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Influence of Delayed Initiation of Cyclosporine on Everolimus Pharmacokinetics in *de Novo* Renal Transplant Patients

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We quantified the influence of delayed initiation of cyclosporine on everolimus pharmacokinetics in order to provide dosing guidance for kidney transplant patients.

In a randomized multicenter study, 56 *de novo* kidney transplant patients received everolimus, basiliximab, corticosteroids and either immediate (n = 40) or delayed (n = 16) initiation of cyclosporine based on renal function. Everolimus and cyclosporine predose blood levels (Cmin) were obtained over the first 3 months post-transplant.

Everolimus Cmin averaged 9–11 ng/mL in the immediate cyclosporine group over the first 3 months. In the delayed cyclosporine group, average everolimus Cmins were significantly lower by 2.9-fold in the absence vs. presence of cyclosporine: 2.9 ± 2.8 vs. 8.3 ± 3.7 ng/mL ($p < 0.001$). Likewise, the within-patient ratio of everolimus Cmins in the presence/absence of cyclosporine averaged 2.9 (range, 0.7–5.6).

Both everolimus and cyclosporine blood concentrations need to be monitored in kidney transplant patients with delayed graft function during the period when cyclosporine is withheld and shortly after its initiation. Dosing of everolimus needs to be adjusted to take into account an average threefold increase in everolimus exposure when cyclosporine is added to the regimen.

Key words: Cyclosporine, drug interactions, everolimus, immunosuppression, kidney transplantation

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Introduction

Everolimus is a macrolide immunosuppressant which inhibits T-lymphocyte proliferation signaling in response to organ allografting (1). Everolimus is intended for use in combination with cyclosporine and has demonstrated similar efficacy to mycophenolate mofetil in phase 3 kidney transplant trials (2) and superior efficacy to azathioprine in a phase 3 heart transplant trial (3). Both everolimus and cyclosporine are extensively metabolized via cytochrome CYP3A with elimination of metabolites via the bile. In addition, everolimus is a substrate of the countertransporter P-glycoprotein, while cyclosporine is both a substrate and an inhibitor. Because of these shared pathways, there is the potential for drug–drug interactions between the agents.

With respect to the influence of everolimus on cyclosporine pharmacokinetics, a crossover study in maintenance kidney transplant patients did not identify any significant change in cyclosporine steady-state profiles upon the addition of everolimus to the regimen for 1 month (4). Over the first year after kidney transplantation, the blinded phase 3 trials noted that 10% lower cyclosporine doses were used in everolimus-treated patients to achieve similar cyclosporine blood concentrations as in mycophenolate mofetil-treated patients (5). Taken together, these data indicate that there is a minor influence of everolimus on cyclosporine, which would likely be compensated for in the context of routine cyclosporine therapeutic drug monitoring.

Uncovering an influence of cyclosporine on everolimus, however, has been more difficult inasmuch as everolimus was always administered in a combined regimen with cyclosporine in drug development trials. The pharmacokinetic data from the phase 3 studies demonstrated that as cyclosporine blood levels were down-titrated over the first year post-transplant, everolimus blood levels remained stable (5,6). Only in the case of a crossover healthy subject study could the complete absence of cyclosporine on everolimus be quantified. That study demonstrated that the AUC of everolimus after a single 2-mg dose given alone increased 2.7-fold when a single 175-mg dose of cyclosporine (Neoral, Novartis) was coadministered (7). Together these studies suggest that as cyclosporine exposure is varied within the normal therapeutic blood level range, there is no differential influence on everolimus

exposure. However if cyclosporine is removed from the regimen, a change in everolimus blood levels may occur.

An opportunity to test this hypothesis at steady state in kidney transplant patients came in the context of a development study in patients at increased risk of developing delayed kidney graft function in whom cyclosporine therapy was withheld in the early days after transplantation and initiated later when renal function recovered. We compared everolimus and cyclosporine dosing histories and blood concentrations over the first 3 months post-transplant in patients with immediate and delayed cyclosporine in order to quantify the influence of cyclosporine on everolimus and to provide practical guidance on the use of this drug combination under these clinical conditions.

Methods

Study design

This was a 1-year, multicenter, randomized, open-label study in *de novo* kidney transplant recipients at increased risk of delayed graft function. The study protocol was reviewed at Ethics Committees at each clinical center and patients gave written informed consent to participate in the trial. The clinical results of the study will be reported separately. This communication presents the pharmacokinetic results from the first 3 months of the trial.

Patients received an immunosuppressive regimen consisting of everolimus (Certican, Novartis Pharmaceuticals, Basel, Switzerland), basiliximab (Simulect, Novartis), corticosteroids, and cyclosporine (Neoral, Novartis). Everolimus was administered orally 1.5 mg twice daily. Basiliximab was given by intravenous bolus injection of 20 mg before surgery and again on day 4 after transplantation. Prednisone or equivalent was dosed according to local center practice. Cyclosporine was administered orally twice daily simultaneously with everolimus. Cyclosporine doses were individualized to achieve predose trough concentrations of 150–350 ng/mL in weeks 1–2, 150–300 ng/mL in weeks 3–4, 100–250 ng/mL in months 2–6, and 100–200 ng/mL in months 7–12.

Patients were assigned to three groups based on post-transplant renal function, as follows: Patients with well-established renal function within 24 h of transplantation, as measured by urine output >2 L, were not randomized but remained in the study in a stand-alone arm and received the same treatment as patients in the immediate cyclosporine group. Patients with urine output <2 L in the first 24 h were considered to be at increased risk of developing delayed graft function and were randomized on a 1 : 1 basis to receive either early or delayed initiation of cyclosporine. In the early cyclosporine group, cyclosporine was initiated not later than 48 h after transplantation with a half-dose regimen allowed up to day 7 for patients who subsequently developed delayed graft function. In the delayed cyclosporine group, cyclosporine was initiated when renal function recovered (serum creatinine <3.4 mg/dL) or by day 14 at the latest. If by day 14, renal function had not adequately recovered, cyclosporine was initiated at half the conventional dose and increased when function recovered.

Pharmacokinetic assessments

Venous blood samples for the determination of everolimus and cyclosporine whole blood concentrations were obtained before the morning drug doses on day 2; at least three times weekly in the first 2 weeks; and at protocol-scheduled clinic visits in months 1 and 3. Blood samples were drawn into EDTA-containing collection tubes, and the tubes were gently inverted several times, and frozen at –20 °C.

Bioanalytics

Bioanalytics were performed at a central laboratory. Everolimus blood concentrations were determined with an enzyme-linked immunosorbent assay (ELISA) after extraction with *tert*-butylmethylether. Assay performance was assessed by a five-point quality control concentration range from 2 to 80 ng/mL. The interassay coefficient of variation ranged from 9.5% to 38.3% and the bias from –8.5% to 16.6%. The lower limit of quantification was 2 ng/mL. Cyclosporine blood concentrations were determined by a radioimmunoassay (RIA) method using the specific reagents of the Incstar Cyclo-trac SP-whole blood radioimmunoassay kit (Diasorin, Stillwater, MN) according to the manufacturer's instruction manual. The assay involves methanol extraction, incubation with radiolabeled cyclosporine and a cyclosporine-specific mouse monoclonal antibody, and a double-antibody separation technique. The calibration and quality control blood samples were prepared by spiking drug-free human blood with cyclosporine instead of using the samples supplied with the kit. The four-point quality control concentration range was 50–1800 ng/mL. The interassay coefficient of variation ranged from 13.1% to 30.4% and the bias from –8.0% to –4.5%. The lower limit of quantification was 50 ng/mL.

Statistical analysis

Data are presented as mean ± standard deviation unless otherwise noted. Everolimus blood levels were compared in the absence and presence of cyclosporine by an independent *t*-test for groups with unequal variances. A p-value of 0.05 was taken as significant.

Results

Study population and evaluation groups

Evaluable concentration data were obtained from 56 patients at 13 clinical centers. Initially the pharmacokinetic data were evaluated based on the three treatment assignments: not randomized, early initiation of cyclosporine, and delayed initiation of cyclosporine. The first two groups yielded similar results, indicating that the decisive factor to take into account was the timing of cyclosporine initiation. Therefore, for the purposes of this clinical pharmacology evaluation, patients were categorized into two evaluation groups according to the day of cyclosporine initiation. Those who began cyclosporine therapy within 2 days of the start of everolimus were designated the *immediate cyclosporine group*; hence, this group included patients in the nonrandomized and early cyclosporine arms of the study. Patients who began cyclosporine 3 days or later after the start of everolimus were designated the *delayed cyclosporine group*.

There were 40 patients in the immediate cyclosporine group, 30 men and 10 women, aged 55 ± 11 years and weighing 71 ± 13 kg. The delayed cyclosporine group was demographically similar with 16 patients, 11 men and five women, aged 54 ± 13 years and weighing 77 ± 12 kg.

Drug dosing

Cyclosporine was initiated in the immediate cyclosporine group by day 2 in all patients. Doses were 330 ± 154 mg/day in the first 2 weeks and 295 ± 133 mg/day at month 1. By month 3 the conventional dose-reduction after the *de novo* period was evident with an average dose of

238 ± 112 mg/day. The average day on which cyclosporine was initiated in the delayed cyclosporine group was day 11.1 ± 3.5 with a range from day 4 to day 15. Consequently, cyclosporine average doses were lower in this group compared with the immediate cyclosporine group for all visits in the first 2 weeks. For example, average doses were 28 ± 83 mg/day at day 6, 192 ± 192 mg/day at day 12, and 264 ± 218 mg/day at day 16. Thereafter, cyclosporine doses were similar in both groups, averaging 346 ± 128 mg/day at month 1 and 228 ± 98 mg/day at month 3.

The protocol-specified everolimus dose of 1.5 mg twice daily was maintained in the majority of patients. An everolimus dose reduction to 1 mg twice daily was made in one patient in the immediate cyclosporine group and two patients in the delayed cyclosporine group in the first 3 months post-transplant.

Pharmacokinetics

There was a total of 374 evaluable everolimus-cyclosporine concentration pairs. Concentration pairs from day 2 (n = 35) were included in plots of the data but omitted from inferential evaluation because everolimus was not yet at steady state at this visit. The main pharmacokinetic evaluation was therefore based on 339 concentration pairs collected from day 4 to month 3. There were 6.2 ± 2.0 concentration pairs per patient in the immediate cyclosporine group (range, 1–9 pairs) and 6.9 ± 1.7 concentration pairs per patient in the delayed cyclosporine group (range, 4–9 pairs). Within the delayed cyclosporine data set, pairs were evenly divided between the period when cyclosporine was withheld (3.6 ± 1.5 pairs per patient) and the period after cyclosporine was initiated (3.3 ± 2.4 pairs per patient).

Figure 1 compares the mean cyclosporine trough trajectories in patients receiving immediate and delayed cyclosporine. In patients receiving immediate cyclosporine, mean troughs rose over the first week post-transplant and then remained relatively stable from week 2 to month 1.

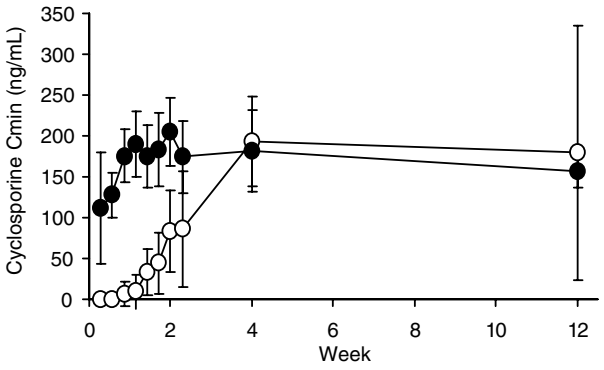

Figure 1: Mean cyclosporine trough blood levels (Cmin) in patients in the immediate cyclosporine group (●) and those in the delayed cyclosporine group (○). Bars represent 95% confidence intervals.

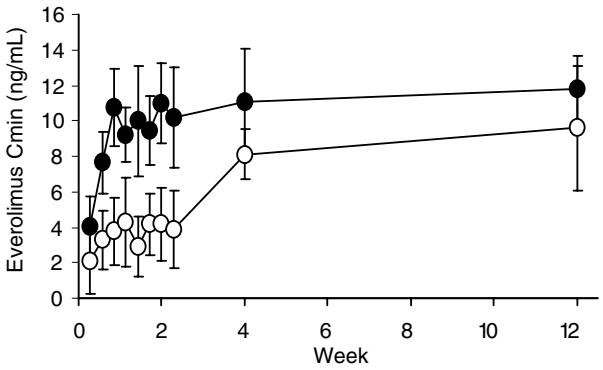

Figure 2: Mean everolimus trough blood levels (Cmin) in patients in the immediate cyclosporine group (●) and those in the delayed cyclosporine group (○). Bars represent 95% confidence intervals.

Thereafter, with cyclosporine dose reduction, troughs declined slightly by month 3. In patients receiving delayed cyclosporine, the earliest initiation was day 4 with progressively more patients in this treatment group on cyclosporine at each successive visit. By month 1, the mean cyclosporine level was similar to that of the immediate cyclosporine group.

Everolimus mean trough trajectories are shown in Figure 2. In patients receiving immediate cyclosporine, everolimus reached steady state between days 4–6. Thereafter, levels remained relatively stable with means between 9 and 11 ng/mL in the first 3 months post-transplant. The average everolimus trough progressively rose over the first 14 days after transplantation in patients receiving delayed cyclosporine. During this period, everolimus troughs were approximately two- to threefold lower in this group compared with troughs in the immediate cyclosporine group. By month 1 and thereafter, the everolimus troughs were similar in the two treatment groups.

Influence of cyclosporine on everolimus

Three evaluations were performed on the everolimus trough data from patients receiving delayed cyclosporine to assess the drug interaction of cyclosporine on everolimus: (1) a graphical assessment generating a time-shifted plot of the serial troughs by patient relative to the visit at which cyclosporine was added to the regimen; (2) a population comparison comparing all troughs across the population before vs. after initiation of cyclosporine; and (3) an intraindividual comparison based on the individual ratios of exposure before and after initiation of cyclosporine.

As shown in Figure 3, each patient's everolimus trough trajectory was plotted by a relative visit, whereby 'visit 0' was the visit at which cyclosporine was started, negative visits were before cyclosporine initiation with decreasing number signifying sequentially earlier visits, and positive visits were after cyclosporine initiation with increasing number

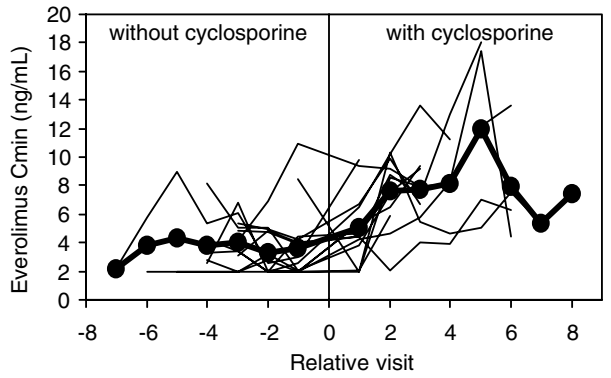

Figure 3: Individual everolimus trough blood level (Cmin) trajectories in patients in the delayed cyclosporine group (–). Mean Cmin trajectory (●). Data are standardized by relative visit whereby *visit 0* is the start of cyclosporine therapy, negative visits are while cyclosporine was withheld, and positive visits are those after the initiation of cyclosporine. Cmins less than the assay quantification limit were set to 2 ng/mL for inclusion in the plot.

signifying progressively later visits. Patients had different numbers of total visits depending on how long cyclosporine was withheld and how many evaluable troughs they provided. Everolimus troughs were notably lower before the initiation of cyclosporine averaging between 2 and 4 ng/mL. At the first visit after the start of cyclosporine, everolimus troughs rose but did not stabilize until the second visit. Thereafter, they remained relatively stable in the group as a whole averaging approximately 8 ng/mL. The delay in stabilization was likely the result of an everolimus accumulation to reach a new steady state in the presence of cyclosporine. Inasmuch as the visits were separated by 2 days, it took approximately 4 days to attain a new steady state as a result of the drug interaction.

Given the indication that everolimus troughs were not at steady state at visit 1 after the initiation of cyclosporine, these data were omitted from this analysis, yielding a total of 96 everolimus-cyclosporine concentration pairs in patients with delayed cyclosporine. The everolimus troughs were divided into two groups yielding the following population average troughs: 2.9 ± 2.8 ng/mL ($n = 57$) without cyclosporine vs. 8.3 ± 3.7 ng/mL ($n = 39$) with cyclosporine present ($p < 0.001$). The ratio of the means was 2.9, indicating that everolimus troughs increased 2.9-fold when cyclosporine was added to these patients' regimens.

Using the same dataset described earlier, each patient's median trough was determined from the values collected before the initiation of cyclosporine and after initiation of cyclosporine. The magnitude of the interaction was calculated as the postcyclosporine/precyclosporine ratio for each patient. This could be derived in 10 patients; in the other six patients either no postcyclosporine data were available or the precyclosporine levels were unquantifiable (<2 ng/mL). Of the 10 evaluable patients, three had essentially unaltered everolimus exposure when cyclosporine was initiated as evidenced by ratios of 0.7, 0.8, and 1.2. For the remaining seven patients, ratios were generally clustered between 1.7 and 3.8 with two outliers at 4.7 and 5.6. The overall median ratio for all 10 patients was 2.9.

Discussion

A single-dose study in healthy subjects has demonstrated that coadministration of cyclosporine as the microemulsion formulation (Neoral) increases everolimus blood levels by an average 2.7-fold (7). As phase 3 everolimus drug development trials in kidney and heart transplantation used this drug combination from the *de novo* through maintenance periods after transplantation, this pharmacokinetic influence of cyclosporine on everolimus is incorporated in the proposed standard dose regimen and in the range of concentrations proposed for everolimus therapeutic drug monitoring (8). In the case of renal transplant patients with delayed kidney graft function, however, the initial withholding of cyclosporine needs to be taken into account in dosing everolimus. We quantified the pharmacokinetic influence of cyclosporine on everolimus in this study to provide guidance for the use of everolimus under these clinical conditions.

This study revealed that when everolimus is used in an immunosuppressive regimen without cyclosporine, everolimus exposure is significantly lower than in a regimen with cyclosporine. The basis for this difference is a drug interaction of cyclosporine on everolimus, likely by their shared CYP3A and/or P-glycoprotein disposition pathways. Delayed kidney graft function itself is unlikely to have played a role in this interaction inasmuch as elimination of everolimus by the kidney is negligible (9).

When cyclosporine was added to an everolimus-based regimen, everolimus concentrations were increased on average by 2.9-fold; however, the increase was highly variable between patients ranging in this study from no change to a 5.6-fold increase. The average change in everolimus exposure in the presence of cyclosporine observed in these patients is in agreement with the previous healthy subject crossover study in which the AUC of everolimus increased in all 12 subjects by an average 2.7-fold with an individual range of 1.5- to 4.7-fold (7). Similar observations have been made for sirolimus. When a single 10-mg dose of sirolimus was coadministered simultaneously with 300 mg of cyclosporine microemulsion, the sirolimus AUC increased 3.3-fold (10).

The clinical implications of our study are that the dosing of everolimus needs to be adjusted to take into account an average threefold increase in blood levels when cyclosporine is added to the regimen. Given the wide variability in the

magnitude of this drug interaction, both everolimus and cyclosporine blood concentrations need to be carefully monitored in delayed graft function patients, especially in the period when cyclosporine is withheld and shortly after its initiation.

Acknowledgment

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