Proteinuria and Mammalian Target of Rapamycin Inhibitors in Renal Transplantation

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Abstract

Mammalian target of rapamycin (mTOR) inhibitor use in renal transplant recipients has been associated with more proteinuria compared with calcineurin inhibitors. This overview will focus on mTOR inhibitor-associated proteinuria in various situations after kidney transplantation: de novo treatment with mTOR inhibitor in combination with a calcineurin inhibitor, de novo mTOR inhibitor-containing and calcineurin inhibitor-free treatment, early conversion from a calcineurin inhibitor-based regimen to mTOR inhibitor-based regimen and late conversion. Some possible mechanisms of mTOR inhibitor-induced proteinuria will also be reviewed. **(Trends in Transplant. 2011;5:139-43)**

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Key words

Kidney transplantation. Proteinuria. mTOR inhibitors. Everolimus. Sirolimus.

Introduction

The mammalian target of rapamycin (mTOR) inhibitors everolimus (EVR) and sirolimus (SRL) are used in transplant medicine as immunosuppressive drugs, especially in kidney transplantation. These drugs have been associated with a significantly higher incidence of proteinuria compared with calcineurin inhibitors (CNI)[1-7]. Proteinuria is a well-known phenomenon in renal kidney transplant patients, and measurement of 24-hour urine protein excretion or urinary protein/creatinine ratio is part of the assessment during posttransplant follow-up. Moreover, proteinuria is a prognostic factor for graft and patient survival[8].

This review will focus on mTOR inhibitor-associated proteinuria in different settings after kidney transplantation:

– *De novo* mTOR inhibitor treatment in combination with a CNI;

– *De novo* mTOR inhibitor treatment free of a CNI;

– Early conversion from a CNI-based regimen to an mTOR inhibitor-based regimen;

– Late conversion from a CNI-based regimen to an mTOR inhibitor-based regimen.

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Mammalian Target of Rapamycin Inhibitor with Calcineurin Inhibitor *De Novo*

Dantal, et al. evaluated 139 patients with a high risk of delayed graft function, defined as at least one of the following risk factors: donor over 55 years of age, cold ischemia time between 24 and 40 hours, or previous kidney transplant[9]. At the same time, these are risk factors for developing proteinuria[10]. The incidence of delayed graft function was compared in immediate EVR (IE; cyclosporine in combination with low-dose EVR *de novo*) and delayed EVR treatment (DE; cyclosporine in combination with mycophenolate sodium *de novo* and conversion from mycophenolate to EVR after week four). Patients in both arms received induction with basiliximab and also steroid treatment. Proteinuria at one year was not significantly different between the two groups, 280 mg/day in the IE arm compared with 265 mg/day in the DE arm; however, the number of patients with available values was less than 30% in both arms. Urinary protein per creatinine analysis showed a similar result: 0.3 vs. 0.3 g/g, with approximately half of the patients with available values.

In the US and global Rapamune trials (SRL in combination with a CNI), proteinuria was not measured as part of the routine assessment of kidney function. However, it also was not mentioned as an adverse event in these early studies on the CNI and mTOR inhibitor combination[11].

Mammalian Target of Rapamycin Inhibitor without Calcineurin Inhibitor *De Novo*

The first studies using mTOR inhibitors without a CNI in the *de novo* setting were performed in the 1990s. Although data on 24-hour urine proteinuria were not collected, proteinuria was not reported more frequently as an adverse event in the SRL arms[12,13].

A more recent study was performed by Büchler, et al. A total of 145 patients were randomized to receive *de novo* treatment with antithymocyte globulin plus mycophenolate mofetil plus prednisolone (the latter for only six months) with either cyclosporin A (CsA; n = 74) or SRL (n = 71). Patient and graft survival in the CsA and SRL groups were 97 vs. 97%, and 93 vs. 90%, respectively (p = ns).

Acute rejection rates were 8.6 vs. 14.3% (p = ns). Glomerular filtration rate (GFR) was 57 vs. 60 ml/min (p = ns). At one year, the incidence of proteinuria > 0.5 g/day was 5.6 vs. 38.8% (p < 0.001) in the CsA vs. the SRL arm. Mean 24-hour proteinuria was 01.8 ± 0.3 g/day in the CsA arm vs. 0.64 ± 0.8 g/day in the SRL arm (p < 0.001)[14].

From these studies one can conclude that *de novo* CNI-free treatment with an mTOR inhibitor can be associated with a higher degree of proteinuria. It remains to be determined if this increase is clinically relevant in terms of its influence on long-term transplant function.

Preventive Conversion from Calcineurin Inhibitors to Mammalian Target of Rapamycin Inhibitors during the First Posttransplant Year

In the ZEUS study, Budde, el al. evaluated one-year kidney graft function in patients who received an immunosuppressive protocol consisting of basiliximab induction with CsA, mycophenolate sodium, and steroids. Of 300 patients, 155 were converted from CsA-based to CNI-free EVR-based treatment at

4.5 months after transplantation. Graft function was clearly better in the EVR group at one year (71.9 vs. 61.3 ml/min; p < 0.0001; not significant difference at baseline). Mean proteinuria was significantly higher in the EVR group (455 ± 510 vs. 284 ± 472 mg/day)[15].

Similar results were found in the Spare-the-Nephron study[16].

In the CONCEPT study, all patients were treated with daclizumab induction, CsA, mycophenolate mofetil, and steroids *de novo*. At 12 weeks, 96 patients were converted from CsA to SRL, whereas 97 patients remained on CsA treatment. After eight months, steroids were withdrawn. Kidney graft function at one year was superior in the SRL arm (modification of diet in renal disease, 61.2 vs. 53.9 ml/min; p = 0.002). Proteinuria was significantly different at six months after transplantation (0.6 vs. 0.3 g/day; n = 65 and 79, respectively; p < 0.05). However, this difference disappeared at one year: 0.4 g/day in the SRL arm (n = 54) vs. 0.3 g/day in the CsA arm (n = 69). Again it remains to be determined if this tendency towards a higher proteinuria might have some influence on outcome despite better kidney function[17].

Late Conversion, Mainly for Slowly Declining Allograft Function

In the CONVERT, study Schena, et al. randomized 830 kidney transplant patients between six months and ten years after transplantation to either continue on their CNI treatment in combination with an antimetabolite and steroids (n = 275), or to withdraw the CNI and convert to SRL (n = 555). Enrolment was halted in patients with a GFR < 40 m/min due to safety reasons. In the patients with GFR > 40 ml/min, there was no difference in patient survival, graft survival, or acute rejection rate at two years.

There was also no difference in graft function in the intent-to-treat analysis. In the SRL group, proteinuria increased from 0.35 g/day at baseline to 0.87 g/day at two years after baseline, whereas it increased from 0.28 to 0.42 g/day in the CsA group.

The difference between the proteinuria in the SRL group and the CsA group was significant: p < 0.001 after two years. Moreover, proteinuria at baseline was a predictive factor for improvement of graft function after conversion (the difference after two years between the proteinuria in the SRL group and the CsA group was significant; p < 0.001). Furthermore, proteinuria at baseline was a predictive factor for improvement of graft function after conversion[18].

Other non-randomized conversion studies have shown an increase of proteinuria in late conversion patients, and suggested a predictive value of proteinuria at conversion for long-term graft function post-conversion[19-21].

Possible Mechanisms of Mammalian Target of Rapamycin Inhibitor-Associated Proteinuria

It was speculated that at least part of the increase of proteinuria after withdrawal of the CNI and subsequent introduction of an mTOR inhibitor could be explained by possible hemodynamic changes due to the withdrawal of the CNI. Calcineurin inhibitors are known to exert anti-proteinuric effects, partly by increasing the resistance of the afferent arteriole and thus reducing intra-glomerular pressure. This effect could be demonstrated by Saurina, et al.[22]. However, a later study of conversion from mainly an azathioprine-based regimen to SRL for skin cancer revealed a marked increase of proteinuria after conversion to SRL from a non-CNI-containing regimen, suggesting that there

is a genuine proteinuria-causing effect of mTOR inhibition[23].

Various authors suggested the vascular endothelial growth factor (VEGF) system to be implicated in mTOR inhibitor-associated proteinuria. Letavernier, et al. exposed primary cultures of human podocytes to therapeutic-range concentrations of sirolimus[24]. They observed that VEGF synthesis and Akt phosphorylation were decreased by SRL exposure. Cell viability was not affected after two days of exposure to the drug, but changes in cell phenotype and cytoskeleton reorganization were observed. Since mTOR inhibition is associated with reduced VEGF secretion and blockage of the VEGF signaling pathway[25], a disrupted VEGF balance at the podocyte level could contribute to proteinuria.

Oroszlan, et al. found that application of either of the two mTOR inhibitors SRL and EVR in proximal tubular epithelial cells resulted in decreased albumin uptake and down-regulated cubilin and megalin expression. Interestingly, these effects could be significantly reversed by angiotensin-converting enzyme inhibition or angiotensin receptor blockade, suggesting that mTOR inhibition induced proximal tubular epithelial cell dysfunction and reduced receptor-mediated albumin uptake through an angiotensin II-dependent mechanism[26].

Conclusion

Several studies show an association of mTOR inhibition and proteinuria after kidney transplantation. This effect could be observed to different extents in different settings. It seems to be most prominent in late conversion from a CNI to SRL performed for chronic allograft dysfunction and much less important in early conversion in well-functioning kidneys during the first year posttransplantation.

In the late conversion setting, baseline proteinuria is predictive of graft function after conversion. In the early conversion setting, graft function is better in converted patients, despite a trend towards higher proteinuria. The long-term effect after five or ten years remains unknown.

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