Renal Function and Histology in Kidney Transplant Patients Receiving Tacrolimus and Sirolimus or Mycophenolate Mofetil

Nassim Kamar,[1] Tuan Tran Van,[2] David Ribes,[1] Anne Modesto,[3] Olivier Cointault,[1] Laurence Lavayssière,[1] Jean Louis Ader,[2] Dominique Durand,[1] Lionel Rostaing[1]

Objective: **The aim of this study was to assess the effects of tacrolimus in combination with either sirolimus (n = 10) or mycophenolate mofetil (n = 7) on renal function and renal histopathologic factors 6 and 12 months after kidney transplantation.**

Materials and Methods: **Renal function was assessed by the glomerular filtration rate (as measured by the inulin clearance rate) and by determining renal functional reserve. A renal allograft biopsy was performed at the time of transplantation and 6 and 12 months later.**

Results: **Serum creatinine levels tended to be higher in the sirolimus group 12 months after transplantation. In contrast, inulin clearance and renal functional reserve were similar in both groups 6 and 12 months after transplantation. With respect to histopathologic findings, only mononuclear-cell interstitial inflammation was significantly higher in the sirolimus group than in the mycophenolate mofetil group 12 months after transplantation. However, the progression of tubular atrophy, interstitial fibrosis, and vascular fibrous intimal thickening within the first postoperative year was significantly greater in the sirolimus group.**

Conclusions: **In the long term, the addition of sirolimus to treatment with tacrolimus in de novo renal transplant patients might more adversely affect renal allograft survival than might the addition of mycophenolate mofetil to tacrolimus therapy.**

Key words: *Sirolimus, Mycophenolate mofetil, Inulin clearance, Renal functional reserve, Histology*

[1]*Nephrology, Dialysis, and Transplantation; Service d'Explorations Physiologiques Rénales, and* [3]*Pathology, CHU Rangueil, Toulouse, France*

Address reprint requests to: *Nassim Kamar, MD, PhD, Department of Nephrology, Dialysis, and Transplantation, CHU Rangueil, 1 Avenue J. Poulhès, TSA 50032, 31059 Toulouse Cedex 9, France*

Phone*: 00 33 5 61 32 26 84* ***Fax:*** *00 33 5 61 32 28 64* ***E-mail:*** *kamar.n@chu-toulouse.fr*

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Recent clinical trials have shown the efficacy of a combination of tacrolimus with either sirolimus (SRL) or mycophenolate mofetil (MMF) in preventing acute rejection after kidney transplantation [1, 2]. MMF is widely used in patients who have undergone kidney transplantation because of its beneficial effect in preventing both acute rejection and chronic allograft nephropathy [3-5]. In a recent randomized study, Stallone and colleagues showed that the conversion from calcineurin inhibitors (CNIs) to SRL improves renal allograft survival and retards the progression of allograft injury in renal transplant patients with chronic allograft nephropathy [6]. However, when given with CNIs, SRL seems to enhance CNI-induced nephrotoxicity. An analysis of the Scientific Renal Transplant Registry by Meier-Kriesche and colleagues showed a lower incidence of renal allograft survival in patients treated with tacrolimus and SRL than in those who received tacrolimus and MMF [7]. The aim of this study was to assess the effects of 6 and 12 months of treatment with both combinations of drugs on the glomerular filtration rate (GFR) as measured by inulin clearance, on renal functional reserve, and on histologic findings in renal transplant patients.

Materials and Methods

Data from 21 de novo renal transplant patients from our institution were analyzed. Earlier data from those patients were presented in a recently published multicenter randomized study [1]. At the end of that study period (ie, 6 months after transplantation), 17 patients who had completed the study underwent maintenance therapy with the same immunosuppressive regimen including tacrolimus, steroids, and either SRL or MMF and were included in our study after having provided informed consent. Our study conformed with the ethical guidelines of the 1975 Helsinki Declaration. In addition to tacrolimus and steroids, 5 patients received a fixed dosage of SRL 0.5 mg/d, 5 others received a fixed dosage of SRL 2 mg/d, and the 7 remaining patients received MMF 1

g/d. The target tacrolimus trough levels were 8 to 16 ng/mL from day zero to day 14 and 5 to 15 ng/mL thereafter. No patient received induction therapy. All patients tested negative for antihepatitis C virus, hepatitis C virus RNA, HBs antigen, and hepatitis B virus DNA. The patients' characteristics are summarized in Table 1.

Table 1. Demographic data of the study subjects

Variables	SRL 0.5-mg group (n = 5)	SRL 2-mg group (n = 5)	MMF group (n = 7)	*P* Value
Recipient				
Age (y)	47 ± 5	39 ± 6.5	46 ± 5	ns
Sex (M/F)	4/1	4/1	4/3	ns
PRA level at RT (%)	0 (0-0)	0 (0-0)	0 (0-0)	ns
Duration of hemodialysis (mo)	54 ± 19.5	31 ± 9	43 ± 9	ns
Donor				
Age (y)	41 ± 7	43 ± 3.5	43 ± 4	ns
Sex (M/F)	4/1	1/4	4/3	ns
Death related to CV disease: Yes/No	4/1	3/2	5/2	ns
Serum creatinine level (μmol/L)	116 ± 25	90 ± 16	89 ± 13	ns
Creatinine clearance (mL/min)	96 ± 15	89 ± 10	105 ± 16	ns
Cadaveric/living donor				
HLA-A, HLA-B, or HLA-DR compatibility	5/0	5/0	7/0	ns
	3 (2-6)	2.8 (1-6)	3 (1-6)	ns
Cold ischemia time (h)	18.6 ± 2.6	12 ± 1.9	15.7 ± 1.3	ns
Warm ischemia time (min)	53 ± 11	48 ± 7	42 ± 5	ns
***Cytomegalovirus* status**				
D positive/R negative	0	1	1	ns
R positive	1	1	4	ns
D negative/R negative	4	3	2	ns

SRL, Sirolimus; MMF, mycophenolate mofetil; M, male; F, female; PRA, panel-reactive antibodies; RT, renal transplantation; CV, cardiovascular disease; Y, yes; N, no; HLA, human leukocyte antibodies, D, donor; R, recipient; ns, not significant.

Six and 12 months after transplantation, the GFR of the study subjects was determined by measuring the clearance of inulin and creatinine. Renal functional reserve also was determined. In addition, a renal allograft biopsy was performed 6 and 12 months after transplantation. Histopathologic findings were compared with the results of a kidney biopsy performed before kidney reperfusion. The biopsy results were classified according to the Banff classification [8].

A posttransplant increase in the serum creatinine level was always investigated by kidney biopsy after urinary obstruction or overt tacrolimus overdosage had been ruled out. Acute cellular rejection as defined in the Banff classification was treated with steroid pulses (prednisolone 10 mg/kg/d) for 3 days. In patients at high risk for *cytomegalovirus* (CMV) infection (ie, seropositive donors and seronegative recipients), systematic sequential prophylaxis with intravenous ganciclovir 10 mg/kg/d (the dosage was adapted to renal function) was administered for the first 2 posttransplantation weeks, after which oral valacyclovir 3 g/d (the dosage was adapted to renal function) was given for the next 3 months. In the other patients, close monitoring of CMV DNAemia was performed with real-time polymerase chain reaction. With respect to CMV infection, reactivation was defined as at least 1 episode of viremia during the first 6 months after transplantation. Patients who exhibited CMV reactivation or disease received intravenous ganciclovir 10 mg/kg/d (the dosage was adapted to renal function) for 14 to 21 days. All patients received prophylaxis (sulfamethoxazole-trimethoprim at a 400:80 mg ratio every other day) against infection with *Pneumocystis jiroveci* during the first 6 months after transplantation.

For each patient, we assessed the donor's characteristics (age, sex, cause of death, serum creatinine level, calculated creatinine clearance, CMV status); the duration of hemodialysis; the panel-reactive antibody level at transplantation; the number of HLA-A, HLA-B, and HLA-DR compatibilities; cold and warm ischemia times; and CMV status. One, 3, 6, and 12 months after transplantation, we assessed the following parameters in each patient: blood pressure level; renal function (serum creatinine level, calculated creatinine clearance [according to the Cockcroft and Gault formula], and 24-hour microalbuminuria levels); hematologic status (levels of hemoglobin, white blood cells, polymorphonuclear leukocytes, lymphocytes, and platelets); liver parameters (levels of aspartate, alanine aminotransferase, and gamma-glutamyl transpeptidase); fasting glycemia value; levels of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride; the dosage and trough levels of immunosuppressive drugs; the use of antihypertensive therapy; the use of angiotensin-converting enzyme inhibitors and/or angiotensin-receptor antagonists; and the use of statins. We also assessed the number of acute rejections, as well as the interval between renal transplantation and the first episode of acute rejection, the number of CMV infections, and the occurrence of de novo diabetes mellitus.

Laboratory procedures and calculations

Functional studies were performed on recumbent renal transplant recipients whose blood pressure was monitored every 10 minutes with a Dynamap 1846 SX (Critikon, Tampa, Fla, USA). At the beginning of the study, each patient drank 300 mL of water during a

15-minute period. Two antecubital veins were cannulated for intravenous infusions and blood samplings. During an initial 90-minute study period, urine obtained by spontaneous voiding was collected, and venous and arterial blood samples were withdrawn at the midpoint. Then, a loading dose of 750 mg polyfructosan-S (inulin; Laevosan Gesellschaft, Linz, Austria), 3 mL in 57 mL of 5% dextrose solution, was slowly administered intravenously before starting polyfructosan-S intravenous administration, 1750 mg or 7 mL in 500 mL of 5% dextrose solution, at a constant infusion rate of 3 mL/min to maintain a plasma concentration of inulin at 200 mg/L throughout the study. The second indwelling venous cannula was used to deliver a 5% dextrose solution into the other arm at an infusion rate of 2 mL/min and to perform blood withdrawals. After a 30-minute equilibration period, a steady urine flow was obtained. Two 30-minute clearance periods involving similar urine collections and venous blood samplings were also performed.

The GFR was equated with the clearance of inulin. Plasma and urine levels of inulin were determined by a photocolorimetric method, and the absorbances were read on a Novaspec II spectrophotometer (Pharmacia Biotech, Cambridge, UK). Renal functional reserve was equated with the difference between the highest GFR value measured during amino acid infusion and the baseline GFR value. Plasma and urine creatinine levels were determined by the Jaffe reaction with picric acid and by kinetic methods (Beckman creatinine reagent kits, Beckman Coulter, Galway, Ireland). Absorbances were read on a Beckman creatinine analyzer 2 (Fullerton, Calif, USA), and the clearance rate of creatinine was calculated.

Statistical analysis

Reported values represent either the mean ± SE or the median (range). Proportions were compared with the chi-square or the Fisher exact test. Quantitative variables were compared with the Kruskal-Wallis test and the nonparametric Mann-Whitney *U* test. In each group, quantitative variables were compared with the nonparametric Friedman test for repeated measurements and the Wilcoxon test. *P* values of less than .05 were considered statistically significant.

Results

The patients' characteristics were similar in the 3 groups. Because biologic parameters, concomitant medications, renal function, and histopathologic renal findings were not significantly different between the 2 SRL groups at transplantation and 6 and 12 months later (data not shown), those 2 groups were pooled and were compared with the MMF group.

Renal function

The serum creatinine level was similar in the 2 groups 6 months after transplantation (120 ± 5 μmol/L in the SRL group vs 106 ± 7 μmol/L in the MMF group; ns). In contrast, that level tended to be significantly higher in the SRL group 12 months after transplantation (124 ± 8 μmol/L in the SRL group vs 106 ± 8 μmol/L in the MMF group; $P = .09$). The creatinine clearance was similar in both groups 6 and 12 months after transplantation (91.8 ± 4.8 and 96.6 ± 10 mL/min/1.73 m^3, respectively, in the SRL group vs 102 ± 8.2 and 104 ± 11 mL/min/1.73 m^3, respectively, in the MMF group; ns). Inulin clearance was also similar in both groups 6 and 12 months after transplantation (63.8 ± 6.3 and 59.8 ± 5 mL/min/1.73 m^3, respectively, in the SRL group vs 65.3 ± 15.4 and 65.6 ± 15.4 mL/min/1.73 m^3, respectively, in the MMF group; ns). Renal functional reserve did not differ significantly between the 2 groups 6 months after transplantation (10.6 ± 2 in the SRL group vs 6.56 ± 3.8 mL/min/1.73 m^3 in the MMF group; ns) or 12 months after transplantation (8.3 ± 1.8 in the SRL group vs 4.8 ± 1.7 mL/min/1.73 m^3 in the MMF group; ns). Six and 12 months after transplantation, the 24-hour microalbuminuria levels were similar in both groups at, respectively, 0.5 mg/d (range, 0-20 mg/d) and 20 mg/d (range, 0-59 mg/d) in the SRL group and 0 mg/d (range, 0-8 mg/d) and 14.5 mg/d (range, 3-200 mg/d) in the MMF group.

Histopathologic findings

Renal histologic patterns, according to the Banff classification, were similar in the 2 groups at the time of transplantation and 6 months later. Twelve months after transplantation, all histologic patterns except for mononuclear cell interstitial inflammation were also similar in both groups. Mononuclear cell interstitial inflammation was significantly higher in the SRL group (0.5 ± 0.19) than in the MMF group (0); $P = .048$. Six patients (4 treated with SRL and 2 treated with MMF) exhibited mononuclear cell interstitial inflammation (score ≥ 1), and the 11 remaining patients did not exhibit that characteristic (score = zero). Interstitial inflammation was not due to previous acute rejection episodes, because among patients with mononuclear cell interstitial inflammation, only 1 had experienced an acute rejection episode. Two acute rejection episodes had occurred in patients who did not exhibit mononuclear cell interstitial inflammation (P = ns). However, as shown in Table 2, the progression of tubular atrophy, interstitial fibrosis, and vascu-

lar fibrous intimal thickening within the first year after transplantation was greater in the SRL group.

Table 2. Histopathologic patterns during the first year after renal transplantation in the study subjects

Variables	At RT	Month 6 After RT	Month 12 After RT	*P* Value*
SRL group				
t	0	0	0.12 ± 0.12	.37
i	0	0.5 ± 0.19	0.75 ± 0.31†	.1
g	0.11 ± 0.11	0	0	.37
v	1.22 ± 0.28	0.25 ± 0.25	0	.37
ah	1.22 ± 0.28	0.88 ± 0.3	1.25 ± 0.3	.26
ci	0.44 ± 0.17	0.62 ± 0.18	1.5 ± 0.27	.06
cg	0	0	0.25 ± 0.16	.37
ct	0.22 ± 0.14	0.5 ± 0.19	1.5 ± 0.27	.04
cv	0.66 ± 0.17	0.87 ± 0.29	1.37 ± 0.32	.06
mm	0.33 ± 0.17	0.62 ± 0.18	0.62 ± 0.18	.37
MMF group				
t	0	0	0	1
i	0	0	0.33 ± 0.21†	.37
g	0.2 ± 0.2	0	0	.37
v	0	0	0	1
ah	1 ± 0.45	0.67 ± 0.2	0.83 ± 0.47	.6
ci	0.4 ± 0.24	0.66 ± 0.21	1 ± 0.25	.1
cg	0	0	0.33 ± 0.21	.37
ct	0.2 ± 0.2	0.67 ± 0.2	1 ± 0.25	.5
cv	0.6 ± 0.24	1.16 ± 0.6	1.33 ± 0.49	.1
mm	0.2 ± 0.2	0.5 ± 0.2	0.83 ± 0.47	.22

*Comparison of histologic parameters (by means of the Friedman test for repeated measurements) at the time of renal transplantation versus 6 and 12 months after transplantation. †$P = .048$

SRL, Sirolimus; RT, Renal transplantation; t, tubulitis; i, mononuclear cell interstitial inflammation; g, glomerulitis; v, intimal arteritis; ah, arteriolar hyaline thickening; ci, interstitial fibrosis; cg, allograft glomerulopathy; ct, tubular atrophy; cv, vascular fibrous intimal thickening; mm, mesangial matrix increase; MMF, mycophenolate mofetil; ns, not significant.

Efficacy and safety data

The number and type of acute rejections, the number of CMV infections, the number of serious infections requiring hospitalization, and the number of cases of de novo diabetes mellitus were similar in both groups (Table 3). Although the number of antihypertensive therapies, including the use of angiotensin-converting enzyme/angiotensin-receptor antagonist II inhibitors, was similar in the 2 groups, diastolic blood pressure values were significantly higher in the SRL group (86 ± 23 mm Hg) than in the MMF group (74.3 ± 4.8 mm Hg; $P = .04$). Six and 12 months after transplantation, the liver and hematologic parameters, cholesterol and triglyceride levels, and the use of statins were similar in both groups. One month after transplantation, however, the values for liver parameters were higher in the SRL group and the lymphocyte and platelet counts were lower in the MMF group (data not shown).

Table 3. Complications during the first year after transplantation in the study subjects

Variables (Yes/No)	SRL Group (n = 10)	MMF Group (n = 7)	*P* Value
Acute rejection	1/9	2/5	.5
Corticosensitive acute rejection	1/9	2/5	.5
CMV infection	1/9	1/6	1
CMV primoinfection	1/9	0/6	1
CMV reactivation	0/10	1/6	.4
Severe infection	0/10	0/7	1
De novo diabetes mellitus	2/8	2/5	1

SRL, Sirolimus; MMF, Mycophenolate mofetil; CMV, *Cytomegalovirus*; ns, not significant.

Discussion

In our study, we compared the GFR (as assessed by inulin clearance), the renal functional reserve, and renal allograft histologic factors 6 and 12 months after transplantation in 2 groups of patients receiving tacrolimus and either SRL or MMF. Despite the small number of patients enrolled in each group, our data show that even though renal function was similar between the 2 groups 6 and 12 months after transplantation, the histologic findings indicate that the combination of tacrolimus and SRL is associated with the acceleration of renal allograft injury to a greater degree than is the combination of tacrolimus and MMF.

Both MMF and SRL have been shown to prevent chronic allograft nephropathy [4, 6]. The beneficial effect of SRL in preventing chronic allograft nephropathy has been reported only in patients receiving CNI-free SRL-based regimens. Flechner and colleagues reported that 2 years after transplantation, lower grades of chronic allograft nephropathy were found in de novo renal transplant patients treated with the combination of SRL, MMF, and steroids than in those treated with cyclosporin A, MMF, and steroids [9]. In maintenance patients, Stallone and coworkers also found lower grades of chronic allograft nephropathy in patients whose treatment was converted from CNIs to SRL than in those who continued to receive low doses of CNIs in addition to MMF and steroids [6].

In a recent large multicenter study by Vitko and coworkers [1], the administration of tacrolimus with either 0.5 or 2 mg of SRL was found to be as effective as the combination of tacrolimus with MMF [1]. Those investigators found that the incidence of 6-month graft survival was similar in all 3 of their study groups. The incidence of biopsy-proven acute rejection was significantly lower in the group treated with SRL 2 mg; however, in that group, the incidence of adverse effects such as hypertension, hyperlipidemia, and new-onset diabetes mellitus was significantly higher than that in the 2 other groups studied. Renal function at 6 months was similar in all 3 groups, but more patients in the 2-mg SRL group discontinued the study, and only the renal function data for

patients who remained in the study were analyzed [1]. Therefore, Vitko and colleagues concluded that the incidence of impaired renal function could have been underreported in their high-dosage SRL group [1].

In our study, even though serum creatinine levels tended to be higher in the SRL group than in the MMF group, renal function (assessed by inulin clearance) and renal functional reserve 6 and 12 months after transplantation were similar in both groups. In contrast, the progression of tubular atrophy, interstitial fibrosis, and vascular fibrous intimal thickening during the first year after transplantation was significantly greater in the SRL group. For that reason, the renal allograft survival rate in patients treated with tacrolimus and SRL was poorer than that in patients treated with tacrolimus and MMF [7].

SRL has been shown to enhance cyclosporin A nephrotoxicity by increasing blood and renal concentration through pharmacokinetic interactions in salt-depleted rats, a model that has been reported to demonstrate functional and histopathologic abnormalities that resemble the dose-dependent impairment of renal function observed in renal transplant patients treated with cyclosporin A [10]. The mechanism of increased tacrolimus nephrotoxicity associated with SRL therapy is not well known. Another strategy of combined tacrolimus-SRL immunosuppressive therapy has also been used: low-dosage tacrolimus (eg, trough levels below 6 ng/mL 1 year after transplantation) and high-dosage SRL (eg, trough levels of 8 ng/mL 1 year after transplantation). Recently, Ciancio and colleagues reported the 3-year effects of that combination and compared them with the results of classic tacrolimus-MMF therapy [2]. Patient and graft survival rates were not significantly different, but renal function tended to be better in the MMF group. Biopsy-proven chronic allograft nephropathy was similar in both groups [2]. In our study, the difference observed in renal histologic factors between the SRL and the MMF groups might be associated with the use of high doses of tacrolimus. Mota and colleagues showed that withdrawing cyclosporin A from an SRL-cyclosporin A steroid regimen 3 months after transplantation did not significantly affect renal histology 1 year after transplantation [11] but did improve renal histology and function 3 years after transplantation, when the Chronic Allograft Damage Index scores were significantly better in patients so treated [11]. Finally, Meier-Kriesche and colleagues showed poorer renal allograft survival in patients treated with high doses of CNIs combined with SRL than in patients who received CNIs combined with MMF [7].

In conclusion, in the long term, the use of a low dosage of SRL with a standard dosage of tacrolimus might be more harmful to renal allograft function than would the combination of tacrolimus and MMF. The long-term effects of a high dosage of SRL combined with a very low dosage of tacrolimus have yet to be evaluated.

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