a long elimination half-life ($\sim$62 h) and new steady-state concentrations are not reached until at least 4 days after starting therapy. As a result, a minimum of 1–2 weeks may be needed for the full effects of rapamycin to wear off, once discontinued [2]. In five patients, renal function recovered once rapamycin was discontinued. In one patient, reintroduction of rapamycin, although at a lower dose, resulted in further increase in serum creatinine. These observations underscore the significant role of rapamycin in these cases.

The mechanism of nephrotoxicity in these patients is not clear. In normal animals, several studies have demonstrated the almost complete absence of haemodynamic-induced renal damage, with minimal morphological signs of toxicity, even when rapamycin was administered at huge doses (1.5–10 mg/kg/day; 20–50 times those necessary for immunosuppression in rats) [3–6]. Similarly, rapamycin (1–13 mg/m^2/day) was given for 14 days to 30 stable renal transplant patients taking cyclosporin A at normal therapeutic doses

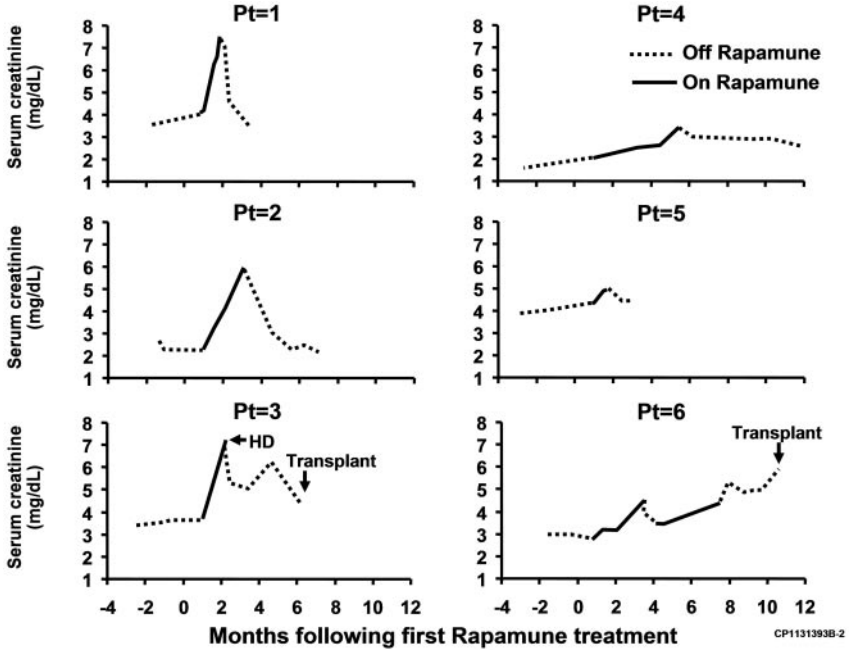

Fig. 1. Serial creatinine measurements in the six patients who developed acute renal failure following treatment with rapamycin. Start and stop dates.

without significant effects on GFR or creatinine clearance [7].

However, salt-depleted rats treated for 14 days with rapamycin at high doses (3 mg/kg/day) developed significant renal dysfunction associated with tubular collapse, vacuolization and nephrocalcinosis. These morphological changes were accompanied by tubular functional changes, namely decreased medullary concentration ability, hypomagnesaemia and increased tubular enzymuria, but without changes in glomerular haemodynamics [8]. *In vitro* studies using cultured mouse proximal tubular cells showed that rapamycin increased apoptosis and decreased the proliferative cell response [9]. The same was observed in the renal tissue of rats subjected to renal artery occlusion. In both cases, the use of rapamycin was associated with a decreased expression of the intracellular signalling protein p70S6 kinase, an enzyme that regulates cell proliferation and is critical to cell-cycle progression [9]. These experiments suggest that rapamycin hinders cell growth and repair after renal injury.

Along this line, a recent article by McTaggart *et al.* [10] provides evidence that rapamycin prolongs the duration of delayed graft function (DGF). These findings are in agreement with reports of increased risk of developing DGF in patients receiving rapamycin on the day of transplant compared with those not receiving the drug [11]. This clinical outcome was predicted based on the *in vitro* studies described above and also by previous animal studies. In a rat model of renal ischaemia, the use of rapamycin impaired recovery compared with the vehicle of MMF [12]. Rapamycin-treated animals 7 days after acute ischaemic injury showed decreased GFR and renal blood flow, together with a more pronounced degree of interstitial fibrosis, than animals treated with vehicle or MMF. Similarly, Lieberthal *et al.* [9] also reported a marked prolongation in the recovery of GFR following acutely induced ischaemic renal failure in rats receiving rapamycin *vs* vehicle-treated animals.

Severe acute renal failure after exposure to a combination of rapamycin and tacrolimus has been reported in two living donor kidney recipients [13]. In both patients, renal allografts functioned immediately post-operatively, but developed oliguric acute renal failure requiring haemodialysis after 2–3 weeks of exposure to rapamycin–tacrolimus. In one patient, renal biopsy showed no acute rejection, acute tubular necrosis (ATN) or thrombotic microangiopathy. Both medications were held and the patient was maintained on steroid therapy and started on MMF. Renal function began improving after 11 days and dialysis was discontinued. He was restarted on low-dose tacrolimus while renal function continued to improve. Two weeks later his serum creatinine was 1.2 mg/dl. The other patient's renal biopsy showed changes consistent with mild ATN, but no acute rejection or microangiopathy. He was managed similarly to the first patient, with discontinuation of rapamycin–tacrolimus, followed by reintroduction of tacrolimus at a lower dose once renal function had recovered, with continuous improvement in serum creatinine. The authors stress the fact that renal function recovery maintained after restarting tacrolimus in the absence of rapamycin underscores the significant role of rapamycin in the two cases [13].

Our observations suggest that rapamycin, at standard treatment doses, is poorly tolerated in patients with chronic glomerulopathies who have moderate

renal dysfunction and proteinuria >1 g/24 h. This is in clear contrast to reports in the renal transplant literature where rapamycin, when administered as primary therapy in combination with azathioprine or MMF, has shown a favourable safety profile compared with cyclosporin A with regards to renal function [14]. In a recent pooled data analysis of renal function parameters from two randomized, multicentre studies conducted by the European Transplant Study Group, mean serum creatinine levels were consistently lower and calculated GFR was significantly higher in the rapamycin group compared with the cyclosporin A-treated group. These differences were observed as early as 8 weeks after transplantation and persisted after 2 years of treatment [14]. However, patients in global/US studies on rapamycin tended to have higher serum creatinine levels than controls, an observation that remains to be adequately explained [15].

How can we reconcile the current literature safety profile on rapamycin with the significant number of side effects observed in our study? First, with the exception of the ischaemia/reperfusion injury, transplanted kidneys are usually morphologically 'intact', while patients in our study had a significant degree of prior renal damage that could predispose to rapamycin toxicity. Second, and also different from patients with stable renal allografts, is the presence of significant proteinuria in our group. It is possible that proteinuria affects the concentration of rapamycin reaching the renal tubules. Alternatively, rapamycin could potentiate the protein toxicity to these nephron segments. Indeed, there is mounting evidence that the secondary process of reabsorption of filtered proteins contributes to renal interstitial injury [16,17]. There is also experimental evidence that proteinuria increases tubular cell turnover as manifested by both increased apoptosis and increased cellular proliferation [18]. It is possible that, by its inhibitory effect on cellular proliferation coupled with an increased rate of apoptosis, rapamycin might actually tip the delicate survival balance, worsening the tubulointerstitial injury.

Regarding this last point, patients with chronic allograft nephropathy who have a 24 h urinary protein excretion >1 g should be closely monitored for an until now unrecognized rapamycin nephrotoxicity. A recent report by Budde *et al*. [19] seems to support this argument. These authors showed that in renal transplant patients with chronic calcineurin-inhibitor nephrotoxicity and proteinuria <1 g/24 h, conversion to rapamycin resulted in improvement of renal allograft function (baseline creatinine: 2.76 ± 0.14 mg/dl *vs* 2.22 ± 0.14 mg/dl after conversion). However, in patients with proteinuria >1.5 g/24 h, conversion to rapamycin resulted in further deterioration of renal function (baseline creatinine: 3.23 ± 0.21 *vs* 4.43 ± 0.34 mg/dl after conversion).

Of note, studies *in vitro* demonstrate that the antiproliferative effect of Rapamycin may be at least in part, mediated by inducing haeme oxygenase-1 (HO-1) [20]. Whether such induction of HO-1 is cytoprotective or injurious is unknown. It is possible that the induction of heme oxygenase-1 by Rapamycin augments renal concentration of iron, the Fenton catalyst for oxidative stress. In the presence of lipiduria, (which accompanies proteinuria), iron-catalyzed lipid oxidation may heighten oxidative stress, resulting, ultimately, in acute tubular injury.

Finally, our conclusion that rapamycin causes nephrotoxicity in patients with chronic glomerulopathies needs to be confirmed by other studies and further studies are needed to determine the exact mechanism of rapamycin toxicity in these patients.

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Conflict of interest statement. None declared.

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