

Sirolimus therapy following early cyclosporine withdrawal in transplant patients: mechanisms of action and clinical results

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Abstract: Cyclosporine (CsA), a member of the family of calcineurin inhibitors, is a cornerstone of the immunosuppressive treatments used after organ transplantation. However, it exhibits significant toxicity, including nephrotoxicity and increased cardiovascular risk factors. CsA withdrawal has been used as a strategy to improve renal allograft function and other CsA-related toxicities. In order to maintain adequate immunosuppression levels, sirolimus may be used in association with CsA withdrawal. Sirolimus is a member of the mammalian target of rapamycin (mTOR) family. It presents a good immunosuppressive efficacy associated with antiproliferative actions. Early withdrawal of CsA with sirolimus is associated with a significant improvement of renal function. Despite numerically a higher incidence of acute rejection episodes, this maneuver seems also to be associated with a better allograft survival in the long-term, and improvement of renal histology and blood pressure. However, CsA withdrawal is only feasible in a selected population. Furthermore, the use of sirolimus is associated with other side-effects including lipid abnormalities, abnormal liver tests, and thrombocytopenia. Other studies are mandatory to define the population who can benefit from this maneuver. Finally, complete CsA avoidance has been already reported and is currently under clinical investigation.

Keywords: cyclosporine, sirolimus, transplantation, nephrotoxicity, withdrawal

Introduction

Since the early 1980s, standard care for immunosuppression in transplant recipients has involved the use of calcineurin inhibitors (CNIs) such as cyclosporine (CsA) and tacrolimus (TAC). CsA inhibits T-cell activation pathways which require a rise in intracellular-free calcium concentration, thus reducing the production of interleukin-2 (IL-2) (Wiederrecht et al 1993). CsA binds specifically and with high affinity to a family of receptors called cyclophilins (Erlanger 1992; Schreiber 1992). This drug-receptor complex inhibits the activation of calcineurin phosphatase which dephosphorylates and activates the nuclear factor of activation of T cells (NF-AT). NF-AT increases transcription of IL-2. Inhibition of IL-2 transcription by CsA therefore stops the proliferation and activation of helper and cytotoxic T cells (Suthanthiran et al 1996). The results of several studies suggest that the use of CsA after renal transplantation reduces the number of acute rejection episodes and enhances short-term allograft survival (The Canadian Multicentre Study Group 1986). However, most of the benefit appears to result from a decrease in the number of acute rejections during the first months after transplantation. CsA is indeed associated with significant nephrotoxic side-effects as well as other side-effects in the short and long term (Myers et al 1988; Schorn et al 1991; Fioretto et al 1995; Bennett et al 1996; Goldstein et al 1997).

In both experimental models and human clinical studies, it has been well established that CsA produces dose-dependent, acute, and reversible vasoconstriction of renal arterioles. CsA-induced acute renal failure may occur as early as a few days or months

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after the initiation of therapy (Flechner et al 1983). The clinical manifestations of renal arteriolar vasoconstriction or acute renal dysfunction include reduction of the glomerular filtration rate, hypertension, hyperkalemia, tubular acidosis, increased reabsorption of sodium, and oliguria (Remuzzi and Perico 1995). The adverse effects on renal hemodynamics are thought to be directly related to blood CsA concentrations. The precise mechanism and the mediators involved in these adverse effects and implicated in the alteration of renal hemodynamics have yet to be identified. Many mediators such as endothelin, thromboxane A₂, renal prostaglandins, and nitric oxide are probably implicated (Bunchman and Brookshire 1991; Lanese and Conger 1993; Bobadilla et al 1994).

Chronic progressive nephrotoxicity is the major toxic effect in the long term and is associated with mild-to-moderate renal dysfunction. The transition from acute hemodynamic changes to chronic injury has not been clearly established. Studies based on biopsies of experimental models, patients with autoimmune diseases, and extrarenal solid organ transplants have demonstrated the specific pathological and morphological changes occurring in CsA-induced chronic progressive nephropathy (Palestine et al 1986; Dische et al 1988; Nizze et al 1988; Mihatsch et al 1995). Histologically, the latter is characterized by arterial wall destruction, myointimal necrosis, and gradual narrowing of the arterial lumen. It is also associated with tubulointerstitial fibrosis with a striped pattern. Its pathogenesis is unclear. Low-grade chronic ischemia due to continuous renal vasoconstriction may be an important cause. However, in an experimental model in salt-depleted rats, no clear relationship between glomerular hemodynamics and the development of histological lesions was found (Elzinga et al 1993). Other experiments led to the conclusions that the renin angiotensin system is activated intrarenally by CsA, which leads to the deposition of excess matrix protein by stimulating its production and/or diminishing its breakdown (Shihab et al 1997). Transforming growth factor β -1 (TGF- β 1) has been suggested to play a role in causing chronic progressive CsA-induced nephropathy, by acting as a profibrotic cytokine.

The incidence of this nephropathy after renal transplantation is difficult to evaluate. Several processes can lead to tubulointerstitial fibrosis and structural and functional deterioration of the kidney. These include chronic rejection, hypertension, hyperglycemia, hyperlipidemia, recurrence of underlying disease, and aging. The major drawback of many studies is the absence of renal histology. Nankivell et al (2003) reported recently a very important approach that enlightens

the natural history of chronic allograft nephropathy. In a prospective study among 120 consecutive patients with type 1 diabetes mellitus who received either a kidney-pancreas transplant (n = 119) or a kidney transplant alone (n = 1). Three or more sequential kidney-transplant-biopsy specimens were available from all these patients. During the first year after transplantation, mild chronic allograft nephropathy was present in 94.2% of patients associated with ischemic injury, and clinical and subclinical rejection. Beyond 1 year, a later phase of chronic allograft nephropathy was characterized by microvascular and glomerular injury. However, chronic rejection, ie, immunological process, was uncommon but nephrotoxicity was almost universal at 10 years even in grafts with excellent early histologic findings. CNI nephrotoxicity was the major cause of late histologic injury and ongoing decline in renal function.

Another approach to evaluating the incidence of chronic allograft CsA-induced nephropathy is to examine the occurrence of this complication among nonrenal transplant recipients (Ojo et al 2003) or in autoimmune disease (Vercauteren et al 1998). After heart transplantation, a 10% prevalence of CNI-induced end-stage renal failure was reported after 5–10 years of immunosuppressive therapy (Greenberg et al 1990). After liver transplantation, in 305 CsA-treated patients, renal dysfunction, defined as a serum creatinine level greater than 140 mmol/L, occurred in 50% of patients, and 15% needed renal support (O'Grady et al 2002). When renal biopsies were performed in liver-transplant recipients because of chronic renal impairment, chronic CsA nephrotoxicity was present in about one third of these biopsies. In auto-immune diseases, Vercauteren et al (1998) reported a difference of 20.9% between the risk of developing nephrotoxicity with CsA therapy and with an alternative therapy.

CsA has been associated also with other toxicities other than renal. The most important is the worsening of many cardiovascular risk factors (Miller 2002). Thus, CsA is associated with hypertension: its incidence among renal transplant recipients was 50% before the CsA era and is now reported to be higher than 80%. Hyperlipemia is another common cardiovascular risk factor in patients treated with CsA. Since cardiovascular disease is the most frequent cause of death, and is also a cause of allograft failure, these issues are becoming increasingly important, as the improvement of immunological complications is observed after renal transplantation.

The balance between preventing immunological allograft failures and managing nephrotoxicity is still an unsolved

issue. In order to include the nonimmunological components of late graft loss or renal dysfunction after kidney transplantation, especially drug-induced nephrotoxicity, the term of “chronic rejection” has been replaced by “chronic allograft nephropathy”. In an attempt to improve the long-term survival of grafts and patients, and to avoid CsA-related adverse effects, conversion from CsA to nonnephrotoxic immunosuppressive drugs has been extensively reported in the literature since 1988. The recent introduction of new immunosuppressive drugs such as mycophenolate mofetil (MMF) or sirolimus (SRL) has strengthened the case for minimizing the use of CsA. The main strategies for reducing potential CsA-induced nephrotoxicity consist of sparing CsA in induction regimens from the day of transplantation or early CsA withdrawal.

Because of its specific properties and the potential synergy in CsA nephrotoxicity, early CsA withdrawal has been explored using SRL treatment. I will focus this review on the reason and aims of this approach and the reports on both short- and long-term results after this maneuver.

Mechanism of action of SRL

SRL, an antifungal macrolide, displays potent antiproliferative activities that produce antitumor and immunosuppressive effects. The search for the mechanism of action of SRL led to the discovery of a protein, target of rapamycin (TOR). TOR is a serine–threonine kinase that plays a critical role in growth factor and nutrient-sensitive signaling pathways that regulate cell growth, integrating signals from the amino acid supply and cellular energy state, and transducing stimuli from a variety of hormonal and cytokine receptors. These actions control autophagy, actin cytoskeletal organization, mRNA transcription, protein turnover, and, particularly, initiation of protein translation (Abe and Thomson 2003; Harris and Lawrence 2003; Lekmine et al 2004).

Structure of TOR

TOR is a member of the phosphatidylinositol 3-kinase (PI3K) superfamily. PI3K has the unique ability to phosphorylate carbon 3 of membrane phosphatidylinositols. TOR is highly conserved from yeast to mammals: yeast TOR1 and TOR2 show 42% and 45% identity, respectively, with the nucleotide sequence of the human genes, and TOR protein (2549 amino acids long) displays 95% identity among humans, mice, and rats (Harris and Lawrence 2003; Bjornsti and Houghton 2004; Mayer et al 2004). Mammalian TOR (mTOR) is a 289-kd serine–threonine kinase. The FKBP12-rapamycin-binding domain of mTOR is a four-helix bundle having

short underhand connections that loop, positioning the NH₂-terminus and COOH-terminus close to each other. Sirolimus forms a complex, simultaneously binding two hydrophobic pockets: one in the isomerase FKBP12 at high affinity (Choi et al 1996) at Ser²⁰³⁵, thereby joining FKBP12 and TOR. SRL is almost completely buried between the macromolecules in the ternary complex. The SRL bridge is critical because, in its absence, the two proteins interact with each other only to a limited extent. Mutations of Ser²⁰³⁵ to Thr render mTOR resistant to the actions of SRL-FKBP12, probably owing to the greater bulk of the substituent residue. The formation of the ternary complex with FKBP12-rapamycin may inhibit TOR kinase activity either directly or by blocking access to substrate partner proteins.

Enzymatic activity of TOR

TOR activation requires upregulation of PI3K and its downstream effector, the serine–threonine Akt. The PI3K pathway not only integrates receptor tyrosine kinase signaling but also the apoptotic network. Akt phosphorylates multiple downstream effectors that stimulate cell cycle progression, alter metabolism, produce changes in mRNA translation, and ultimately control cell death (Abe and Thomson 2003; Beugnet et al 2003; Kristof et al 2003; Caldarola et al 2004) by decreasing transcription of proapoptotic genes through inhibition of forkhead transcription factors. Akt thus increases cell size, suppresses apoptosis, inactivates cell cycle inhibitors, and induces cyclin and cytokine gene expression. In T and B cells, Akt activation, which follows antigen receptor engagement, is greatly boosted by costimulation (Edinger et al 2003; Manning and Cantley 2003). Akt directly phosphorylates TOR at Ser²⁴⁴⁸, thereby dissociating its complex with the proteins coded by the genes mutated in tuberous sclerosis complex (TSC)—hamartin (TSC1, Mr <130 000) and tuberin (TSC2, Mr <200 000) (Gao et al 2002; Inoki et al 2002; Potter et al 2002; Jefferson and Kimball 2003).

mTOR inhibitors cause cell cycle arrest in the G1-phase. mTOR is part of a multisubunit complex that contains the regulatory associated protein of mTOR (RAPTOR) and a protein denoted as GssL (G protein subunit-like protein) (Kim et al 2003; Stromberg et al 2004; Tokunaga et al 2004). RAPTOR links TOR to p70 ribosomal S6 kinase (S6K1) or to an inhibitor of eukaryotic translation initiation factor 4E–eIF-4E binding protein 1 (4E-BP1 also known as PHAS) through a TOR signaling (TOS) motif (Yonezawa et al 2004) in the N terminus of S6K1 or C terminus of 4E-BP1. On stimulation by insulin, other growth factors, cytokines, or nutrients

(Bjornsti and Houghton 2004), mTOR phosphorylates at least two regulators of translational protein synthesis: S6K1 and 4E-BP. By promoting phosphorylation of S6, mTOR activates S6K1.

Phosphorylation of the S6 subunit of 40S ribosomes follows mitogenic stimulation, leading to initiation of protein translation by mRNAs possessing a 5-terminal oligopyear imidine tract (5-TOP) (Tabancay et al 2003; Lekmine et al 2004), including p70 ribosomal proteins and eukaryotic elongation factor 2 (Kim and Chen 2000; Gstaiger et al 2003; Sehgal 2003). All known ribosomal proteins and several abundant elongation factors are encoded by TOP messages (Zhu et al 2003; Caldarola et al 2004).

mTOR-dependent phosphorylation of serine Ser⁶⁵ and N-terminal Thr³⁷, Thr⁴⁶, and Thr⁷⁰ in 4E-BP1 cleaves 4E-BP1 from the translational activator eIF-4E (Ferguson et al 2003). After a final phosphorylation event at Ser65, 4E-BP1 dissociates from eIF-4E, an action that reconstitutes the translationally competent initiation factor (eIF-4F), resulting in translation of a subset of capped mRNAs, which contain highly structured 5-untranslated regions that encode proteins involved in G1- to S-phase progression (Boulay et al 2004). In favorable growth conditions, mTOR phosphorylates 4E-BP1, which binds to and inhibits the action of the mRNA 5 cap recognition element of the translation initiation complex protein eIF-4E (Jefferson and Kimball 2003). The dissociation of eIF-4E thus enhances the translation initiation complex (Raught et al 2004), allowing it to associate with eIF-4G to initiate translation (Tang et al 2002; Lekmine et al 2003; Browne and Proud 2004). mTOR phosphorylates Stat3, which enhances transcription of factors c-myc and cyclin D1, and catalyzes activation of cyclin-dependent kinases by releasing the inhibitory factor p27kip1. These effects promote cell cycle progression, resulting in activation of RNA polymerases I and II as well as polyphosphorylation of retinoblastoma protein.

TOR regulation of cell survival

Nonactivated mature T cells in the G0 phase of the cell cycle require 3 signals for full activation. The first signal is delivered by the binding of presented alloantigen to the T-cell antigen receptor. The second signal is generated by the interaction of CD28–B7 and CD40–CD40 ligand and other less well understood molecules that couple antigen-presenting elements to T cells. These two signals cause T cells to produce cytokines that act in paracrine and autocrine fashion to bind specific high-affinity receptors and drive cells to enter the G1 phase, the third signal, to prepare for

the S phase, leading to nucleic acid synthesis, cell division, and clonal expansion (Abraham and Wiederrecht 1996; Slavik et al 2004). In T cells, mTOR participates in protein translation following sustained upregulation of cytokine transcription by CD28 engagement. Coengagement of the T-cell receptor by major histocompatible complex peptides and CD28 by B7 ligands provides the requisite combination to promote survival and initiate cytokine production (Abe and Thomson 2003). Subsequently, these cytokines, particularly interleukin-2, provide a strong proliferation and survival signal that enables T-cell clonal expansion. Thus, in T cells and also in B cells, stimulation via the antigen receptor and other mitogenic sites causes phosphorylation and activation of S6K1 in a PI3K dependent manner (Abe and Thomson 2003; Deane and Fruman 2004).

Biochemistry of mTOR inhibitors

SRL inhibits the catalytic activity of mTOR by virtue of its high-affinity binding to the isomerase FKBP12-rapamycin, thereby preventing the association of RAPTOR with mTOR. This association reduces mTOR-catalyzed phosphorylation of RAPTOR-dependent S6K1 and 4EBP1 but not RAPTOR-independent substrates. Further, SRL blockade of mTOR accelerates the turnover of cyclin D1, leading to a deficiency of active CDK4–cyclin D1 complexes, all of which may cause G1-phase arrest (Stromberg et al 2004). CD28-mediated degradation of I B and translocation of c-Rel to the nucleus is also inhibited by SRL (Lai and Tan 1994).

Increased nephrotoxicity after simultaneous use of SRL and CNIs

The different renal adverse events observed in clinical practice and suggested to be related to SRL therapy include potentiation of CNI nephrotoxicity, prolongation of delayed graft function when used in de novo patients, proteinuria, and acute nephrotoxicity.

SRL enhances CNIs nephrotoxicity

Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMA) registration trials have shown that in combination with CsA, SRL significantly lowered acute rejection incidence when compared with azathioprine (AZA), MMF, or placebo. However, impaired renal function was demonstrated in the CsA–SRL–steroid group compared with CsA–AZA–steroid, CsA–placebo–steroid, or CsA–MMF–steroid groups.

In a phase III trial designed to investigate the impact of the addition of SRL (2 or 5 mg daily), compared with AZA, to a CsA and prednisone regimen in renal transplant patients, Kahan (2000) demonstrated that combination therapy with CsA and SRL resulted in significantly higher serum creatinine concentrations and lower creatinine clearance than in patients treated with CsA and AZA after 6 and 12 months. Whereas acute rejection rates were lower in the two SRL groups than in the AZA group, calculated creatinine clearances were 62.29 ($p < 0.01$ vs AZA), 59.15 ($p < 0.001$ vs AZA), and 68.78 mL/min at 6 months and 61.95 ($p < 0.05$ vs AZA), 55.48 ($p < 0.001$ vs AZA), and 67.51 mL/min at 1 year in the 2 mg/day SRL, 5 mg/day SRL and AZA groups respectively. In a similar double-blind, multicenter, placebo-controlled study, MacDonald (2001) investigated the ability of 2 different doses of SRL (2 mg and 5 mg) to prevent acute rejection when added to a regimen of CsA and corticosteroids after renal transplantation. Significant differences among the treatment groups in mean creatinine clearance values were observed at month 3 ($p = 0.006$) and month 6 ($p = 0.041$). At month 3, the 5 mg/day SRL group had lower mean glomerular filtration rate values (54.98 mL/min) than either the 2 mg/day SRL group (59.07 mL/min, $p = 0.05$) or the placebo group (61.12 mL/min, $p < 0.01$). A similar difference was observed between the 5 mg/day SRL group and the placebo group at month 6 (56.42 vs 62.58 mL/min, $p = 0.05$) (MacDonald 2001). These results are now confirmed after 24 months of follow-up (Kahan 2003). These studies resulted in the EMEA recommendation to withdraw CsA from SRL at 3 months. In a retrospective study including 23 016 renal transplant patients (between January 1988 to July 2003), Meier-Kriesche et al (2004) have compared the outcomes of patients who were initially treated with CsA+MMF with those initially treated with CsA+SRL. Multivariate analysis showed that CsA+SRL was associated with a significantly increased risk for graft loss, death-censored graft loss, and decline in renal function (HR=1.22, $p = 0.002$; HR=1.22, $p = 0.018$; HR=1.25, $p < 0.001$). Finally, in nonrenal transplantation, a prospective study has compared everolimus, another mTOR inhibitor, with AZA in 634 heart transplant patients treated with CsA and steroids (Eisen et al 2003). Patients receiving the association CsA and everolimus experienced a significant increase in serum creatinine levels, beginning as early of the first month post-transplantation, whereas CsA blood concentrations were similar in the two groups.

Experimental data and possible mechanisms

There is no evidence in the literature that therapeutic doses of SRL alone may cause significant nephrotoxicity in most animal studies. Nevertheless, at high concentrations (3 mg/kg/day) in rats, SRL caused renal dysfunction, tubular collapse, vacuolization, nephrocalcinosis, and magnesium wasting (Andoh, Burdmann, et al 1996). Several animal or in vitro models have shown that SRL, when used in specific situations, such as in association with CNI or after ischemic damage, induces functional or morphological alterations of the kidneys.

Nephrotoxic interaction between SRL and CNIs

Andoh et al studied the effects of combining CsA and SRL on renal structure and function in a rat model of chronic CsA nephropathy. Associated with placebo, or various CsA dose, SRL at a subtherapeutic dose of 0.1 mg/kg worsened glucose metabolism and potentiated chronic nephrotoxicity induced by CsA at 8 mg/kg in terms of both renal function and structural injury (Andoh, Lindsley, et al 1996). In another study, Sprague-Dawley rats were treated with either CsA (15 mg/kg/day), TAC (3 mg/kg/day), or SRL (0.4 mg/kg/day) monotherapy or in different combinations over a 2-week period in order to examine the effects on glomerular function, as well as the possible morphological changes in the kidneys (Nielsen et al 2003). The greatest glomerular filtration rate (GFR) deteriorations were observed in the groups where CsA was associated with either SRL or TAC. Interestingly, CsA whole-blood concentrations did not differ significantly between the two groups treated or not with SRL. The result indicates that SRL has a pronounced synergistic nephrotoxic effect on the GFR and on a number of morphological fibrotic changes when combined with CsA. These results were confirmed more recently in a similar animal model of chronic CsA nephrotoxicity (Shihab et al 2004a). When combined with low-dose CsA (half the dose that produces nephrotoxicity in their model), SRL resulted in changes in renal structure and function similar to the changes observed with full-dose CsA.

Pharmacokinetic interaction

The major explanation for the enhancement of CsA nephrotoxicity by SRL is a pharmacokinetic interaction. SRL is thought to increase the exposure to CsA. This hypothesis was supported by the observation that significantly lower CsA doses were required to achieve whole-blood targeted concentrations among SRL-treated patients than AZA-treated

patients in the US pivotal trial. The hypothesis of a local interaction within the kidney between SRL and CsA was suggested. Napoli et al (1997) demonstrated in a rat model that a pharmacokinetic interaction between SRL and CsA increased the whole-blood and, especially, the renal-tissue concentrations of each agent. More recently, a second study by Podder et al (2001) confirmed these results. A salt-depleted rat model was used to dissect the pharmacokinetic component of the toxicity produced by CsA–SRL combination. At each CsA dose level, concomitant administration of SRL produced a significant pharmacokinetic interaction, increasing further the CsA concentration by approximately 2-fold above that found in hosts treated with CsA alone. They also found that at each CsA dose level, intra-renal CsA concentration increased when animals were also treated with SRL. It may be hypothesized that a pharmacokinetic interaction between SRL and CsA may alter CsA exposure, more importantly within the kidney cells than in the blood.

The mechanisms and the targets of this interaction, which may ultimately affect SRL and/or CsA drug exposure, are largely unknown. However, the cytochromes P450 3A (CYP3As) isozymes and the P-glycoprotein (P-gp) are candidates, since CsA and SRL share common metabolic and transport pathways by these proteins. Recent data suggest that P-gp may be especially involved in the SRL–CsA interaction. SRL and CsA are both substrates for P-gp, the product of the multi-drug resistance (*MDR1* also called *ABCB1*) gene. Since P-gp acts as a transmembrane efflux pump involved in energy-dependent export of xenobiotics from inside the cells, it is thought that P-gp plays a central role in cellular detoxication. Using an in vitro model of normal human renal epithelial cells (HRECs) in primary culture, we have shown that CsA exhibited a cytotoxic effect on HRECs in a concentration-dependent manner and that P-gp inhibition led to a significant increase of CsA cellular concentration and to an increase of its cytotoxicity (unpublished data).

Another hypothesis implicates TGF- β 1 which is a well known factor in the pathophysiology of chronic CsA nephrotoxicity. There is evidence to suggest that SRL can independently increase TGF- β 1 expression in renal proximal tubular epithelial cells (Swinford et al 2002). Moreover, it has been shown that patients treated with SRL have an enhanced production of TGF- β 1 and type III collagen early after kidney transplantation (Oliveira et al 2002; Saunders et al 2003). In a salt-depleted rat model of CsA chronic nephrotoxicity, Shihab et al (2004b) used low doses of CsA to examine the impact of SRL co-administration on kidney structure and function and on the expression of TGF- β 1, PAI-

1, and ECM proteins in the kidney. They demonstrated that SRL alone significantly increased TGF- β 1 production and potentiated TGF- β 1 production by 3-fold in combination with low-dose CsA. Using 2 doses of CsA in combination with SRL, Shiab et al showed that SRL in combination with low doses of CsA was associated with a significant augmentation of TGF- β 1 production and that the net result was a TGF- β 1 expression that was similar to what was observed with full doses of CsA.

Finally, the role of apoptosis has been investigated. Using a mouse model, Lieberthal et al (2001) showed that SRL increases the apoptosis of renal tubular epithelial cells. The induction of pro-apoptotic genes may impair the ability of the kidney to remodel effectively in response to injury. As a result, SRL, when associated with CsA, may inhibit tissue repair not only by this proapoptotic effect but also by its antiproliferative effect.

Short and long-term effects of early CsA withdrawal in presence of SRL

Feasibility of CsA withdrawal with SRL in stable patients

A systematic review of CNIs has been published recently (Mulay et al 2005). We will focus on the 5 studies which used CsA, one of them having been published several times according to the length of the follow-up (Johnson et al 2001; Gonwa et al 2002; Baboolal 2003; Stallone et al 2003; Jardine 2004). Most trials involved low-risk patients with a large proportion of Caucasians, very few diabetics, and the majority of patients undergoing primary transplantation. Only 1 study was performed in the US, with the remainder from Canada, Europe, and Australia. Delayed graft function and highly sensitized patients were sometimes used as noninclusion criteria of some patients. Most trials also required that the patient had adequate and stable renal function with no significant acute rejection in the preceding month. The characteristics of the studies are summarized in Table 1.

The first study, the so-called Tricontinental study, came from a group of 57 renal transplantation centers in Europe, Canada, and Australia (Johnson et al 2001; Oberbauer, Kreis, et al 2003; Kreis et al 2004; Oberbauer et al 2005). The patients enrolled in this prospective, randomized, open-label trial received SRL, CsA, and steroids up to the time of randomization at month 3 after transplantation. In this study, to be randomized, patients had to have experienced no

Table 1 Demographic characteristics of CsA withdrawal studies

Characteristics	Johnson et al (2001)	Gonwa et al (2002)	Baboolal (2003)	Stallone et al (2003)	Jardine (2004)
Year	2001	2002	2003	2003	2004
Sample size	215/215	100/97	42/45	20/20	10/105
Primary transplant (%)	90/92	100/100	NR	NR	NR
Caucasian (%)	94/95	80/73	93/98	100/100	NR
Diabetes (%)	8/7	8/9	5/0	NR	NR
Deceased donor (%)	88/88	100/100	85/91	100/100	NR
Mean donor age (years)	42/44	NR	NR	40/46	NR
Follow-up	12 ^a	12	6	12	12
Criteria for CsA withdrawal	Cr < 400 Absence of severe rejection (4 weeks)	Stable function Absence of rejection in 3 weeks	Cr < 400 Absence of severe rejection	NR	NR
Exclusion criteria	Planned antibody induction	DGF > 7 days Repeat transplant or live donors	Planned antibody induction	NR	NR
Time of withdrawal post-transplantation (months)	3	2	3	3	3
Complete withdrawal (%)	93	78	81	NR	NR

Adapted from Mulay et al (2005).

^aPublished follow-up at 48 months (Oberbauer et al 2005).**Abbreviations:** Cr, creatinine; DGF, delayed graft function; NR, not reported.

grade 3 acute or vascular rejection during the 4 weeks before randomization, and to have a serum creatinine level that never exceeded 400 mmol/L. In all, 525 patients were enrolled but only 430 (82%) met the criteria for randomization at 3 months. In another recent controlled, randomized study (Gonwa et al 2002), 246 renal transplant recipients were enrolled, and 197 of them were randomly assigned to receive either conventional-dose CsA combined with 2 mg/day SRL (n=97) or reduced-dose CsA with concentration-controlled SRL, at doses adjusted to maintain trough concentration levels of 10–20 ng/mL (n=100). Among them 49 were not randomized because of the absence of renal function recovery by 48 hours post surgery. Patients in the CsA reduced-dose group were eligible for CsA withdrawal if their renal function was stable at the end of month 2 after transplantation, if they had not been treated for acute rejection in the preceding 3 weeks, and if their SRL trough concentrations were 10–20 ng/mL. A total of 82 patients were eligible for CsA elimination. This maneuver was successfully completed in 76 patients among 246 initial patients. Overall, CsA withdrawal was completed in 78–93% of randomized patients (Table 1).

Regarding the regimen used to withdraw CsA, in the Tricontinental study, patients were randomly assigned, either to remain on triple-drug therapy including SRL (trough levels >5 ng/mL) and CsA (trough levels: 75–200 ng/mL), or to

CsA withdrawal. Those in the withdrawal group had their daily SRL dose adjusted to maintain trough concentrations of 20–30 ng/mL, and the CsA dose was gradually decreased and then eliminated over 4–6 weeks. The median CsA elimination period was 41 days (Johnson et al 2001). In the bicontinental study, CsA was withdrawn completely during the third month after transplantation (25% dose reduction per week for 4 weeks). At month 12, the mean daily dose of SRL in the latter group was 6.45 ± 0.43 (Gonwa et al 2002).

Graft and patient survival

CsA withdrawal had no effect on patient death. In the Tricontinental study, patient survival was similar between groups. After 48 months of follow-up, this survival was 95.3% in the CsA elimination group vs 92.1% ($p=0.232$) (Oberbauer et al 2005). Interestingly, during the same follow-up, the incidence of malignancies was lower in the elimination group (7.4% vs 12.1%, $p=0.14$).

Regarding graft survival, all the studies report in the short term the absence of difference between patients in the withdrawal group compared with patients still receiving CsA. The most striking data come from the long-term follow-up of the Tricontinental study (Oberbauer et al 2005). The results after 48 months have been reported recently. In this study, the primary endpoint was noninferiority of graft survival in

the CsA elimination group. Indeed the graft survival was not inferior. Because of the figures observed on the 95% confidence interval (difference -11.2%, CI=-18.5, -3.8), it is possible to conclude that graft survival was superior in the elimination group. This survival was better both when death with a functioning graft was included (91.5% vs 84.2%, $p=0.024$) or when it was censored (96.1% vs 90.6%, $p=0.026$). However, the major drawback of this study is that the association of SRL and CsA as the control group is not optimal and that it is difficult to draw any clear conclusion in the long term, since most patients in the control group were not receiving the protocol regimen during all the study period.

Acute rejection

Regarding the risk of acute rejection after CsA withdrawal in the context of SRL treatment, there was a constant trend toward a higher incidence following CsA withdrawal (Mulay et al 2005). The pooled estimate including the trial with TAC showed a significant increase in the risk of acute rejection. The absolute risk difference was 6.0%. In the Tricontinental study, withdrawal was associated with a small but significant increase in the incidence of acute rejection. After randomization at 3 months post-transplantation, 9.8% of the patients in the withdrawal group experienced a biopsy-proven acute rejection episode versus 4.2% of those who continued on CsA ($p=0.035$) (Johnson et al 2001). This increase seems to appear during the early period after CsA withdrawal, especially in patients with low SRL trough concentrations. Indeed, very few rejections occurred after the first year. After 48 months of follow-up, the incidence of acute rejection after randomization was 10.2% in the elimination group vs 6.5% in the control group ($p=0.223$) (Oberbauer et al 2005).

Renal function

Renal function was assessed in all studies using serum creatinine and calculated creatinine clearance. In all studies, CsA withdrawal was associated with a significant increase in the creatinine clearance (weighted mean difference of 7.78 mL/min, $p<0.001$) and a significant lower serum creatinine (weighted mean difference -0.19 mg/dL, $p<0.001$) (Mulay et al 2005).

In the Tricontinental study, this difference was observed as early as one month after randomization. The GFR progressively improved during month 12 in the CsA withdrawal group. Although some patients who remained on CsA also improved, a larger proportion improved in the CsA

withdrawal group (72.2% vs 40.4, respectively, $p<0.001$). When patients were grouped according to baseline serum creatinine (ie, last value measured before randomization), all groups potentially benefit from CsA elimination at 3 months, irrespective of their serum creatinine level at this time (Mota et al 2004). These results have been recently confirmed (Russ et al 2005). The benefit was more marked in patients with a baseline calculated GFR of less than 45 mL/min. On the other hand, more than 25% of the patients in the CsA withdrawal group experienced no improvement in renal function. After 2 years of follow-up, serum creatinine was still significantly lower in the CsA-withdrawal group. One important finding in this study is the long-term follow-up (up to 48 months) which has been recently reported (Oberbauer et al 2005). This improvement is still observed even after a long follow-up. Using the calculated GFR, the slope was significantly negative in the intent-to-treat (ITT) analysis for the CsA maintenance group (-2.71 mL/min/year, $p<0.001$) and was positive in the CsA elimination group (0.53 mL/min/year, $p=0.151$). The difference between slopes was statistically significant (-3.24 mL/min/year, $p<0.001$) in favour of the CsA elimination group.

In another study, at 12 months, renal function assessed by serum creatinine and calculated GFR values using Nankivell's formula had improved significantly in the CsA withdrawal group. This improvement was greater in the patients who had remained rejection-free, but was still significant when those who experienced a rejection were included in the analysis. Unfortunately, specific information on renal function in the "rejection"-group is not available (Gonwa et al 2002).

Renal pathology

Various studies including the one from Nankivell have demonstrated the value of protocol biopsies to determine the true incidence of chronic allograft nephropathy, to detect early signs of such chronic damage, and to differentiate between chronic rejection and CNIs nephrotoxicity (Nankivell et al 2003). Pathologic allograft lesions have been evaluated in 2 different studies.

Ruiz et al (2004) have analyzed pretransplant and 1-year renal allograft biopsies from 64 patients enrolled in the Tricontinental study. A higher proportion of patients showed progression of chronic interstitial (ci), tubular (ct), and vascular (cv) lesions in patients with CsA continuation (70% vs 40.9%, 70% vs 47.8%, and 29.4% vs 25%, respectively). However, progression of chronic glomerular lesions and arterial hyalinosis was not different between the groups. When the Banff chronicity index was used, progression was

present in 76.5% of patients in CsA continuation vs 31.3% in the CsA elimination group ($p=0.01$) (Racusen et al 1999). Similar results were found when using another classification of chronic lesion (CADI score) (Isoniemi et al 1994), with more frequent progression of both interstitial fibrosis (F) and tubular atrophy (T). Finally, using the Banff classification, the diagnosis of chronic allograft nephropathy (CAN) (any grade) at 1 year was higher, but not statistically in the group of patients remaining on CsA (70.8% vs 59.3%, $p=0.25$).

The results from the whole Tricontinental population were reported elsewhere (Mota et al 2004). Two pathologists blindly evaluated 484 biopsies performed at engraftment and at 12 and 36 months after transplantation. The results are shown in Table 2. The global analysis of all patients with biopsy readings shows that the mean CADI score at 36 months was significantly lower in the CsA elimination group (4.70 vs 3.20, $p=0.003$). This was also true for the mean tubular atrophy score (0.77 vs 0.32, $p<0.001$). All 6 components of the CADI score were numerically lower in the CsA elimination group. Furthermore, inflammation and tubular atrophy scores decrease significantly between 12 and 36 months.

Stallone et al (2003) report the results of routine biopsies in 40 patients at 12 months after transplantation. CAN was diagnosed in 55% of all patients, of whom 64% were in the CsA continuation group and 36% in the CsA elimination group. These findings were even more notable when moderate

to severe lesions were analyzed (respectively 90% vs 32%) and for vascular lesions (90% vs 38%).

Risk of malignancy

As already stated, SRL, in contrast to CsA or TAC, has been shown to inhibit rather than promote cancer in experimental models. SRL alone, or combined with CsA, prevented metastatic tumor progression and prolonged survival of mice inoculated with either mouse renal cancer or human T24 human bladder cancer (Luan et al 2002). Additionally, SRL alone or plus CsA reduced the number of human renal cell carcinoma metastases in SCID mice, whereas CsA alone increased the number of metastases (Luan et al 2003). In humans, 2 recent studies have reported very important data on this matter. First, Kauffman et al (2005) have analyzed the post-transplant malignancies reported in the Organ Procurement and Transplantation Network (OPTN) among 33249 renal transplant recipients. In a multivariate analysis, the relative risk associated with mTOR inhibitors immunosuppression for any *de novo* cancer was 0.39 ($p=0.0002$) or for *de novo* solid cancer was 0.44 ($p=0.0092$). Finally, Campistol et al (2006) reported the risk of developing cancer in the Rapamune Maintenance Regimen trial. At 5 years, the median time of a first skin carcinoma was delayed (491 vs 1126 days, $p=0.007$) and the risk of an event was significantly lower (relative risk 0.346, $p<0.001$). The risk of both basal and squamous cell carcinomas and nonskin cancer were reduced (9.6% vs 4.0%, $p=0.032$).

Table 2 Chronic lesions assessed by the Chronic Allograft Damage Index (CADI) score

	Baseline		12 mo		36 mo	
	CsA continuation	CsA withdrawal	CsA continuation	CsA withdrawal	CsA continuation	CsA withdrawal
All patients						
Mean $\pm$ SD	1.27 $\pm$ 1.37	1.23 $\pm$ 1.36	3.56 $\pm$ 1.92	3.45 $\pm$ 1.79	4.39 $\pm$ 1.84	3.59 $\pm$ 1.98
N	97	82	95	86	47	48
Bx BL–12 mo						
Mean $\pm$ SD	1.27 $\pm$ 1.38	1.15 $\pm$ 1.35	3.64 $\pm$ 1.82	3.63 $\pm$ 1.82		
N	79	68	79	68		
Bx BL–36 mo						
Mean $\pm$ SD	1.17 $\pm$ 1.51	1.02			4.50 $\pm$ 1.94 ^a	3.32 $\pm$ 1.24 ^a
N	40	37			40	37
Bx 12–36 mo						
Mean $\pm$ SD			3.62 $\pm$ 2.04	3.50 $\pm$ 1.65	4.55 $\pm$ 1.92	3.63 $\pm$ 2.02
N			35	42	35	42
Bx BL 12 and 36 mo						
Mean $\pm$ SD	1.26 $\pm$ 1.60	0.99 $\pm$ 0.92	3.74 $\pm$ 1.84	3.53 $\pm$ 1.66	4.70 $\pm$ 1.86 ^a	3.20 $\pm$ 1.85 ^a
N	31	32	31	32	31	32

Adapted from Mota et al (2004).

^a $p<0.005$ ANCOVA

Abbreviations: BL, baseline; Bx, biopsy; mo, months.

Adverse events profile

Hypertension

Some of the adverse events were less frequent in the CsA withdrawal group. CsA withdrawal was associated in all studies with a significant reduction in the risk of hypertension (relative risk 0.56, $p < 0.001$) and with a significant decrease of both systolic and diastolic blood pressure (Mulay et al 2005).

This was demonstrated specifically in the Tricontinental study (Johnson et al 2001). Hypertension was reported in 7.0% of the patients in the withdrawal group vs 16.3% in the triple therapy group. In addition, both diastolic and systolic blood pressure were significantly lower in the CsA withdrawal group (-3 mmHg and -6 mmHg, respectively) despite significantly less frequent use of antihypertensive medication in this group. At 2 years, systolic blood pressure remained significantly lower in the CsA withdrawal group (Oberbauer, Kreis, et al 2003). These differences were still present after 48 months of follow-up (Oberbauer et al 2005).

Lipids profile

Another question is the lipid profile of these patients. After randomization and high dose SRL, a transient increase in serum triglycerides averaging about 0.4 mmol/L was observed (Oberbauer et al 2005). However, there was no difference between the CsA withdrawal and continuation groups for mean concentrations of total cholesterol, calculated LDL cholesterol, HDL cholesterol, and triglycerides. Noteworthy, in the 2 groups, which both continued with SRL, 70% of patients were taking statins and 23%, fibrates. When all the studies are pooled together, CsA elimination was not associated with any significant effect on total cholesterol or triglyceride. However, there was a trend toward more statin use in the CsA elimination group.

Quality of life

Health-related quality of life was specifically compared in SRL-treated kidney transplant patients after CsA elimination and in patients who were still treated with CsA at 2 years in the Tricontinental study (Oberbauer, Hutchison, et al 2003). Of the 361 patients studied, those in the CsA-withdrawal group improved significantly more than others, according to the replies in the "Kidney Transplant Questionnaire" (KTQ) (Laupacis et al 1993; Jacobs et al 1998) fatigue and appearance domains. SF-36 vitality score was also better in the CsA-withdrawal group. The authors reported an improvement in the fatigue and appearance scores but no

differences in physical symptom scores. Other important findings are the consistent treatment differences on measures of fatigue and vitality. From month 3 after transplantation (the time of randomization) the patients in the CsA elimination group reported further improvements in fatigue whereas in the CsA continuation group, patients still reported increase in fatigue. These differences in the health related quality of life and the SF36 vitality scores are considered to be clinically meaningful.

Various side-effects

CsA withdrawal was associated with improved uric acid and magnesium levels. On the other hand, SRL has been associated with other adverse events. Indeed, in the CsA withdrawal group treated with high trough SRL levels, more thrombocytopenia, hypokaliemia, and abnormal liver function test were reported (Johnson et al 2001). In other studies, SRL-related side-effects, such as thrombocytopenia, abnormal liver function tests, hypokaliemia, ileus, abnormal wound healing, infectious pneumonia, and stomatitis were more frequent in patients randomized to CsA withdrawal. It is, however, difficult to assess precisely this risk since patients in both the treatment and in the control groups were receiving SRL.

Discussion and conclusions

Although CsA has been associated with a constant improvement in the results of renal transplantation, its use is also responsible for various short- and long-term toxicities, including nephrotoxicity, and for the worsening of cardiovascular risk factors. Another potential benefit might be a lower risk of long-term cancer, because low-dose CsA has already been linked to the development of carcinologic complications. Furthermore, concomitant use of CsA and SRL is associated with an increased risk of nephrotoxicity. On the other hand, SRL use may have a potential effect on tolerance induction, decreased fibrosis associated with chronic allograft dysfunction, and anti-tumoral effect. Therefore, it is important to assess the best way to use this immunosuppressive agent after organ transplantation. After CsA withdrawal, SRL seems to be associated with better prevention of acute rejection episodes than mycophenolate mofetil, since the increase of the incidence of such rejection when CsA was withdrawn 3 months after transplantation was not statistically significant. To identify the regimen that achieves the most beneficial metabolic outcomes, it is clear that prospective, randomized studies in which SRL-steroids are compared with MMF-steroids as maintenance therapy are

of major importance for renal transplantation. One important point, when considering the results from the Rapamune Maintenance Regimen trial (Oberbauer et al 2005), is that the SRL blood concentration target levels are higher than the one used nowadays. It is well known that since the introduction of CsA in the early 1980s, much has been learned about the use of immunosuppressive treatments. This knowledge often leads to a decrease in drug dosage and therefore an improvement of the possible side-effects.

The next problem is to define the population of patients that can benefit from CsA withdrawal. It is noteworthy that in the SRL trial, only 82% of the patients were eligible for CsA withdrawal 3 months after transplantation. In both the MMF and the SRL trials, CsA was effectively withdrawn from 93% and 97% of patients, respectively.

Finally, besides CsA withdrawal, there is increased interest in the design and implementation of avoidance protocols, ie, immunosuppressive regimens designed to avoid treatment with CsA or eliminate it completely. The major disadvantage of CsA sparing or withdrawal is that patients must be exposed to CsA in the first place. There is evidence that CsA-associated renal toxicity may occur early, and progress in the native kidney of pancreas and lung transplant recipients and in patients with uveitis, even after dose reductions. Another concern is the effect of calcineurin inhibitors on the induction of immune tolerance. CNIs have been shown to limit T-cell activation by blocking susceptibility to apoptosis and activation-induced cell death. Many preliminary studies of CsA avoidance have been reported. Flechner et al (2002) have administered a combination of anti-CD25 antibody, MMF, steroids, and SRL, a regimen that provided comparable 1-year patient and graft survival. In addition, at 1 year, the incidence of acute rejection episodes reaches an impressive 6.4% compared with 16.6% in the controls on a CsA-based regimen. Renal function, measured by the creatinine clearance, was also significantly better in the CsA-free group (81.1 mL/min vs 61.1 respectively) and did not tend to worsen during the first year after transplantation.

In any case, not all patients could benefit from these new approaches and new concepts, which more than ever illustrate the need for immunological and nonimmunological markers to enable immunosuppression to be tailored to the requirements of specific patient populations.

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