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Sirolimus-Associated Proteinuria and Renal Dysfunction

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Contents

Abstract

Sirolimus is a novel immunosuppressant with potent antiproliferative actions through its ability to inhibit the raptor-containing mammalian target of rapamycin protein kinase. Sirolimus represents a major therapeutic advance in the prevention of acute renal allograft rejection and chronic allograft nephropathy. Its role in the therapy of glomerulonephritis, autoimmunity, cystic renal diseases and renal cancer is under investigation. Because sirolimus does not share the vasomotor renal adverse effects exhibited by calcineurin inhibitors, it has been designated a 'non-nephrotoxic drug'. However, clinical reports suggest that, under some circumstances, sirolimus is associated with proteinuria and acute renal dysfunction. A common risk factor appears to be presence of pre-existing chronic renal damage. The mechanisms of sirolimus-associated proteinuria are multifactorial and may be due to an increase in glomerular capillary pressure following calcineurin inhibitor withdrawal. It has also been suggested that sirolimus directly causes increased glomerular permeability/injury, but evidence for this mechanism is currently inconclusive. The acute renal dysfunction associated with sirolimus (such as in delayed graft function) may be due to suppression of compensatory renal cell proliferation and survival/repair processes. Although these adverse effects occur in some patients, their occurrence could be minimised by knowledge of the molecular effects of sirolimus on the kidney, the use of sirolimus in appropriate patient populations, close monitoring of proteinuria and renal function, use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers if proteinuria occurs and withdrawal if needed. Further long-term analysis of renal allograft studies using sirolimus as *de novo* immunosuppression along with clinical and laboratory studies will refine these issues in the future.

Sirolimus (or rapamycin) is an immunosuppressant drug with potent anti-trophic effects on immune as well as intrinsic renal cells through its ability to specifically inhibit the rapamycin-sensitive (or raptor-containing) mammalian target of rapamycin (mTOR) protein kinase complex.[1] The unique properties of sirolimus, as well as its theoretical advantages over calcineurin inhibitors, make it a

novel candidate drug, along with everolimus and other mTOR inhibitors,[2] to improve the therapy of a variety of kidney diseases, including the prevention of renal allograft rejection, chronic allograft nephropathy, post-transplantation malignancies,[3] immune-initiated glomerulonephritis,[4-6] and cystic renal diseases.[7] Sirolimus-eluting endovascular stents for the management of renal artery stenosis and adjunctive treatment of primary genitourinary malignancies are also other potential applications of sirolimus in renal medicine.[1]

However, there are a number of clinical reports suggesting that sirolimus is associated with proteinuria and renal dysfunction, and this has caused significant controversy.[8,9] The aim of this article is to review the available evidence and address two specific questions: (i) does sirolimus cause proteinuria? and (ii) does sirolimus cause acute and/or chronic renal dysfunction? A search for relevant articles was conducted on MEDLINE (1966 to March 2006) using the keywords: 'sirolimus'; 'rapamycin'; 'proteinuria'; 'creatinine'; 'renal failure'; 'renal dysfunction'; and 'fibrosis'. In addition, the abstracts of international meetings in nephrology and transplantation (2004–6) were reviewed. The molecular mechanisms and pharmacology of sirolimus are reviewed elsewhere[1,2] and are not detailed in this article.

1. Does Sirolimus Cause *De Novo* Proteinuria?

The vast majority of the literature regarding sirolimus-associated proteinuria is derived from patients with chronic allograft dysfunction (CAD) in which a calcineurin inhibitor was withdrawn and converted to sirolimus.[10] With one exception (discussed in this section), all of the reported studies were either case series or case reports, measuring proteinuria pre- and post-conversion to sirolimus following withdrawal of a calcineurin inhibitor, without control groups. The incidence of post-conversion proteinuria in these studies ranged between 30% and 92% (with a lower incidence of nephrotic-range proteinuria and nephrotic syndrome of up to 10%).[10-12] The onset of proteinuria was within weeks or months of conversion and typically glomerular in origin (albumin with a IgG/albumin ratio of 0.15).[11] In some (but not all) studies, the increase in post-conversion proteinuria was limited to a cohort of patients with pre-conversion proteinuria >2 g/day.[13]

However, these studies have design flaws. Because proteinuria is a feature of CAD,[3] and because rebound proteinuria can occur following calcineurin inhibitor withdrawal in a similar timeframe in patients with CAD[14,15] and other glomerular diseases,[16] it is not possible to conclude that sirolimus *per se* is responsible for the exacerbation of proteinuria in these studies because of the absence of a control group. The hypothesis that the proteinuria is, at least partly, due to calcineurin inhibitor withdrawal is supported by data showing that post-conversion patients develop glomerular hyperfiltration,[11] and that the proteinuria is reversible following angiotensin blockade or reinstitution of calcineurin inhibitors.[13] Moreover, in a randomised controlled study design,[17] proteinuria was not affected by conversion to sirolimus. In this study, patients had renal allografts (6 months to 8 years) with impaired renal function (baseline creatinine was 190–400 μmol/L). Calcineurin inhibitors were withdrawn abruptly and converted to sirolimus. At the follow-up of 12 months, proteinuria did not increase in the sirolimus-converted arm and was similar to the control group. The authors suggested that the negative results of their study (in contrast to the others) might relate to the slightly lower sirolimus target levels (5–15 ng/mL as opposed to 8–12 ng/mL in previous studies), minimal pre-conversion proteinuria and/or the inclusion of a control group.[17]

In contrast to CAD, few studies have examined the effect of sirolimus on proteinuria in patients with pre-existing glomerular disease in native kidneys. Senior et al.[18] reported in a retrospective case series that three of 62 clinical islet-transplant recipients developed proteinuria when converted from a calcineurin inhibitor-based regimen to a calcineurin inhibitor-free/sirolimus-based treatment. At least two of the three patients had underlying diabetic nephropathy, which progressed following islet transplantation. Another five patients developed microalbuminuria. The proteinuria improved with increased doses of calcineurin inhibitor, suggesting that the mechanisms of the proteinuria may be similar to that described earlier for patients with CAD

(that is, partly due to calcineurin inhibitor withdrawal).

Two small studies have evaluated the efficacy of sirolimus on proteinuria in idiopathic focal segmental glomerulosclerosis (FSGS). Cho et al.[19] studied six patients who had previously been exposed to a calcineurin inhibitor (but not at the time of sirolimus initiation), and had heavy baseline proteinuria (8.4 g/day) with significant renal impairment (baseline glomerular filtration rate, [GFR] of 52 mL/min/1.73m^2). In this study, sirolimus-exacerbated proteinuria and GFR declined after 6 months of therapy, and both parameters returned to baseline when sirolimus was discontinued. In contrast, Tumlin et al.[6] reported that sirolimus reduced the mean proteinuria from 8.8 to 2.1 g/day in patients with steroid-resistant FSGS and stabilised renal function. Of note, subgroup analysis revealed that the 'responder' population were those that had the shortest duration of disease.[6] Patel et al.[20] also reported that a combination of tacrolimus with sirolimus was beneficial in reducing proteinuria and inducing remission in a single patient with a long history of refractory minimal change nephropathy.

There are several possible hypotheses to explain the divergent effects of sirolimus on proteinuria in FSGS/minimal-change disease. Firstly, sirolimus may be anti-proteinuric when administrated in the early stages of FSGS through control of immune-mediated mechanisms.[5-7] Secondly, sirolimus may impair compensatory glomerular endothelial and mesangial cell repair mechanisms leading to glomerulosclerosis,[21] over-riding any beneficial effects in patients with more advanced glomerular lesions.

The available clinical studies do not provide evidence to suggest that sirolimus is associated with proteinuria in 'normal' native or transplanted kidneys. In a small cohort of renal-allograft recipients treated with sirolimus *de novo* (mainly with a calcineurin inhibitor), Nungaray et al.[22] reported that 15 patients did not develop proteinuria after 7 years of follow-up (censored for graft failure and death).[22] In addition, proteinuria was not reported in large, randomised controlled trials of renal transplant recipients in which sirolimus was administered *de novo* without calcineurin inhibitors or following calcineurin inhibitor elimination at 3 months[2] (although a caveat that should be considered is that proteinuria may not have been adequately assessed, with 24-hour urine collection and/or spot urine albumin to creatinine ratio, in these trials). These results are also identical to studies of renal transplant recipients in which calcineurin inhibitors were eliminated and converted to either azathioprine or mycophenolate mofetil.[14,23] In these studies, proteinuria did not occur when the calcineurin inhibitor was withdrawn early,[23] but was exacerbated if there was underlying CAD.[14] Similarly, in sirolimus-treated, liver transplant recipients or when sirolimus was used for other indications (such as psoriasis), proteinuria was not reported.[24,25]

Even if proteinuria is properly assessed in future randomised controlled trials of renal-transplant recipients, the interpretation of the data might be difficult if the non-sirolimus arm contains a calcineurin inhibitor, as proteinuria could be suppressed in this group and thereby make it appear that the sirolimus group has developed proteinuria. Similarly, the inclusion of a calcineurin inhibitor in both sirolimus- and non-sirolimus groups could also be problematic, because concurrent administration of a calcineurin inhibitor with sirolimus exacerbates calcineurin inhibitor nephrotoxicity and proteinuria. In fact, this was demonstrated in a randomised controlled trial comparing two dosages of everolimus (1.5 or 3 mg/day) with mycophenolate mofetil (2 g/day) in a ciclosporin/corticosteroid regimen in 583 *de novo* renal allograft recipients, where the incidence of proteinuria of >1000 mg/day was increased 5-fold in the everolimus groups (11.6% and 11.4% vs 2.3%, p = 0.028).[26] Proteinuria was assessed by urinalysis only, and the temporal pattern and details (particularly whether it varied according to ciclosporin levels) were not discussed. Calcineurin inhibitor nephrotoxicity occurred in both everolimus arms requiring study modification. Therefore, it was difficult to determine whether higher doses of the calcineurin inhibitor in the mycophenolate mofetil arm masked the proteinuria in that group or whether calcineurin inhibitor nephrotoxicity in both the everolimus groups accounted for the proteinuria.

Does sirolimus aggravate or induce certain types of chronic glomerular injury? Many investigators argue that the temporal onset and rapid exacerbation of proteinuria after the introduction of sirolimus is

inconsistent and not always associated with calcineurin inhibitor withdrawal, and therefore have suggested that the drug might directly exacerbate underlying post-transplantation glomerular damage or induce *de novo* glomerulosclerosis.[13,15] A small number of CAD patients with post-conversion sirolimus-associated proteinuria in the various case series underwent biopsy, particularly if the proteinuria was in the nephritic range.[27-29] A variety of patterns of *de novo* glomerulonephritis have been reported, including FSGS and membranoproliferative glomerulonephritis, following sirolimus therapy.[27-29] However, given the high prevalence of various forms of glomerular disease in patients with post-transplantation nephrotic syndrome, it is impossible to directly prove that sirolimus has a causal role in this process, based on this evidence alone.

Animal models of glomerulonephritis provide some interesting insights and show that sirolimus has divergent effects on pre-existing glomerular injury. In Heymann nephritis, a model of membranous nephropathy,[5] therapeutic administration of sirolimus in established disease reduced proteinuria and renal hypertrophy. In the puromycin aminonucleoside model of FSGS,[30] everolimus reduced proteinuria when given prophylactically. In the adriamycin nephropathy model of FSGS, the progression of glomerulosclerosis was reduced by sirolimus, but proteinuria was unaffected.[31]

In contrast, in rats with mesangioproliferative glomerulonephritis induced by the anti-thy1.1 antibody, high-dose everolimus reduced endothelial cell proliferation and vascular endothelial growth factor (VEGF) expression, impaired capillary repair and, interestingly, led to microaneurysm glomerular capillary dilatation, glomerulosclerosis, crescent formation and renal impairment in a dose-dependant manner.[21] The relevance of the doses used in this study to humans is not known, but it is noteworthy that endothelial cell proliferation is a feature of allograft rejection and some glomerular diseases.[32] In contrast to these findings, a renal biopsy from a patient with CAD and post-sirolimus proteinuria showed that the glomerular expression of VEGF was increased, and the authors speculated that this might increase glomerular permeability to plasma proteins.[33] The reasons for the divergent effects on glomerular VEGF expression in these studies are not known.

Therefore, the question as to whether sirolimus causes or aggravates glomerular toxicity in humans remains unresolved. The foregoing animal studies highlight that the podocyte-endothelial cell-angiogenic cytokine axis should be a focus of attention in future experiments. Another factor also to consider is the possibility of an idiosyncratic reaction (similar to many other drugs, such as non-steroidal inflammatory agents) causing glomerular injury in humans. The latter will not be revealed in animal studies.

Does sirolimus alter the tubular cell handling of filtered proteins? Nephrotic-range proteinuria is a feature of delayed graft function in renal transplant recipients due to reduction in the tubular processing of filtered proteins.[34] A single case report presented a patient with delayed allograft dysfunction (DGF) following a living-related transplant, who developed heavy nephrotic-range proteinuria (up to 12 g/day) by day 4 and peaking on day 8 post-transplant.[35] Withdrawal of sirolimus and substitution with tacrolimus reduced the proteinuria. Light microscopy showed no podocyte foot effacement and fluorescein isothiocyanate-labeled albumin staining was reduced in tubular cells compared with a transplant recipient who developed recurrent FSGS.[35]

Similarly, Morelon and Kreis[27] also found that rapid onset proteinuria occurred in 14 of 17 patients treated with sirolimus (20mg loading dose; 8mg maintenance dose; trough levels 12–20 ng/mL) in a calcineurin inhibitor-free *de novo* regimen (that included induction with basiliximab, mycophenolate mofetil and prednisone) in patients with renal allografts. It is noteworthy that 65% of these patients had DGF in this study[27] and, consistent with this, the mean proteinuria was 2.73g on day 1 and 2.53g on day 7, progressively decreasing to 0.33 g/day by 4 months. The interpretation of these studies is difficult in the absence of control groups. Experimental studies raise the hypothesis that sirolimus could exacerbate proteinuria through tubular mechanisms;[36] however, further studies in humans are needed.

2. Does Sirolimus Cause Renal Dysfunction?

Probably the most common cause of renal dysfunction with sirolimus is a drug interaction with a calcineurin inhibitor. The use of sirolimus with calcineurin inhibitors is often associated with increased serum creatinine due to potentiation of acute and chronic calcineurin inhibitor nephrotoxicity in both renal transplant recipients[37] and other patient populations.[25] Blood and tissue levels of sirolimus and ciclosporin are increased when they are used in combination, possibly because both drugs are metabolised by similar routes involving cytochrome P450 3A4 and P-glycoprotein.[37] Therefore, dose reduction of the calcineurin inhibitor and close drug-level monitoring should be performed when mTOR inhibitors are administered with a calcineurin inhibitor.

Sirolimus may prolong the duration of DGF. At least three single-centre studies[38-40] and a retrospective analysis of 27 772 primary kidney transplants in the US Renal Data System[41] reported that sirolimus was associated with a 2-fold dose-dependant increase in the duration of delayed graft function in cadaveric renal transplants. One-year graft survival and function was not affected by sirolimus,[40,41] although long-term studies are still needed. The limitations of a retrospective analysis and single-centre studies need to be considered when reviewing these data.[42] Some studies have also suggested that sirolimus may increase the incidence of DGF;[39,41] however, this has not been a consistent finding.

Experimental data have suggested the mechanisms by which sirolimus could prolong DGF. Sirolimus exacerbated renal dysfunction in rats with renal ischaemia by suppressing tubular cell proliferation, regeneration and survival when administered for 4 days post-ischaemia at doses that produce therapeutic immunosuppression (0.2 mg/kg by intraperitoneal administration).[43] These results were confirmed in a rat model of renal ischaemia and kidney transplantation.[44] In contrast, Inman et al.[45] found that 0.2 mg/kg by oral administration at the time of ischaemia did not alter GFR in rats 7 days later. The oral absorption of sirolimus in rats is <10% and, therefore, the discrepancy in these experimental data could be explained by differences in the dose of sirolimus.[46] In humans, sirolimus-treated patients with DGF had markedly reduced expression of the mTOR target phosphorylated p70 S6 kinase.[47] Smith et al.[38] also found that sirolimus-treated patients with DGF developed intratubular cast nephropathy analogous to myeloma cast nephropathy. The potential mechanisms were postulated to be suppression of the cell cycle, a reduction in tubular cell survival and promotion of cast formation, leading to intranephronal obstruction and acute renal failure, as in animal models of renal ischaemia.[43]

Sirolimus is rarely associated with thrombotic microangiopathy. Unlike calcineurin inhibitors, sirolimus is only rarely associated with *de novo* thrombotic microangiopathy in renal allograft patients.[9] Sirolimus-induced reduction in VEGF and endothelial cell proliferation may play a role in this process.[48] Preceding endothelial cell injury, such as due to acute cellular rejection, may be a predisposing factor but the overall risk of this complication appears to be low.

Is there an increased risk of renal dysfunction in proteinuric renal diseases with sirolimus use? Fervenza et al.[49] examined the safety and efficacy of sirolimus in a group of 11 patients with proteinuric chronic glomerular disease (>1 g/day) and progression (increase in serum creatinine) over the previous 12 months. In this population, six of 11 patients developed acute renal failure. The renal failure improved upon discontinuation of sirolimus in four patients, recurred on rechallenge in one and required temporary haemodialysis support leading to permanent reduction in GFR in another.

As discussed earlier, sirolimus appears to have divergent effects in FSGS. In the small study by Cho et al.,[19] GFR declined after 6 months of therapy and returned to baseline when sirolimus was discontinued. Tumlin et al.[6] reported partial or complete remission in 57% of 21 patients with steroid-resistant FSGS treated with sirolimus for 6 months. Treatment with sirolimus was continued for another 6 months in the responders, and during that time the GFR remained stable and proteinuria decreased further. In contrast, GFR declined slightly at the end of the 6 months in the 43% of non-responders and was significantly lower compared with baseline creati-

nine at 12 months. Because of the lack of a control group, it is not possible to differentiate whether sirolimus exposure accelerated the renal dysfunction in this group. Interestingly, the duration of FSGS was significantly longer in the non-responders (57 ± 34 [mean ± SEM] vs 17 ± 4 and 12 ± 3 months, respectively, in the partial and complete remission groups) and a greater number had been treated with calcineurin inhibitors (24% vs 9%, although this was not statistically different). With the exception of a case report demonstrating beneficial effects of sirolimus in a patient with long-standing refractory minimal change disease,[20] the foregoing studies therefore suggest that sirolimus could cause renal dysfunction in patients with a long duration of chronic glomerular disease.

The mechanisms of renal dysfunction in glomerular diseases and its interaction with proteinuria with sirolimus are not clear. It is well known that nephrotic glomerular diseases are associated with increased tubular cell proliferation probably in part to compensate for the increased intraluminal protein load.[8,36] In support of this hypothesis, suppression of tubular cell proliferation with sirolimus in rats with acute protein-overload nephropathy caused intratubular cast nephropathy and acute renal failure but without alteration in cell survival (unlike renal ischaemia).[36]

Proteinuria is a predictor of renal function decline in a variety of kidney diseases, possibly through sublethal, inflammatory and fibrotic effects on the tubulointerstitium.[8] For this reason, it is not surprising that many of the studies in which sirolimus exacerbated proteinuria in CAD were associated with worsening renal dysfunction.[10,13,15] However, the proteinuria at least is partially is amenable to therapy through the concurrent administration of an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker, which probably should be mandatory in this patient population.[2,15]

Several experimental studies have examined the effect of sirolimus on the normal tubulointerstitium of the kidney. Treatment with sirolimus preserved glomerular filtration and renal blood flow in normal, salt-depleted and spontaneously hypertensive rats.[50] At high parenteral doses (2.5 mg/kg/day administered subcutaneously) or when administered orally at lower bioequivalent doses (3.5 mg/kg/day), sirolimus caused mild alterations in tubular structure (vacuolisation, nephrocalcinosis) and function (increased magnesium excretion). However, no changes in renal structure or function occurred at doses relevant for immunosuppression (0.1–0.2 mg/kg administered subcutaneously or intravenously) in normal rats fed a low-salt diet.[50] In humans, alterations in urinary electrolyte and tubular enzyme excretion have been reported, but the data were based on small numbers and the study was inadequately controlled.[51]

Some animal studies also suggest that sirolimus paradoxically has profibrotic effects on the tubulointerstitium, which are lesser in magnitude but synergistic with that of the calcineurin inhibitors. *In vitro*, therapeutic concentrations of sirolimus (1–20 ng/mL) increase transforming growth factor (TGF)-β1 messenger RNA (mRNA) in rat tubular epithelial cells.[52] In addition, sirolimus upregulated α-smooth muscle actin expression in cultured rat fibroblasts but reduced cell number.[53]

These *in vitro* observations were corroborated by an *in vivo* model of isogenic kidney transplantation in which sirolimus treatment for 14 days increased TGF-β1 expression in proximal tubular cells and α-smooth muscle actin-positive cell infiltration in the cortex.[54] These effects were observed only with the highest and less-relevant dosages (6.5 mg/kg/day orally), but there was a trend for an increase in these parameters with lower dosages (0.5–3.2 mg/kg/day). In addition, the administration of sirolimus (0.3 mg/kg/day subcutaneously) for 28 days in normal rats fed a low-salt diet, increased the number of TUNEL-positive cells and favoured a proapoptotic environment (increased mRNA for p53, reduced Bcl-xl mRNA).[55] Finally, in transgenic rats overexpressing renin and angiotensinogen, everolimus upregulated connective tissue growth factor and TGF-β1 mRNA as well as collagen deposition in the kidney.[56]

In contrast, the chronic administration of sirolimus reduced tissue fibrosis in Heymann nephritis,[5] as well as in other non-renal models of fibrosis.[57] This may be due to the fact that sirolimus attenuates the downstream effects of TGF-β1;[58] *in vitro* sirolimus directly reduced collagen I production in human fibroblasts;[59] and/or that sirolimus

partially suppressed epithelial-mesenchymal transition in peritoneal cells.[60]

The reasons for the divergent effects of sirolimus on fibrosis in animal models[5,57] remain unclear. In humans, short-term data so far have been favourable. Three randomised controlled trials in renal transplant recipients have demonstrated that at 1–2-year follow-up, GFR is higher and better preserved in an immunosuppressant regimen containing sirolimus compared with one that contains a calcineurin inhibitor. In one small study, chronic allograft nephropathy indices were also reduced.[2] Similar results were obtained when calcineurin inhibitors were completely avoided.[2] In the absence of a non-nephrotoxic control group, the conclusion to draw from these studies (similar to the animal data) is that sirolimus is 'less nephrotoxic' than calcineurin inhibitors, but they do not prove that it is completely non-nephrotoxic. The other limitation is the relatively short follow-up of these studies; clearly data beyond 5–10 years will be important to conclusively determine whether the short-term benefits of sirolimus in this cohort of patients are sustained.

Sirolimus may also reduce the progression of renal dysfunction in cystic and autoimmune-related renal diseases. The proliferation of tubular epithelial cells is increased in cystic renal diseases, and antiproliferative therapies may prevent progression. In support of this hypothesis, sirolimus reduced cyst growth and progression in the Han : SPRD rat model of polycystic kidney disease.[7] Confirmation in other models of polycystic kidney disease and humans is awaited. Sirolimus also has beneficial effects in mediating autoimmunity, and studies have shown that it expands regulatory T cells and reduces autoantibody titres.[61,62] Therefore, sirolimus could be beneficial as part of induction therapy in immune-mediated proliferative glomerulonephritides and autoimmune diseases, and thereby prevent chronic renal failure arising from these diseases. These hypotheses are under evaluation in clinical studies in humans.

3. Conclusion and Directions for Future Research

The discovery and development of sirolimus represents a major advance in the management of patients with renal disease. However, in recent years, significant controversy has arisen because of clinical and experimental studies suggesting that sirolimus is associated with proteinuria and/or renal dysfunction. The vast majority of the available evidence suggests that the proteinuria is mediated by glomerular haemodynamic mechanisms due to calcineurin inhibitor withdrawal in a kidney with chronic glomerular injury (as in CAD). The issue as to whether sirolimus directly causes proteinuria and/or mediates direct glomerular toxicity remains unresolved and requires further study. With regard to renal dysfunction, short-term follow-up (1–3 years) of renal allograft recipients indicates better GFR preservation in sirolimus-treated groups compared with control groups. The only exceptions to these observations are patients with pre-existing acute and chronic renal injury and heavy proteinuria where the antiproliferative effects of sirolimus may have adverse effects on renal function, as well as the rare induction of *de novo* thrombotic microangiopathy.

As demonstrated in this review, the current literature regarding sirolimus-associated proteinuria and renal dysfunction does not allow definitive conclusions to be reached. Further research is needed and possible studies in the future might include: (i) a large randomised controlled trial on the effects of sirolimus on post-conversion proteinuria and GFR in CAD patients (currently underway); (ii) investigation of the effects of sirolimus/everolimus on the glomerular endothelial-podocyte-angiogenic cytokine axis in both normal and chronically injured kidneys; (iii) accurate assessment of proteinuria in all randomised controlled trials involving sirolimus/everolimus; (iv) more long-term follow-up data (>5–10 years) of renal function and structure in sirolimus-treated patients; (v) investigation of profibrotic biomakers and correlation with renal structure in the tubulointerstitium in clinical studies; and (vi) efficacy of sirolimus in autoimmunity, cystic renal diseases and glomerular diseases. Carefully performed animal and epidemiological studies over the next few years should further clarify the significance and mechanisms of these adverse effects.

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