Immunosuppressive treatment and progression of histologic lesions in kidney allografts

JOSE M. MORALES
Renal Transplant Unit, Nephrology Department, Hospital 12 de Octubre, Madrid, Spain

Immunosuppressive treatment and progression of histologic lesions in kidney allografts. Renal transplantation is the best therapeutic option for patients with end-stage renal disease. Although short-term results are excellent, long-term graft survival has not improved substantially in recent times. Chronic allograft nephropathy (CAN) and death with a functioning graft are the most important causes of graft loss. Recent evidence shows that nephrotoxicity of calcineurin inhibitors contributes to CAN, and the introduction of non-nephrotoxic drugs such as mycophenolate mofetil (MMF) and mammalian target of rapamycin inhibitors may provide new immunosuppressive strategies to improve long-term results after renal transplantation. MMF decreases the risk of developing chronic allograft failure and is useful for treating established CAN, because it has a beneficial effect on allograft fibrosis. Treatment with sirolimus (SRL), a basic immunosuppressive drug given in association with MMF, may offer better renal function, decrease the prevalence of CAN, and downregulate expression of genes responsible for the progression of CAN than treatment with cyclosporine A (CsA). SRL also permits an early elimination of CsA from SRL-CsA-steroid regimens and shows better renal function and improved renal histology without risk of rejection. Notably, this approach improves graft survival at 4 years. Further multicenter studies are needed to determine whether both approaches produce similar results by comparing immunosuppression caused by SRL-based and tacrolimus (TAC)-based treatments. Because TAC is the most commonly used anticalcineurin drug, it is important to compare the effects of steroid-TAC-SRL treatment with and without elimination of TAC. Finally, although caution is needed, the use of non-nephrotoxic immunosuppressive treatment may change the natural history of CAN.

Renal transplantation is the therapy of choice for patients with end-stage renal disease [1]. The short-term outcome of renal transplantation has improved dramatically in the past 20 years. Introduction of cyclosporine (CsA) and newer, more efficient immunosuppressive drugs such as tacrolimus (TAC), mycophenolate mofetil (MMF), and rapamycin (sirolimus [SRL]) has reduced the prevalence of acute rejection and increased the rate of graft survival from 90% to 95% at 1 year in most units [2]. However, long-term graft survival has not improved substantially [3]. The most important causes of late allograft failure are chronic allograft nephropathy (CAN), death of the functioning graft, and de novo or recurrent disease [4]. Chronic CsA-TAC nephrotoxicity can contribute to CAN, which is the second most common cause of graft loss (after death with a functioning graft) [5]. CAN is characterized by progressive renal dysfunction accompanied by interstitial fibrosis, tubular atrophy, vascular occlusive changes, and glomerulosclerosis [6]. Despite the use of potent immunosuppressive combinations, biopsies show that the rate of CAN may be as high as 94% in the first year [7]. The long-term presence of anticalcineurin nephrotoxicity is almost universal [7]. The introduction of MMF and target of rapamycin (TOR) inhibitors, a new class of antirejection drugs, may provide new immunosuppressive strategies to prevent, decrease, or slow the progression of graft failure.

In this review, we discuss the most important recent data about the effects of non-nephrotoxic immunosuppression in the treatment of chronic renal dysfunction and focus particularly on the progression of histological lesions in kidney allografts.

CAN IN PATIENTS RECEIVING CsA OR TAC

Histopathologic results of 2-year protocol biopsies from 144 patients enrolled in the United States FK506 kidney study showed that acute rejection occurred in 9% of patients treated with TAC and 9.2% of patients treated with CsA. CAN was also similar with the two treatments (73.2% and 62%, respectively), showing that nephrotoxicity and acute rejection are the most significant predictors of CAN. The authors concluded that non-immunologic factors, such as CsA or TAC nephrotoxicity, may play an important role in CAN [8].

Taking protocol biopsies regularly after transplantation, Nankiwell et al evaluated the natural history of CAN in a prospective study of 120 recipients of pancreas–kidney transplantation [7]. They described two distinct

Key words: kidney transplantation, immunosuppressive treatment, progression of chronic kidney disease.

© 2005 by the International Society of Nephrology
phases of injury. The initial phase of early tubulointerstitial lesion arises because of ischemic injury, prior severe rejection, and a subclinical rejection showing that grade I CAN was present in 94.2% of patients. Notably, the combination TAC plus MMF eliminated subclinical rejection and reduced tubulointerstitial damage at 12 months [9]. Compared with TAC, CsA increases chronic interstitial fibrosis, and MMF is more protective than azathioprine (Aza), independent of their nephrotoxic and immunologic properties [10]. The later phase of CAN occurs after 1 year and is characterized by microvascular and glomerular injury. The presence of CsA and TAC nephrotoxicity (e.g., arterial hyalinosis, ischemic glomerulosclerosis, and tubulointerstitial damage) implicated in the late permanent injury approaches 100% at 10 years. Severe CAN is present in 58.4% of patients at 10 years [6]. This study clearly demonstrated the contribution of nephrotoxicity by anticalcineurin drugs in the development and progression of CAN.

Recent studies have compared the effect of anticalcineurin drugs on the development of CAN. Moreso et al [11] performed a case-control study to compare histological lesions in protocol biopsies from patients treated with TAC + MMF with those treated with CsA + MMF. Protocol biopsies were performed 4 to 6 months after transplantation. Although subclinical acute rejection occurred less frequently in TAC patients (14.2% vs. 34.7%), the total chronic score and the prevalence of CAN did not differ between groups (38.8% in CsA and 34.6% in TAC). Jurwicz [12], Murphy et al [13], and Baboolal et al [14] used randomized trials to compare treatment with CsA microemulsion and TAC, and reported that treatment with CsA was associated with an increased allograft fibrosis. Baboolal et al also suggest that the development of allograft fibrosis is mediated by an upregulation of profibrotic growth factors [14].

NON-NEPHROTOXIC IMMUNOSUPPRESSION IN THE TREATMENT AND PROGRESSION OF HISTOLOGICAL LESIONS IN KIDNEY ALLOGRAFTS

Azathioprine

A recent article described an extended follow-up of an open-label, randomized trial that examined conversion to Aza as early as 3 months after transplantation. One hundred twenty-eight patients were enrolled in a single center in 1983 and randomly assigned to continue CsA therapy (N = 68) or were converted to Aza (N = 60). At 15 years, graft survival tended to be lower in the CsA group (64% vs. 76.5%). The relative risk (RR) of CAN was significantly higher in patients receiving CsA (RR, 4.3: 95% confidence interval, 1.4–12.9), and the incidence of CAN was lower in patients with Aza (28% vs. 62%). These findings suggest a role of CsA nephrotoxicity in the development of CAN in a study started more than 20 years ago. The authors recommended the same immunosuppressive strategy using MMF instead of Aza [15].

MYCOPHENOLATE MOFETIL

MMF, the morpholinoethyl ester of mycophenolic acid, inhibits purine metabolism by inhibiting the enzyme inosine monophosphate dehydrogenase. MMF inhibits the proliferation of T and B cells and the synthesis of antibodies by B cells [16]. MMF administration in association with steroids, CsA, or TAC reduces acute rejection [17, 18]. Notably, MMF is not nephrotoxic and does not have any hypertensive effect [17, 18]. The Federal Drug Administration approved its use in renal transplantation in 1995. Currently, nearly 80% of patients receive MMF in various immunosuppressive combinations after renal transplantation in the United States [19].

Ojo et al analyzed 66,774 renal transplant recipients from the US renal transplant registry and showed that MMF decreased the RR of developing CAN by 27% (RR, 0.73) [20]. Notably, this effect was independent of acute rejection, suggesting that, through its antiproliferative effects, MMF could be useful in protecting against vascular damage. In animal models of chronic allograft arteriolopathy, MMF decreases vascular intimal hyperplasia and the development of lesions similar to that seen in patients with CAN. Censored graft survival with MMF was significantly better than that with Aza at 4 years (85.6% vs. 81.9%). The most relevant conclusion of this important study is that MMF decreases the risk of developing chronic allograft failure. Reporting for the Tricontinental Mycophenolate Mofetil Renal Transplant Study Group, Mathew compared 3-year graft survival in two groups of renal transplant patients receiving MMF (1 g or 2 g) or Aza in association with CsA and steroids, and found significantly higher graft survival in the MMF group [21].

The Spanish Cooperative Study Group of Chronic Allograft Nephropathy prospectively explored the potential therapeutic role of MMF in patients diagnosed with CAN [22]. One hundred-twenty patients with biopsy-proven CAN on double or triple therapy with CsA were treated with MMF. Before the introduction of MMF, patients showed progressive deterioration of renal function (glomerular filtration rate [GFR] 54.8 mL/min vs. 39.7 mL/min). After MMF started, renal function remained stable (GFR 39.7 mL/min vs. 41.3 mL/min) with a significant change in the slope of the GFR from −0.0144 to +0.00045. This positive reduction in the slope of the GFR was also seen in patients in whom CsA blood levels remained unchanged during follow-up. More recently, Dudley et al demonstrated that in patients with progressive deterioration renal function (“creeping creatinine”) secondary to CAN, addition of MMF followed by
withdrawal of CsA results in a significant improvement of renal function without the risk of acute rejection [23]. These and other center-based studies [24] have demonstrated that MMF is clinically useful for medium-term treatment of CAN.

Little is known about whether MMF can also improve histological lesions associated with CAN. However, two MMF trials (American and tri-continental trials) analyzed sequential protocol biopsies from several centers. The chronic allograft damage index (CADI) was evaluated in biopsies taken at baseline, 12 months, and 36 months [25]. The CADI increased from 1.3 at baseline to 3.3 at 1 year and 4.1 at 3 years. Unfortunately, the effect of immunosuppression on the progression of histological lesions was not compared between treatment with MMF and Aza/placebo. In the study by Nankiwell that analyzed immunosuppression and interstitial fibrosis [10], TAC and MMF were more potent than other conditions in reducing early chronic interstitial fibrosis. MMF was more protective than Aza in preventing a worsening of the chronic interstitial fibrosis score. The beneficial effect of MMF on allograft fibrosis might result from the reduction in tubular destruction by immunologic mechanisms or by a positive effect on fibrogenesis.

**SIROLIMUS**

SRL, a mammalian TOR inhibitor, suppresses graft rejection by interfering with cytoplasmic, biochemical cascades that transduce signals from the cell membrane to the nucleus. In animal models of organ transplantation, SRL exhibits potent anti-rejection activity and the ability to prolong allograft survival [26, 27]. Because SRL does not inhibit calcineurin, SRL should lack the nephrotoxic profile of calcineurin inhibitors. Preclinical studies have also indicated that SRL has no deleterious effects on renal function [27, 28], and that the combination of SRL and MMF is particularly promising, because it suppresses transforming growth factor-beta and had no effect on glomerular filtration. SRL also inhibits growth factor-stimulated smooth muscle cell proliferation and migration [26] and reduces neointimal hyperplasia by its inhibitory effect on arterial smooth muscle [29]. Additionally, despite elevating serum lipid concentrations, SRL inhibits atherosclerosis in experimental mouse models of atherogenesis [30].

Clinical studies have confirmed the success of SRL as a prophylaxis for acute renal transplant rejection when used concomitantly with existing therapies. Early studies included a phase 2 trial [31] and two phase 3 trials that compared an SRL-CsA combination with Aza [32] or placebo [33] and demonstrated reduced acute rejection rates. However, the phase 3 trials demonstrated that SRL exacerbated the nephrotoxic effects of CsA.

Two early phase 2 studies directly compared the effects of SRL and CsA in Europe [34–36]. Patients received either CsA or SRL in combination therapy with either corticosteroids and Aza or corticosteroids and MMF. SRL did not exhibit the nephrotoxic properties of CsA, and renal function was enhanced after CsA-free, SRL-based therapy. The 2-year data continued to show more favorable outcomes for risk factors such as hypertension and renal function in response to SRL therapy than with CsA therapy [36]. Although the benefits of decreased hypertension and improved renal function were evident with SRL therapy, the poor lipid profiles were a side effect that had to be managed with increased use of lipid-lowering therapy.

Flechner et al directly compared SRL and CsA and reported improved outcomes by adding an induction agent and reducing the doses of SRL to minimize side effects [37]. Their randomized phase 2 trial used basiliximab as an antilymphocyte-induction agent, and SRL or CsA in addition to MMF and steroids in primary renal allograft recipients. The results showed comparable outcomes for patient survival, graft survival, and biopsy-confirmed acute rejection and significantly better renal function in SRL-treated patients. Although higher than at baseline, fasting lipid concentrations were similar in both the SRL and CsA groups at all times studied in the 1-year period. Subsequent analysis at 2 years demonstrated that patients on SRL exhibited better renal function, lower prevalence of CAN (normal biopsies, 66% vs. 20.8%), and downregulated expression of genes linked to the pathways of tissue injury–remodeling and immune or inflammatory responses responsible for the progression of CAN [38]. The authors concluded that this treatment may provide an alternative to the natural history of CAN with improved graft survival.

Because SRL exacerbated CsA nephrotoxicity in phase 3 trials, a new phase 3 trial of early CsA withdrawal, the Rapamune Maintenance Regimen (RMR) study, was conducted [39]. The regimen comprised the administration of SRL (2 mg), CsA, and steroids after transplantation, followed by randomization to CsA withdrawal 3 months with SRL through concentrations targeted at 20 ng/mL to 30 ng/mL (immunoassay) until month 12, and 15 ng/mL to 25 ng/mL thereafter. The results of this study confirmed that CsA withdrawal provides the benefits of improved renal function and lower blood pressure. At 12 months [40], both groups showed similar patient and graft survival rates. Acute rejection rates were 4.2% and 9.8% for SRL-CsA-steroid (ST) and SRL-ST, respectively ($P = 0.035$).

Ruiz et al reported histological data at 1 year on 64 patients from Spain and Portugal enrolled in this trial, and showed that the percentage of patients in whom chronic lesions progressed was lower in the CsA elimination group than in the SRL group [40]. Significant
Table 1. Histological changes at 3 years in patients receiving sirolimus-based therapy after early CsA elimination

<table>
<thead>
<tr>
<th>Score (mean ± SD)</th>
<th>Engraftment 12 months</th>
<th>36 months</th>
<th>SRL+SRL</th>
<th>SRL+SRL</th>
<th>SRL+SRL</th>
<th>SRL+SRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADI</td>
<td>1.26 ± 1.60</td>
<td>1.27 ± 1.50</td>
<td>1.09 ± 1.50</td>
<td>1.59 ± 1.60</td>
<td>1.70 ± 1.60</td>
<td>1.80 ± 1.60</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.11 ± 0.35</td>
<td>0.10 ± 0.27</td>
<td>0.11 ± 0.27</td>
<td>0.10 ± 0.27</td>
<td>0.10 ± 0.27</td>
<td>0.10 ± 0.27</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>0.08 ± 0.27</td>
<td>0.08 ± 0.27</td>
<td>0.08 ± 0.27</td>
<td>0.08 ± 0.27</td>
<td>0.08 ± 0.27</td>
<td>0.08 ± 0.27</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.24</td>
<td>0.10 ± 0.24</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>0.33 ± 0.41</td>
<td>0.33 ± 0.41</td>
<td>0.33 ± 0.41</td>
<td>0.33 ± 0.41</td>
<td>0.33 ± 0.41</td>
<td>0.33 ± 0.41</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>0.27 ± 0.34</td>
<td>0.27 ± 0.34</td>
<td>0.27 ± 0.34</td>
<td>0.27 ± 0.34</td>
<td>0.27 ± 0.34</td>
<td>0.27 ± 0.34</td>
</tr>
<tr>
<td>Intimal proliferation</td>
<td>0.37 ± 0.52</td>
<td>0.37 ± 0.52</td>
<td>0.37 ± 0.52</td>
<td>0.37 ± 0.52</td>
<td>0.37 ± 0.52</td>
<td>0.37 ± 0.52</td>
</tr>
</tbody>
</table>

Abbreviations are: CsA, cyclosporine A; SD, standard deviation; SRL, sirolimus; CADI, chronic allograft damage index. Data from Mota, *Am J Transplant* 4:953–961, 2004.

Analysis of the RMR data at 36 months [43] showed a continuing beneficial trend in graft survival in the SRL-ST group. Acute rejection rates from randomization to month 36 did not differ significantly between the SRL-ST and SRL-CsA-ST groups, although serum creatinine concentration was significantly lower, and overall renal function improved or remained stable in the SRL-ST group. Blood lipid measures and cumulative use of statins were similar between the groups.

Histological data at 3 years was available from 484 biopsies performed at engraftment at 12 and 36 months [44]. Two pathologists blindly evaluated all biopsies to obtain the CADI scores. At 36 months, among 63 patients with serial biopsies, the SRL-ST group had a significantly lower main CADI score (4.7 vs. 3.2, *P* = 0.003) and mean tubular atrophy score (0.77 vs. 0.32, *P* < 0.001). All six components of the CADI score (inflammation, tubular atrophy, intimal proliferation, glomerular sclerosis, mesangial matrix, and fibrosis) were numerically lower in the SRL-ST group. The inflammation and tubular atrophy scores decreased significantly between 12 and 36 months in the SRL-ST group (0.82 to 0.50, and 0.56 to 0.32, respectively) (Table 1). The calculated GFR was significantly higher in SRL-ST patients (68.2 mL/min vs. 54.8 mL/min). This study clearly demonstrated that withdrawing CsA from the SRL-CsA-ST regimen results in improved renal function and renal histology.

At 48 months in the RMR study [45], graft survival censored for loss to follow-up was significantly better after CsA withdrawal, either when including death with a functioning graft as an event (84.1% vs. 91.5%, *P* = 0.024, Fig. 1) or when excluding it (90.5% vs. 96.1%, *P* = 0.025). The incidence of death (7.9% vs. 4.7%) or biopsy-proven acute rejection after randomization (7.0% vs. 10.2%) did not differ significantly between the SRL-CsA-ST and SRL-ST groups. As shown in Figure 2, the calculated GFR, which included values from discontinued patients and set GFR to 0 for functional graft loss, was significantly higher (43.8 mL/min vs. 38.3 mL/min, *P* < 0.001) in the CsA withdrawal group. Mean arterial blood pressure (101.0 mm Hg vs. 97.6 mm Hg, *P* = 0.046) was lower in the CsA withdrawal group than in the SRL group (< 0.001 between groups, analysis of covariance, change 12 to 36 months).
CONCLUSION

The introduction of the non-nephrotoxic drugs MMF and mammalian TOR inhibitors may provide new immunosuppressive strategies to improve long-term results after renal transplantation. MMF decreases the risk for developing chronic allograft failure, is useful for treating established CAN, and has a beneficial effect on allograft fibrosis.

SRL as a basic immunosuppressive agent associated with MMF, steroids, and basiliximab may offer better renal function, reduce the prevalence of CAN, and downregulate expression of genes responsible for the progression of CAN compared with CsA treatment. SRL may also permit an early elimination of CsA from ST-CsA-SRL regimes and improve renal function and renal histology without increasing the risk of rejection. Notably, this approach increased graft survival at 4 years. An extension of other trials with SRL to assess the long-term safety of SRL with CsA and to compare this with SRL treatment without CsA showed that patients on SRL-based therapy exhibited long-term improvement in renal function with no increase risk of late acute rejection [51].

Because TAC is the most frequently used anticalcineurin drug, further multicenter studies are needed to determine whether SRL-based treatment produces similar results to those of TAC-based immunosuppression, and to compare TAC elimination from ST-TAC-SRL with a regimen that maintains TAC. A pilot Spanish study showed encouraging results, with better function in patients when TAC was eliminated from the ST-TAC-SRL regimen [52, 53], although no histological data are available. Whether these protocols based on SRL can also be extended to high-risk patients is currently unknown. Finally, although caution is needed, it can be speculated that the use of non-nephrotoxic immunosuppression might change the natural history of CAN.

lower in the SRL-ST group despite significantly less antihypertensive therapy \( (P < 0.001) \). The cumulative 4-year data demonstrated superior outcomes for renal function, stabilized lipid values, and the ongoing benefit of lower blood pressure in patients receiving SRL-ST. This was the first trial in the new immunosuppressive era to demonstrate that the protocol based on SRL and eliminating CsA 3 months after transplantation leads to significantly better graft survival, renal function, and renal histology than the CsA and SRL protocol.

Others have explored the conversion from anticalcineurin inhibitors to SRL in patients with chronic allograft dysfunction [46, 47]. Diekmann et al showed that proteinuria <800 mg/day at conversion was the only independent predictor for a positive outcome in conversion from anticalcineurin drugs to SRL in patients with chronic allograft dysfunction [46]. These limited data suggest that early conversion to SRL in patients with moderate insufficiency could be useful for treating chronic allograft dysfunction [47].

The antiproteinuric and antiproliferative actions of angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists might be useful in combination with MMF or SRL in patients with CAN. A rat model of renal allograft showed that losartan dramatically reduces nephrotic proteinuria [48], diminishes transforming growth factor-beta in patients with CAN [49], and, when combined with MMF, fully protects against chronic rejection [50]. A prospective study exploring the value of including losartan in the ST-TAC-MMF protocol to prevent CAN is ongoing. The role of statins as renoprotective drugs in CAN remains to be determined [4].

![Fig. 1. Graft survival, censored for loss to follow-up, in the RMR study (with permission from Morales JM, Kidney Int 67:S69–S73, 2005).](image1)

![Fig. 2. Calculated GFRs (Nankivell method) in the RMR study intention-to-treat analysis. (with permission from Morales JM, Kidney Int 67:S69–S73, 2005).](image2)
REFERENCES


17. Ruiz JC, Campistol JM, Grinyo JM, et al.: Early cyclosporine A withdrawal in kidney-transplant recipients receiving sirolimus...
S-130

Morales: Progression of histologic lesions in allografts

prevents progression of chronic pathologic allograft lesions. Transplantation 78:1312–1318, 2004


