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Nephrotoxicity of rapamycin: an emerging problem in clinical medicine

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Introduction

Rapamycin represents substantial progress as a maintenance immunosuppressive agent to prevent rejection episodes and to decrease steroid and calcineurin inhibitor (CNI) exposure [1–3]. This drug is commonly administered in combination with mycophenolate mofetil, an inhibitor of inosine monophosphate dehydrogenase, or with azathioprine, a purine antimetabolite. Furthermore, rapamycin is prescribed as a substitute for or in combination with CNI.

The macrocyclic lactone rapamycin or sirolimus (Rapamune®) and its more polar derivative everolimus (Certican®) exhibit a similar mode of action but a different pharmacokinetic behaviour. Everolimus shows a shorter elimination half-life (~30 *vs* 60 h) and a higher bioavailability [4,5].

In the absence of concomitant use of CNI, rapamycin was shown repeatedly to spare renal function. To the surprise of the transplant community, evidence for rapamycin-associated nephrotoxicity has been accumulating in the last few years. In line with such observations, Crew *et al*. [6] describe two patients with thrombotic microangiopathy as a result of rapamycin exposure and suggest potential mechanisms of the disorder in this edition of *NDT*. Thus, definitely, the question has to be addressed whether nephrotoxicity of rapamycin represents a relevant problem in clinical practice.

Evidence of rapamycin nephrotoxicity in kidney transplantation

Randomized phase-III trials (US trial and global trial) conducted over 24 months and comprising almost 1300 renal allograft recipients treated with cyclosporin investigated the efficacy and safety of the concurrent administration of sirolimus in doses of 2 or 5 mg/day *vs* placebo or azathioprine [7]. Mean values of serum creatinine concentrations in patients treated with sirolimus were higher than in subjects treated with placebo or azathioprine (175 *vs* 137 μmol/l) [7]. The addition of sirolimus revealed a pharmacokinetic interaction, since ~20% lower doses of cyclosporin were required to achieve target levels in patients treated with cyclosporin and rapamycin than in patients exposed to cyclosporin plus azathioprine or placebo. Nevertheless, the 1 year graft survival rates in patients on rapamycin were similar and the rejection rates lower, suggesting nephrotoxicity of rapamycin rather than under-immunosuppression.

In a prospective study, high-risk renal allograft recipients (79% African-Americans, 59% delayed graft function, high proportion of marginal kidneys) were randomized to receive, following thymoglobulin induction, either standard tacrolimus (10–15 ng/ml) plus reduced sirolimus (5–10 ng/ml; group I, $n = 16$) or reduced tacrolimus (5–10 ng/ml) plus standard sirolimus (10–15 ng/ml; group II, $n = 23$) [8]. Although the outcome at 6 months was identical between the two groups, 38% of patients from group I needed discontinuation of tacrolimus due to biopsy-proven tacrolimus nephrotoxicity. Therefore, a reduced quality of the grafts may render them more susceptible to nephrotoxicity of this drug combination.

Furthermore, two cases of acute oliguric renal allograft failure due to suspected acute tubular necrosis (one case biopsy-confirmed) associated with the combined use of tacrolimus and sirolimus were described [9].

In a recent retrospective analysis, the influence of immunosuppression on delayed graft function (DGF) was investigated in 144 kidney transplant patients treated with tacrolimus with or without rapamycin [1]. DGF occurred almost three times more often in subjects treated with rapamycin than without (25% *vs* 8.9%), and was correlated positively with drug dose

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(odds ratio: 1.1 per mg). In addition, a subset of rapamycin-treated patients developed cast nephropathy, reminiscent of myeloma cast nephropathy. These observations are in line with the results from another retrospective clinical study examining 132 renal allograft recipients who developed DFG (23.4%) from a total of 563 adults [10]. Recipients of sirolimus were half as likely to resolve DGF or twice as likely to remain on dialysis as subjects without this agent [10].

In a recent 12 month analysis of 634 cyclosporin-treated heart transplant patients, the concurrent administration of everolimus and cyclosporin led to a significant increase in serum creatinine levels beginning on day 28, as compared with the use of azathioprine [11]. Importantly, cyclosporin drug concentrations were not different among the different groups.

Thus, in patients with an allograft, the combination of a CNI (cyclosporine) and sirolimus/everolimus is definitely associated with higher serum creatinine concentrations than a CNI combined with the other immunosuppressive agents.

Nephrotoxicity of rapamycin in glomerulonephritis

Reports of rapamycin-associated nephrotoxicity in native kidneys are sparse due to its preferential use in transplantation medicine. The largest clinical study included 11 patients suffering from chronic glomerulonephritis (GN) with moderate kidney failure and proteinuria ($\geq$1 g/day) [12]. Within the first 6 weeks of rapamycin therapy (5 mg/day), six subjects developed acute kidney failure with improvement of kidney function after discontinuation of rapamycin in five cases. However, no biopsies were performed in these patients and the mechanism of rapamycin-associated nephrotoxicity remained unclear.

In addition, a recent investigation described four patients with chronic allograft dysfunction who developed biopsy-confirmed post-transplantation GN with nephrotic-range proteinuria 2–9 months after conversion of a CNI-containing regimen to rapamycin [13]. Stabilization of kidney function and complete remission of proteinuria occurred in all cases after reintroduction of CNI.

Mechanisms of rapamycin-associated nephrotoxicity

Rapamycin may display direct tubular or, to a lesser degree, glomerular toxicity and may also potentiate CNI nephrotoxicity.

Sirolimus and everolimus bind to FKBP12 and inhibit mTOR, later identified as FRAP kinase [4], a key regulator of cell proliferation, growth, apoptosis and survival in response to growth factors and cytokines [1,4,14]. FRAP kinase belongs to the phosphatidylinositol kinases, crucial for the progression of the cell cycle from G1 to S phase by signalling through the p70 S6 kinase and eIF4E-binding protein [4].

Rapamycin exposure leads primarily to cell-cycle arrest in the early G1 phase of T and B lymphocytes [4,10]. Following transplantation, rapamycin prevents acute rejection by inhibition of cytokine and growth factor-mediated lymphocyte proliferation, especially the proliferation and clonal expansion of interleukin-2-stimulated T lymphocytes [15]. Importantly, other cells are also a direct target of this drug, including endothelial, smooth muscle, mesangial and renal tubular cells [1,12,16]. Furthermore, rapamycin may promote the 'default way' of apoptosis by inhibiting the activity of 'survival factors' and of p70 S6 kinase [15,17].

Importantly, rapamycin inhibits growth factor-induced proliferation of cultured proximal tubular cells and supports their apoptosis by blocking the survival effects of the same growth factors [15]. Accordingly, rapamycin impaired recovery from experimental acute renal failure induced by renal artery occlusion due to the combined effects of increased tubular cell loss (apoptosis) and inhibition of regenerative proliferation [15]. These effects were attributable to the inhibition of p70 S6 kinase [15].

In addition, rats given rapamycin (3 mg/kg/p.o.) for 2 weeks on a low-salt diet developed magnesium wasting and structural renal lesions consisting of tubular collapse, vacuolization and nephrocalcinosis [18].

Thus, during periods of renal allograft injury, rapamycin may be harmful due to its negative effects on tubular cell regeneration and survival [1,15].

The same may be true at the glomerular level in inflammatory states that require intact cell proliferation for repair and/or compensatory mechanisms [12]. In addition, rapamycin may potentiate tubular toxicity of proteinuria *per se* [12].

Toxicity of rapamycin, indeed, depends upon the type of GN, as first reported by our group in experimental GN models [19]. We demonstrated that everolimus highly accentuated acute mesangial proliferative anti-Thy1.1 nephritis, reflected by severe glomerular destruction with areas of necrosis, kidney failure and increased proteinuria. In contrast, a significant reduction of proteinuria was achieved as a result of everolimus exposure in the non-proliferative acute puromycin aminonucleoside nephrosis [19]. In addition to the antiproliferative effect, everolimus also inhibits mesangial cell migration, mediated by preventing the decrease in the CDK-inhibitor p27 KIP1 induced by growth factors [20]. In this respect, particular caution may be warranted for the use of rapamycin in antibody-mediated forms of proliferative GN.

Pharmacokinetic and possibly also pharmacodynamic interactions of CNI and sirolimus are another potential mechanism of nephrotoxicity. Because of the shared metabolism by the P-450 (CYP) 3A4 and competition for the extrusion by the p-glycoprotein mechanism, blood and tissue levels of both cyclosporin and rapamycin can be increased if administered concurrently [1,7,21–24]. Possibly, the same is true for the tacrolimus and rapamycin combination with respect to an intracellular accumulation resulting

in direct tissue injury [1,8,12,25]. However, unlike the cyclosporin/sirolimus combination, sirolimus/tacrolimus pharmacokinetics appears to remain essentially unchanged following their simultaneous administration [23,24].

In addition, a possible potentiation of CNI nephrotoxicity by sirolimus or everolimus *per se* unrelated to changes in drug levels was proposed also [11]. This may be especially true in cases of tissue regeneration following injury.

Conclusions

Rapamycin and its derivative everolimus appear not to be nephrotoxic to the healthy, predominantly quiescent kidney. However, *in vitro* studies and observations in animals and humans indicate that these agents are nephrotoxic in disease states. Evidence is growing that the same mechanisms that lead to excellent immunosuppression may also impair recovery of tissue injury. Therefore, the administration of rapamycin may exacerbate pre-existing or newly occurring renal lesions.

This might explain why renal function deteriorates in patients with kidneys in the process of repairing tubular cells (delayed graft function and acute tubular necrosis), endothelial cells (thrombotic microangiopathy and GN) or mesangial cells (GN).

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[See related article by Crew *et al*. (this issue pp. 203–209)]

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