Favorable Results of Concomitant Tacrolimus and Sirolimus Therapy in Taiwanese Renal Transplant Recipients at 12 Months

Kuo-Hsin Chen,1,2 Meng-Kun Tsai,3* I-Rue Lai,1 Fe-Lin Lin Wu,3,4 Rey-Heng Hu,1 Po-Huang Lee1

**Background/Purpose:** Combined therapy of sirolimus and cyclosporine has been found to exacerbate cyclosporine-related nephrotoxicity and to imperil graft renal function. We hypothesized that tacrolimus could bring about better renal function than cyclosporine when used in combination with sirolimus and corticosteroids for de novo renal transplantation.

**Methods:** A two-arm randomized study was conducted to test the hypothesis. Patients who gave written informed consent and received renal transplantation were randomized to take sirolimus in combination with either tacrolimus or cyclosporine. The primary endpoint of this study was renal function, and the secondary endpoints were acute rejection, graft and patient survival, metabolic side effects and infectious complications.

**Results:** A total of 41 Taiwanese renal transplant patients were randomized to receive cyclosporine (CsA group, n = 20) or tacrolimus (TAC group, n = 21) in combination with sirolimus and corticosteroids. The average estimated glomerular filtration rate (eGFR) was 52.77 ± 3.86 mL per minute for the TAC group at 6 months, and 46.42 ± 3.95 mL per minute for the CsA group (p > 0.05). At 12 months, the average eGFR was 52.04 ± 4.38 mL per minute for the TAC group, and 46.79 ± 4.38 mL per minute for the CsA group (p > 0.05). The biopsy-proven acute rejection rate of the TAC group was 4.76% (1/21), and that of the CsA group was 5.00% (1/20) at 12 months. The 12-month graft survival rates for the TAC and CsA groups were 100% and 90% (p = 0.142), respectively.

**Conclusion:** Our study demonstrated that concomitant tacrolimus and sirolimus therapy resulted in a favorable outcome in Taiwanese renal transplant patients at 12 months. Large-scale clinical trials will be needed to further address the issue of which calcineurin inhibitor, cyclosporine or tacrolimus, provides better renal function and graft survival for renal transplant patients. [J Formos Med Assoc 2008;107(7):533–539]

**Key Words:** renal transplantation, sirolimus, tacrolimus

An abstract containing preliminary data from this study was accepted and published by the World Transplant Congress, Boston, U.S.A., 2006.

Sirolimus (SRL, rapamycin), a macrocyclic lactone that binds FKBP 12 and inhibits the mammalian target of rapamycin-mediated signal-transduction pathway, was found to produce an immunosuppressive synergism with cyclosporine, which inhibits calcineurin and the associated signal transduction of T-cell receptors. The US and global trials on combined sirolimus and...
cyclosporine (CsA) regimens showed that patients with combined sirolimus and cyclosporine therapy had markedly reduced acute rejection rates.\(^2,3\) Interestingly, in these two studies, the mean serum creatinine levels of the sirolimus groups at 6 months were significantly higher than those of the azathioprine group and the placebo group.\(^2,3\) A significant pharmacokinetic interaction between sirolimus and cyclosporine was observed, and sirolimus actually augmented the nephrotoxicity of cyclosporine even at low concentrations.\(^4\)

While tacrolimus (TAC), also a calcineurin inhibitor (CNI), was found to have less pharmacokinetic interaction with sirolimus, tacrolimus could be a better choice to produce immunosuppressive synergism with sirolimus.\(^5\) Animal models have demonstrated that sirolimus could prolong allograft survival without adverse effects on glomerular function or on interstitial fibrosis.\(^6\) Combined tacrolimus and sirolimus therapy, therefore, could provide adequate immunosuppressive effects without overt nephrotoxicity, which could occur when sirolimus was combined with cyclosporine. We hypothesized that tacrolimus could result in better renal function than cyclosporine when used in combination with sirolimus and corticosteroids for de novo renal transplantation. A prospective two-arm randomized study was accordingly conducted to test the hypothesis.

**Methods**

A prospective two-arm randomized study was undertaken to test the hypothesis that tacrolimus could result in better renal function than cyclosporine when used in combination with sirolimus for de novo renal transplantation. The institutional review board approved the protocol, and patients gave written informed consent preoperatively to be enrolled. Adult patients with their first renal transplant were blindly randomized by a randomization sequence to receive either cyclosporine (Neoral; Novartis, Basel, Switzerland) (CsA group) or tacrolimus (Prograf; Fujisawa Healthcare, Deerfield, IL, USA) (TAC group) in combination with sirolimus (Rapamune; Wyeth-Ayerst, Radnor, PA, USA) and corticosteroids. Patients with chronic hepatitis B or C viral infection or any other active infection were excluded.

Patients who were randomized to the CsA group received cyclosporine at an initial dose of 3 mg/kg twice daily. The trough levels were maintained at 100–200 ng/mL. In the TAC group, the initial dose of tacrolimus was 0.05 mg/kg twice daily, and the trough levels were kept at 4–8 ng/mL. Additionally, sirolimus was given to all patients at a loading dose of 6 mg on the day after transplantation, and then at 2 mg daily starting 2 days after transplantation. The trough levels of sirolimus were kept at 4–8 ng/mL. Corticosteroids were given as usual. Every patient took valganciclovir (Valcyte; Roche, Nutley, NJ, USA) at a dose of 450 mg/day for 3 months to prevent cytomegalovirus disease. Valganciclovir was discontinued if significant bone marrow suppression occurred.

The graft function was monitored by regular checks of serum creatinine. The Cockcroft-Gault formula was used for calculation of estimated glomerular filtration rate (eGFR), with a correction factor of 0.85 in women. Acute rejection was suspected when an increase of over 30% in serum creatinine was noted. Graft biopsy was performed in every patient with suspected acute rejection. Post-transplant diabetes was defined as insulin usage for more than 30 days or regular use of hypoglycemic agents in recipients without a history of diabetes prior to transplant. Lipid profiles were regularly checked, and cholesterol levels above 240 ng/mL or triglyceride levels more than 200 ng/mL were considered to indicate dyslipidemia. The primary endpoint was estimated renal function, and the secondary endpoints were biopsy-proven acute rejection, graft survival, patient survival, metabolic side effects and infectious complications. Statistical analyses were performed using NCSS 2000 software (NCSS, Kaysville, UT, USA) for Windows. All values were expressed as mean ± standard deviation (SD).
Results

From March 2002 to February 2004, a total of 41 Taiwanese renal transplant recipients were randomized to receive cyclosporine (CsA group, \( n = 20 \)) or tacrolimus (TAC group, \( n = 21 \)) in combination with sirolimus and corticosteroids for de novo immunosuppressive therapy. The patients were from 22 to 62 years old. The average age of the CsA group was 40.2 ± 2.4 years, and that of the TAC group was 42.7 ± 2.3. More female patients than males were recruited in both groups; 13 of the 20 patients in the CsA group and 16 of the 21 patients in the TAC group were female. In the CsA group, seven of the 20 transplants were from live donation, while it was eight of 21 in the TAC group. The average mismatch between donor and recipient human lymphocyte antigens was 2.8 ± 0.3 for the CsA group and 3.3 ± 0.3 for the TAC group. The incidence of delayed graft function for the CsA and TAC groups were 20.0% and 14.3%, respectively. The demographic characteristics and 12-month outcome of both groups are summarized in the Table.

During the study period, three patients of the CsA group stopped taking cyclosporine and were converted to tacrolimus individually because of hirsutism, rapidly progressing glomerulonephritis and hemolytic uremic syndrome. The average dose and trough level of cyclosporine were 123.33 ± 33.36 mg/day and 86.38 ± 36.72 ng/mL at 6 months, respectively. At 12 months, the average dose of cyclosporine was 93.75 ± 41.46 mg/day, and the average trough level was 50.55 ± 26.04 ng/mL. All the patients in the TAC group were maintained on tacrolimus therapy for the 12-month follow-up period. The average dose and trough level of tacrolimus were 6.00 ± 3.03 mg/day and 5.97 ± 2.01 ng/mL at 6 months, respectively. The average dose of tacrolimus was 5.03 ± 3.16 mg/day, and the average trough level was 4.90 ± 1.94 ng/mL at 12 months. The average dose and trough blood level of tacrolimus and cyclosporine are shown in Figure 1. As for sirolimus, the average daily doses for the TAC group were 1.47 ± 0.56 mg/day and 1.53 ± 0.67 mg/day at 6 and 12 months, respectively. The average trough level for the TAC group was 3.29 ± 1.58 ng/mL at

<table>
<thead>
<tr>
<th>Characteristics and outcome</th>
<th>TAC group (( n = 21 ))</th>
<th>CsA group (( n = 20 ))</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-age (yr)</td>
<td>42.7 ± 2.3</td>
<td>40.2 ± 2.4</td>
<td>0.46</td>
</tr>
<tr>
<td>R-gender (M:F)</td>
<td>5:16</td>
<td>7:13</td>
<td>0.43</td>
</tr>
<tr>
<td>R-weight (kg)</td>
<td>55.1 ± 2.5</td>
<td>55.6 ± 2.5</td>
<td>0.88</td>
</tr>
<tr>
<td>Donor type (C:L)</td>
<td>13:8</td>
<td>13:7</td>
<td>0.84</td>
</tr>
<tr>
<td>D-age (yr)</td>
<td>46.5 ± 3.3</td>
<td>45.4 ± 3.3</td>
<td>0.82</td>
</tr>
<tr>
<td>D-sCre (mg/dL)</td>
<td>1.27 ± 0.13</td>
<td>1.22 ± 0.13</td>
<td>0.76</td>
</tr>
<tr>
<td>HLA mismatches</td>
<td>3.3 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Delayed function</td>
<td>14.3% (3/21)</td>
<td>20.0% (4/20)</td>
<td>0.63</td>
</tr>
<tr>
<td>Biopsy-proven acute rejection</td>
<td>4.76% (1/21)</td>
<td>5% (1/20)</td>
<td>0.96</td>
</tr>
<tr>
<td>Graft loss</td>
<td>0</td>
<td>10% (2/20)</td>
<td>0.14</td>
</tr>
<tr>
<td>Patient death</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Biopsy-proven CNI toxicity</td>
<td>4.76% (1/21)</td>
<td>10% (2/20)</td>
<td>0.61</td>
</tr>
<tr>
<td>CNI-switch</td>
<td>0</td>
<td>15% (3/20)</td>
<td>0.11</td>
</tr>
<tr>
<td>Wound complication</td>
<td>0</td>
<td>5% (1/20)</td>
<td>0.30</td>
</tr>
<tr>
<td>Infection</td>
<td>19.0% (4/21)</td>
<td>15% (3/20)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\( ^* \) Two-tailed t tests and Fisher’s exact tests were used for continuous variables and categorical variables, respectively, and log rank test was used for acute rejection and graft loss. \( R = \) recipient; \( D = \) donor; \( C = \) cadaveric donor; \( L = \) live donor; \( HLA = \) human lymphocyte antigen; \( CNI = \) calcineurin inhibitor.
6 months and 4.24 ± 1.23 ng/mL at 12 months. The average daily doses for the CsA group were 1.57 ± 0.86 mg/day and 2.00 ± 0.82 mg/day at 6 and 12 months, respectively. The average trough level for the CsA group was 5.19 ± 2.86 ng/mL at 6 months and 5.09 ± 1.75 ng/mL at 12 months. The dose and trough level of sirolimus are summarized in Figure 2. Sirolimus was discontinued in four patients in the TAC group and in two patients in the CsA group. The reasons for stopping sirolimus therapy included urine leakage from the percutaneous cystostomy in two patients, elevation of liver enzymes (1 patient), fever of unknown origin (1 patient), hemolytic uremic syndrome (1 patient) and deep vein thrombosis of the lower limbs (1 patient).

After transplantation, the average eGFR was 52.77 ± 3.86 mL per minute for the TAC group at 6 months, and 46.42 ± 3.95 mL per minute for the CsA group (p > 0.05). At 12 months, the average eGFR was 52.04 ± 4.38 mL per minute for the TAC group, and 46.79 ± 4.38 mL per minute for the CsA group (p > 0.05; Figure 3). In each group, one biopsy-proven acute rejection episode (Banff IA) developed and responded to steroid pulse therapy. Toxicity of CNIs was noted in the biopsy specimens in two patients, one in each group. The 12-month acute rejection rate was 4.76% for the TAC group, and 5% for the CsA group. Two patients in the CsA group lost their grafts at 12 months. The causes of graft loss were wound disruption with graft incarceration in one patient.
and progressive focal sclerosing glomerulonephritis in the other. There was no wound complication in the TAC group. The 12-month graft survival rates were 90% for the CsA group and 100% for the TAC group, and the 12-month patient survival rates were 100% for both groups.

Post-transplant diabetes mellitus developed in one patient in each group. Both required insulin injection for control of blood sugar. Three patients in the TAC group and two in the CsA group needed statins because of hypercholesterolemia. One patient in each group took fibrates for hyperlipidemia. The lipid profiles of the two groups are summarized in Figure 4. Four patients in the TAC group developed infections, which included peritoneal dialysis-related peritonitis (1 patient), legionella pneumonia (1 patient), urinary tract infection (1 patient) and fever of unknown origin (1 patient). The infections in the CsA group were quite similar to those in the TAC group, including bacterial pneumonia (1 patient), urinary tract infection (1 patient) and fever of unknown origin (1 patient).

Discussion

Sirolimus has been recognized as a potent immunosuppressive agent with a different adverse event profile from cyclosporine. In early phases of clinical trials, de novo sirolimus therapy achieved comparable results with cyclosporine in terms of acute rejection and graft survival but produced considerable dyslipidemia in the renal transplant recipients. When combined with cyclosporine, sirolimus could significantly reduce the incidence of acute rejection, though sirolimus exacerbated cyclosporine-related nephrotoxicity by a pharmacokinetic interaction. Therefore, it was cyclosporine and the pharmacokinetic interaction with sirolimus that jeopardized the graft renal function of patients on combined sirolimus and cyclosporine therapy. While tacrolimus has been shown to interact less significantly with sirolimus, we demonstrated that tacrolimus could be used in combination with sirolimus for de novo renal transplantation. Large-scale clinical trials will be needed to address the issue of which CNI, cyclosporine

Figure 3. Mean estimated glomerular filtration rates (eGFR) (±SD), as estimated by the Cockcroft-Gault formula, of patients in the CsA and TAC groups.

Figure 4. (A) Mean triglyceride (TG) levels (±SD) of patients in the CsA and TAC groups. (B) Mean cholesterol levels (±SD) of patients in the CsA and TAC groups.
or tacrolimus, provides better renal function and graft survival for renal transplant patients.

The role of sirolimus in the immunosuppressive management of kidney transplantation has varied from a supplement to CNI-based regimens, a replacement for antimetabolites in low-dose CNI-based regimens or the main immunosuppressive agent in CNI-free regimens. In a large multicenter randomized trial using different doses of sirolimus, Vitko et al concluded that a fixed dose of 2 mg sirolimus resulted in a low incidence of acute rejection despite more cases of hyperlipidemia. In another prospective randomized trial of tacrolimus combined with sirolimus or mycophenolate mofetil, Mendez et al found excellent results in both arms with low acute rejection at 6 months (11.4% and 13%, respectively). There was no difference in graft survival but more adverse effects occurred in the tacrolimus + sirolimus group. In our study, we demonstrated that the 12-month acute rejection rate could be reduced to around 5% when sirolimus was combined with either cyclosporine or tacrolimus. Therefore, with the introduction of sirolimus, the acute rejection rate after kidney transplantation has been dramatically reduced. We propose that CNIs and sirolimus are actually working synergistically; CNIs suppress T-cell activation and sirolimus inhibits proliferation. This could be the reason why such a low rejection rate could be achieved with two immunosuppressive agents at low doses.

Sirolimus, especially at high blood levels, was found to produce significant side effects, including poor wound healing, hyperlipidemia and myelosuppression. Lymphocele, wound infection and incisional hernia were reported once in up to 47% of patients. Tacrolimus has been demonstrated to increase the blood levels of sirolimus far less than cyclosporine, and sirolimus when combined with tacrolimus can have a synergistic effect in immunosuppression without the side effects resulting from high sirolimus blood levels. As demonstrated in our study, there were no wound complications after sirolimus therapy except for one patient in the CsA group, who was obese and suffered from wound disruption. Meanwhile, the antiproliferative effect associated with sirolimus is a unique characteristic with potential benefit to patients under immunosuppression. Kauffman et al concluded, in a multivariate analysis of post-transplant malignancy, that a significantly reduced risk of de novo malignancy was noted in patients given sirolimus maintenance therapy.

Taking into account all the factors including graft function, incidence of acute rejection, graft and patient survival, infection and metabolic adverse effects, low-dose tacrolimus combined with de novo sirolimus therapy resulted in a favorable outcome in this study: the graft renal function was well preserved with little renal toxicity, and the incidence of acute rejection was very low. The risk of infection was not high and the metabolic side effects were manageable. A large-scale randomized study will be necessary to demonstrate the superiority of tacrolimus and sirolimus combined therapy in Taiwanese renal transplant recipients.

Acknowledgments

The authors would like to thank Ms Hui-Yin Lin and Ms Ming-Hui Lin for their assistance with data management. This study was supported by a grant from the National Taiwan University Hospital (NTUH-91 S010).

References


