# Management of Side Effects of Sirolimus Therapy

*Giovanni Stallone,[1,3] Barbara Infante,[1] Giuseppe Grandaliano,[2] and Loreto Gesualdo[1]*

Sirolimus (SRL) has been shown to improve long-term graft survival in several calcineurin inhibitor avoidance/minimization protocols. Although SRL has been suggested to reduce the progression of chronic renal graft damage and to prevent the development of neoplasia, two of the most prominent challenges in the field of transplantation, its use is significantly limited by an extremely high incidence of side effects. Some of the side effects are directly linked to the antiproliferative action of SRL, whereas the mechanisms underlying most of the undesired effects of the drug are still far from being clarified. Nevertheless, there is an increasing body of evidence linking most these drug-associated events to SRL dose. In addition, it is now possible to identify well-defined risk factors for most of these effects. Thus, to limit SRL-related side effects the two golden rules are (1) accurate selection of patients to be treated and (2) avoidance of high SRL doses.

**Keywords:** Sirolimus, Side effects, Kidney transplant.

(*Transplantation* 2009;87: S23–S26)

Sirolimus (SRL) is a macrolide lactone with a novel mechanism of immunosuppression (*1*). After administration, SRL enters the cells and binds to a specific cytoplasmic receptor, FK506-binding protein (FKBP-12). The drug-receptor complex blocks the activity of a cytoplasmic serine-threonine kinase known as mammalian target of rapamycin (mTOR). This kinase is the downstream effector of the phosphatidylinositol 3 kinase (PI3K)-Akt signaling pathway, and it is a key check-point in several cell functions, including cell growth and proliferation (*1*). The mTOR is also involved in the modulation of cell metabolism, sensing the availability of extracellular nutrients. A number of different stimuli, including interleukin-2, -15, oncogenic proteins, vascular endothelial growth factor, may activate mTOR. The inhibition of this pivotal cytoplasmic kinase by SRL in T cells hampers their clonal expansion in response to alloantigen and represents the basis of the immunosuppressive effect of the drug. The inhibition of mTOR has been suggested to be involved in the antifibrotic effects of SRL as well as in its ability to reduce the incidence and progression of several posttransplant neoplasia (*1*, *2*).

On the basis of these molecular effects and with the support of several clinical observations, SRL has been suggested as an alternative to calcineurin inhibitors (CNI) in the long-term immunosuppressive regimen of kidney transplantation. The use of SRL, however, has been significantly limited by an extremely high incidence of side effects. Indeed, in most of the clinical trials testing this immunosuppressive drug the rate of drop out caused by side effects was almost invariably higher in the treatment arm including SRL. Although the central role of mTOR in several cell functions may explain this observation, the fine molecular mechanisms underlying most of the SRL-associated undesired effects are still poorly defined.

The aim of this review was to analyze the most common SRL side effects, focusing on their management, in the attempt to define the best approach to use this drug.

## Metabolic Effects

Because mTOR plays a pivotal role in the modulation of cell metabolism it is not surprising that SRL exerts several undesired metabolic effects.

### Hyperlipidemia

The most frequent SRL side effect is represented by hyperdyslipidemia (*3*). The drug may increase the serum levels of total cholesterol, triglycerides, and apolipoprotein C-III. Lipid abnormalities are probably the result of complex interferences of SRL on lipid metabolism (*3*). Several studies demonstrate that SRL may up-regulate apolipoprotein C-III, reduce the catabolism of very low density lipoprotein (VLDL) apo B100-containing lipoprotein, and alter the insulin signaling pathway with increased hepatic synthesis of triglycerides and increased secretion of VLDL (*3*). The co-administration of CsA and corticosteroids may aggravate lipid abnormalities. Long-term studies with SRL showed that hyperlipidemia tends to improve over time (*4*). Immunosuppressive strategies minimizing doses of SRL, CNIs or corticosteroids may help in controlling hyperlipidemia. The administration of statins and fibrates are effective in reducing hypercholesterolemia and hypertrygliceridemia, respectively (*3*).

### Impaired Glucose Tolerance and Posttransplant Diabetes Mellitus

Posttransplantation glucose intolerance or overt posttransplant diabetes mellitus may result from a combination of insulin resistance and dysfunctional insulin secretion, as in the general population. However, the relative contribution of either mechanism may vary largely among patients, in relation to several pathogenic factors, including age, body mass index, ethnicity, time from transplant, and use of different immunosuppressive regimens (*5*). Among immunosuppressant, steroids are well known to affect glucose tolerance by increasing peripheral insulin resistance, whereas CNIs may alter insulin release. A recent report suggests that SRL might have a role in the development of posttransplant diabetes

G.G. and G.S. have received honoraria within the last three years from Wyeth. B.I. and L.G. declare no conflict of interest.

[1] Department of Biomedical Sciences, Section of Nephrology, University of Foggia, Foggia, Italy.
[2] Department of Emergency and Organ Transplantation, Nephrology, Dialysis and Transplantation Unit, University of Bari, Bari, Italy.
[3] Address correspondence to: Giovanni Stallone, M.D., Department of Biomedical Sciences, Division of Nephrology, University of Foggia, Viale Luigi Pinto, 1, 71100 Foggia, Italy.
E-mail: g.stallone@unifg.it

Received 30 October 2008. Revision requested 4 December 2008.
Accepted 3 February 2009.

ISSN 0041-1337/09/8708S-23
DOI: 10.1097/TP.0b013e3181a05b7a

mellitus (*5*). Indeed, mTOR plays a key role in the insulin signaling cascade. A SRL-sensitive pathway has been implicated in the regulation of glycogen synthase kinase 3 and glycogen synthase and in the inactivation of glycogen phosphorylase by insulin (*5*). Moreover, SRL has been shown to abrogate the insulin-mediated increase in GLUT1 protein synthesis, thereby possibly modulating insulin-dependent glucose transport (*6*). Teutonico et al. (*7*) suggested that SRL-induced insulin resistance might be linked to the increase in circulating triglycerides induced by the drug. Indeed, hypertriglyceridemia has been shown to be associated with insulin resistance in the general population. In addition, mTOR and P70S6K signal transduction pathways have been suggested to control beta-cell size and proliferation and insulin release; in this way, the inhibition of P70SS6K activation by SRL might contribute to the onset and development of "insulin resistance" in the beta-cell (*5*). From a clinical standpoint, however, it is important to underline that, according to Johnston et al. (*5*), SRL diabetogenic effect is prominent when the drug is used in association with CNIs.

## Hematological Effects

### Anemia

Anemia may occur in patients treated with SRL; this effect is generally mild and dose-dependent and worsens when the drug is associated with mycophenolate mofetil (*8*). Although anemia might be caused by a direct antiproliferative action of SRL on bone marrow erythroid precursor, a pathogenic link between SRL-induced anemia and the appearance of an inflammatory state was recently suggested (*9*). We demonstrated that SRL-related anemia is independent of the drug antiproliferative effect and does not present the features of inflammation-related anemia, characterized by a functional iron deficiency (*9*). This event may be due to the direct influence of SRL on iron homeostasis (*9*). In addition, Friend et al. (*10*) recently suggested that the direct effect of the drug on anemia, particularly evident in the short term, is "compensated" in the long term by the preservation of renal function, the major independent factor in the pathogenesis of post-transplant anemia.

## Kidney Effects

### Acute Renal Toxicity

SRL has been introduced in the renal transplantation field as an immunosuppressive drug without known nephrotoxic effects. However, experimental studies suggested that SRL may cause acute tubular damage. The drug has been shown to impair the recovery of renal function after ischemia-reperfusion injury by increasing the apoptosis of tubular cells and inhibiting their regenerative response (*11*). This toxic effect, observed in an experimental model of acute renal failure, was confirmed in renal transplant recipients. Indeed, we and others described a deferred recovery from delayed graft function in de novo renal transplant recipients treated with SRL, alone or in combination with low-dose cyclosporine A (CsA) or tacrolimus (*12*, *13*). SRL in this set of patients caused an histologic picture resembling acute cast nephropathy and the tubular damage was most likely due to the inhibition of the akt-S6p70K pathway, the main anti-apoptotic signal (*14*). However, our data would suggest that this impaired recovery from delayed graft function does not influence graft function and survival at 1 year (*12*).

### Proteinuria

Up to 30% of renal transplant recipients may develop proteinuria after conversion from CNI to SRL. Although the appearance of proteinuria was suggested to be caused by the sudden withdrawal of CsA, a well-known antiproteinuric drug, recent data did not confirm this hypothesis. Indeed, Van den Akker et al. (*15*) observed a raise in proteinuria also in kidney transplant recipients converted from azathioprine to SRL. Thus, it is likely that SRL may cause proteinuria by other mechanisms. Saurina et al. (*16*) showed that, after conversion from CSA to SRL, there is an increase in intraglomerular pressure with a concomitant reduction of renal reserve, suggesting that proteinuria may be caused, at least in part, by glomerular hyperfiltration. Recently, a case report suggested a tubular mechanism for increased proteinuria in kidney transplanted patient who received SRL as standard therapy. This report hypothesizes that SRL may induce severe proteinuria through a reduction of proximal tubular protein reabsorption and a subsequent increase in protein loss. Interestingly, Letavernier et al. (*17*) reported that SRL at high dosage may induce de novo focal segmental glomerulosclerosis, a glomerular disease characterized by significant podocyte alterations and proteinuria. The same authors suggested that SRL at high dosage may induce a significant podocyte dysregulation. Diekmann et al. (*18*) suggested that the level of proteinuria before conversion could not only predict the development of nephrotic range proteinuria after conversion, but also the clinical response to SRL introduction. These authors suggested that a cutoff of 800 mg/24 hr may reliably identify responders from nonresponders (*18*).

In addition, it should be taken into consideration that SRL has been shown to reduce interstitial fibrosis and renal damage progression in several experimental models of progressive renal diseases characterized by massive proteinuria (*19*). Thus, the real impact of the raise in urine protein excretion induced by SRL needs to be defined.

Finally, the appearance of proteinuria may be easily controlled in most of the cases with the administration of angiotensin-converting enzyme (ACE)-inhibitors and angiotensin II receptor antagonists and reducing SRL blood levels (trough levels) below 10 to 12 ng/mL.

## Miscellaneous

### Wound Healing and Lymphocele

Impaired wound healing has been observed in patients treated with SRL, probably as a consequence of its antiproliferative activity. However, retrospective studies demonstrate that the only factor associated with delayed wound healing was obesity (*20*). In addition, several observations suggested a key role for corticosteroids in this phenomenon. Thus, reducing obesity before transplantation, delaying SRL introduction in obese patients and minimizing/avoiding corticosteroids can significantly reduce the risk of delayed wound healing.

SRL has also been suggested to increase the incidence of lymphocele formation. This effect may result from the anti-lymphoangogenic effect of SRL, recently observed by Huber

et al. (*21*). Langer and Kahan (*22*) investigated the factors associated with an increased occurrence of clinically significant perinephric fluid collections and lymphoceles among SRL-treated renal transplant recipients. Their conclusion indicated that the addition of SRL to a CsA-corticosteroids regimen resulted in a higher incidence of lymphocele, even if the presence of corticosteroids seems to be an independent risk factor. Also for lymphocele, as for impaired wound healing, the presence of obesity may play a significant role (*20*).

### Interstitial Pneumonia

Several cases of interstitial pneumonia caused by SRL have been reported (*23*). Patients were presented with cough, dyspnoea accompanied by fatigue and, less often, fever. Chest X-ray and computer-assisted tomography show bilateral patchy or diffuse alveolo-interstitial infiltrates with a predilection for the lower lobes. Weiner et al. (*24*) recently reported that impaired graft function and late SRL introduction are two independent risk factors for this serious drug-related adverse event. Dramatic reduction of SRL doses, or in serious cases SRL discontinuation, led to resolution within 2 to 3 weeks (*23*).

### Mouth Ulcers

This side effect is reported in 24% of patients treated with SRL and is one of the major causes of SRL discontinuation (*25*). The development of mouth ulcers also seems to be dose-related, because they usually appear after the loading dose and often improve after a dose reduction. The effect seems particularly frequent and severe in patients given a combination of SRL and mycophenolate mofetil. Chuang and Langone (*26*) observed that the direct application of clobetasol, a high-potency topical steroid, led to prompt resolution of the aphthous ulcers that developed in transplant patients on SRL-based immunosuppression.

### Joint Pain

This side effect is reported in 23% of patients treated with SRL (*25*). Pain may be disabling, but usually resolves with dose reduction. Pain might be caused by changes in circulation in the bone. The concomitant administration of CsA may worsen joint pain, inducing intraosseous vasoconstriction.

### Edema

Eyelid or leg edema may occur in SRL-treated patients (*25*). It is usually moderate and often reversible with SRL dose reduction. The mechanism leading to edema formation is uncertain and open to speculation. Rabbit endothelial cells exposed to SRL have been shown to release increased amounts of prostacyclin, compared with those exposed to tacrolimus (*27*). An excess of prostaglandins can lead to inadequate vasodilatation and thus increase fluid collection in peripheral organs. We described an unusual side effect, tongue edema, in a small number of patients, when ACEi and high doses of SRL (TL 12 ng/mL) were administered. This effect disappeared when SRL doses was reduced (TL 7 ng/mL) or ACE inhibitor discontinued (*28*).

## CONCLUSION

The high incidence of side effects remains the main limit to SRL use in kidney transplantation. Several studies have identified specific risk factors for most of these drug-related adverse events. Thus, delaying/avoiding the use of SRL in high-risk patients as well as removing modifiable risk factors should be the first approach in the attempt to reduce undesired SRL effects. In addition, there is an increasing body of evidence that most of the adverse events caused by SRL are dose dependent. Thus, a careful therapeutic drug monitoring and a reduction in the target SRL trough levels should be considered both to prevent and to treat SRL side effects.

However, further studies are necessary to optimize the use of SRL in kidney transplant recipients, in particular we will need to determine appropriate target concentrations over time, the requirement and dosage of concomitant immunosuppressive therapy, and the best clinical strategies to overcome SRL-related adverse effects.

## REFERENCES


1. Sehgal SN. Rapamune: Mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem* 1998; 31: 335.
2. Stallone G, Schena A, Infante B, et al. Rapamycin for Kaposi's sarcoma in renal transplant patients. *N Engl J Med* 2005; 352: 1317.
3. Kasiske BL, de Mattos A, Flechner SM, et al. Mammalian target of rapamycin inhibitor dyslipidemia in kidney transplant recipients. *Am J Transplant* 2008; 8: 1384.
4. Blum CB. Effects of sirolimus in renal allograft recipients: An analysis using the Framingham risk model. *Am J Transplant* 2002; 6: 551.
5. Johnston O, Rose CL, Webster AC, et al. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. *J Am Soc Nephrol* 2008; 19: 1411.
6. Taha C, Liu Z, Jin J, et al. Opposite translational control of GLUT1 and GLUT4 glucose transporter mRNAs in response to insulin. Role of mammalian target of rapamycin, protein kinase b, and phosphatidylinositol 3-kinase in GLUT1 mRNA translation. *J Biol Chem* 1999; 274: 33085.
7. Teutonico A, Schena FP, Di Paolo S. Glucose metabolism in renal transplant recipients: Effect of calcineurin inhibitor withdrawal and conversion to sirolimus. *J Am Soc Nephrol* 2005; 16: 3128.
8. Kreis H, Cisterne JM, Land W. Sirolimus in association with mycophenolate mofetil induction for the prevention of acute rejection in renal allograft recipients. *Transplantation* 2002; 73: 920.
9. Maiorano A, Stallone G, Schena A, et al. Sirolimus interferes with iron homeostasis in renal transplant recipients. *Transplantation* 2006; 82: 908.
10. Friend P, Russ G, Oberbauer R, et al. Incidence of anemia in sirolimus-treated renal transplant recipients: The importance of preserving renal function. *Transpl Int* 2007; 20: 754.
11. Lieberthal W, Fuhro R, Andry CC, et al. Rapamycin impairs recovery from acute renal failure: Role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol* 2001; 281: F693.
12. Stallone G, Di Paolo S, Schena A, et al. The addition of sirolimus to cyclosporine delays the recovery from delayed graft function but does not affect 1-year graft function. *J Am Soc Nephrol* 2004; 15: 228.
13. Smith KD, Wrenshall LE, Nicosia RF, et al. Delayed graft function and cast nephropathy associated with tacrolimus plus rapamycin use. *J Am Soc Nephrol* 2003; 14: 1037.
14. Loverre A, Ditonno P, Crovace A, et al. Ischemia-reperfusion induces glomerular and tubular activation of proinflammatory and antiapoptotic pathways: Differential modulation by rapamycin. *J Am Soc Nephrol* 2004; 15: 2675.
15. van den Akker JM, Wetzels JF, Hoitsma AJ. Proteinuria following conversion from azathioprine to sirolimus in renal transplant recipients. *Kidney Int* 2006; 70: 1355.
16. Saurina A, Campistol JM, Piera C, et al. Conversion from calcineurin inhibitors to sirolimus in chronic allograft dysfunction: Changes in glomerular haemodynamics and proteinuria. *Nephrol Dial Transplant* 2006; 2: 488.
17. Letavernier E, Bruneval P, Mandet C, et al. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol* 2007; 2: 326.

18. Diekmann F, Budde K, Oppenheimer F, et al. Predictors of success in conversion from calcineurin inhibitor to sirolimus in chronic allograft dysfunction. *Am J Transplant* 2004; 4: 1869.
19. Brunskill NJ. Rapamycin: A new string to the antiproteinuric bow? *J Am Soc Nephrol* 2005; 16: 1878.
20. Flechner SM, Zhou L, Derweesh I, et al. The impact of sirolimus, mycophenolate mofetil, cyclosporine, azathioprine and steroids on wound healing in 513 kidney transplant recipients. *Transplantation* 2003; 76: 1729.
21. Huber S, Bruns CJ, Schmid G, et al. Inhibition of the mammalian target of rapamycin impedes lymphangiogenesis. *Kidney Int* 2007; 71: 771.
22. Langer RM, Kahan BD. Incidence, therapy, and consequences of lymphocele after sirolimus-cyclosporine-prednisone immunosuppression in renal transplant recipients. *Transplantation* 2002; 74: 804.
23. Pham PT, Pham PC, Danovitch G, et al. Sirolimus-associated pulmonary toxicity. *Transplantation* 2004; 77: 1275.
24. Weiner SM, Sellin L, Vonend O, et al. Pneumonitis associated with sirolimus: Clinical characteristics, risk factors and outcome—A single-centre experience and review of the literature. *Nephrol Dial Transplant* 2007; 22: 3631.
25. Montalbano M, Neff GW, Yamashiki N, et al. A retrospective review of liver transplant patients treated with sirolimus from a single centre: An analysis of sirolimus-related complications. *Transplantation* 2004; 78: 264.
26. Chuang P, Langone AJ. Clobetasol ameliorates aphthous ulceration in renal transplant patients on sirolimus. *Am J Transplant* 2007; 7: 714.
27. Yatscoff RW, Fryer J, Thliveris JA. Comparison of the effect of rapamycin and FK506 on release of prostacyclin and endothelin in vitro. *Clin Biochem* 1993; 26: 409.
28. Stallone G, Infante B, Di Paolo S, et al. Sirolimus and angiotensin-converting enzyme inhibitors together induce tongue oedema in renal transplant recipients. *Nephrol Dial Transplant* 2004; 19: 2906.