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ORIGINAL ARTICLE

Reduction of cyclosporine following the introduction of everolimus in maintenance heart transplant recipients: a pilot study

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Summary

Data are scarce concerning the calcineurin inhibitor dose reduction required following introduction of everolimus in maintenance heart transplant recipients to maintain stable renal function. In a 48-week, multicenter, single-arm pilot study in heart transplant patients >12 months post-transplant, everolimus was started at 1.5 mg/day (subsequently adjusted to target C_0 5–10 ng/ml). Mycophenolate mofetil or azathioprine was discontinued on the same day and cyclosporine (CsA) dose was reduced by 25%, with a further 25% reduction each time calculated glomerular filtration rate (cGFR) decreased to <75% of baseline. Of 36 patients enrolled, 25 were receiving everolimus at week 48. From baseline to week 48, there was a mean decrease of 44.5%, 50.9% and 44.6% in CsA dose, C_0 and C_2, respectively. Mean cGFR was 68.9 ± 14.5 ml/min at baseline and 61.6 ± 11.5 ml/min at week 48 ($P = 0.018$). The prespecified criterion for stable renal function was met, i.e. a mean decrease ≤25% of cGFR from baseline. Two patients experienced biopsy-proven acute rejection Grade 3A (5.6%). Between baseline and week 48, there were significant increases in total cholesterol, LDL-cholesterol and triglycerides, and small but significant elevations in liver enzymes. This 1-year pilot study suggests that CsA dose reduction of ca. 40% after initiation of everolimus was associated with a decrease in cGFR, however, based on the prespecified criteria stable renal function was attained.

Introduction

Calcineurin inhibitor (CNI) sparing immunosuppression regimens are an attractive option following heart transplantation, with the potential to restrict long-term CNI-related toxicities such as chronic kidney disease and metabolic or infectious complications that can contribute to cardiac allograft vasculopathy (CAV) [1,2].

Everolimus blocks not only growth-factor-driven proliferation of T cells and B cells [3,4], but also the prolifera-

tion of vascular smooth muscle cells [3,4] that result in the intimal thickening, which characterizes CAV [5]. *De novo* use of everolimus is associated with a significant reduction in intimal thickening and CAV assessed by intravascular ultrasound at 1 year after heart transplantation compared to azathioprine (AZA) [6], with a subsequent reduction in the occurrence of major cardiac adverse events [7].

Because of their different mechanisms of action, everolimus and CNIs suppress immune function in a synergistic manner [8] giving rise to the potential for reductions in CNI exposure without loss of efficacy. Also, importantly, proliferation signal inhibitors do not appear to be associated with direct renal toxicity [9]. Instead, the impaired renal function observed with proliferation signal inhibitor therapy and standard cyclosporine (CsA) treatment [6] is believed to be due to potentiation of the nephrotoxic effect of CsA, possibly through a pharmacokinetic interaction that increases the tissue concentration of CsA [10], although this requires clarification. In a randomized study in 199 *de novo* heart transplant recipients during which patients received everolimus with standard- or reduced-exposure CsA, there was a trend to improved renal function in the reduced-exposure arm with no significant differences in any efficacy endpoint between treatment groups [11]. More recently, another randomized trial in *de novo* heart transplant patients has compared everolimus with reduced-exposure CsA versus mycophenolate mofetil (MMF) with standard-exposure CsA in 176 recipients [12]. Efficacy was similar in both treatment arms; indeed, there were fewer patients with recurrent biopsy-proven acute rejection (BPAR) in the everolimus/reduced-CsA cohort [13], indicating that reduced CsA exposure in everolimus-treated *de novo* heart transplant patients does not compromise efficacy. Creatinine clearance at month 12, and the change in creatinine clearance over the first year post-transplant, did not differ significantly between the two treatment groups [12].

Data are scarce, however, concerning the introduction of everolimus in maintenance heart transplant patients and the associated potential for reduction of CNI exposure. The multicenter CADENCE study (CAnadian pilot study to Determine safe and Effective dosing of Neoral and CErtican in stable cardiac transplant recipients) was undertaken as a pilot study to explore the feasibility of CsA dose reduction in stable cardiac transplant patients after conversion from MMF or AZA to everolimus in preparation for a phase IV study evaluating everolimus in patients with allograft vascular disease. The primary objective of CADENCE was to assess the extent of CsA dose reduction required after introduction of everolimus to maintain renal function within 25% of the calculated GFR (cGFR) at baseline. Results at 48 weeks after conversion are presented here in terms of renal function, efficacy and safety.

Materials and methods

Study design

This was a multicenter, single-arm study in maintenance heart transplant patients. Following a protocol amendment at the request of the study investigators, the study was extended from the initial duration of 3–12 months with additional study visits at weeks 24 and 48. All 3-month analyses were repeated for study completion at 12 months, including data analysis at month 6, and these are reported here.

Patient population

Male or female cardiac transplant recipients between 18 and 70 years of age were eligible to participate in the study. Patients were required to be more than 12-month post-transplant and be receiving a CsA-based immunosuppressive regimen with or without either AZA or MMF, and with or without steroids. Absence of BPAR of ≥Grade 2 within the 12 months before enrollment was an additional inclusion criteria. The major exclusion criteria were: multiple organ transplantation; life-threatening CAV or graft dysfunction (ejection fraction < 30%) with an expected life expectancy of <1 year; baseline cGFR < 40 ml/min [14]; positive tests for HBs-Ag, HCV-Ab or HIV-Ab; severe hypercholesterolemia (≥9.1 mmol/l) or hypertriglyceridemia (≥8.55 mmol/l); hemoglobin < 10 g/dl, white blood cell count ≤ 2500/mm^3 or platelet count <50 000/mm^3.

Treatment

On day 2 of the study, all patients received one tablet of 0.75 mg everolimus (Certican®; Novartis Pharma AG, Basel, Switzerland) taken simultaneously with CsA (Neoral®; Novartis Pharma AG) and again 12 h later, on a consistent schedule with regards to time of day and relation to meals (Fig. 1). At week 1 and thereafter, everolimus dose was increased if the C_0 was below 5 ng/ml and decreased if the C_0 exceeded 10 ng/ml. A follow-up everolimus C_0 value was to be measured 4–6 days after any dose adjustment to ensure that C_0 remained between 5 ng/ml and 10 ng/ml. Everolimus C_0 and C_2 were measured at each study visit except Visit 1. Dose reduction was permitted for those patients who did not tolerate the full dose of everolimus (based on a decrease in platelet count, an increase in cholesterol or triglyceride level, or other adverse events).

There was a protocol-mandated CsA dose reduction of 25% on day 2, with a further 25% reduction each time the

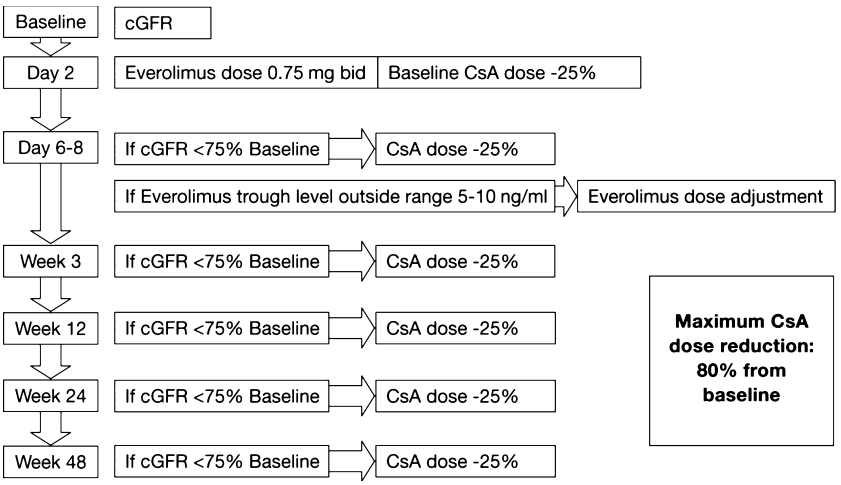

Figure 1 Study design.

cGFR decreased to below 75% of the baseline value. The maximum CsA dose reduction permitted was 80% of the baseline dose. After a preliminary review of the renal function data, the protocol was amended to stipulate that 25% CsA dose reductions would be based on an absolute, rather than a percentage drop in cGFR; this was to address potential safety concerns over using a percentage drop from baseline. The amended protocol required CsA dose reduction if the cGFR fell by more than 3 ml/min from baseline at two independent blood draws taken at least 24 h apart. However, the majority of patients had completed the study by the time of this protocol amendment.

Therapeutic drug monitoring of both everolimus and CsA was required for the duration of the study. MMF or AZA was discontinued on day 1 (the day before the first dose of everolimus). Administration of lipid-lowering therapy was mandatory throughout the study.

Evaluation

Maintenance of renal function was assessed by comparing calculated glomerular filtration rate (cGFR) at week 48 versus baseline. The efficacy and the safety of conversion from standard immunosuppression to everolimus were assessed using a range of parameters including the incidence of acute rejection episode ≥Grade 3A [15], the change in everolimus and CsA as assessed by C_0 and C_2 concentration, premature discontinuation of study treatment, the incidence of serious adverse events, and results of laboratory tests including lipid profiles, hematology and proteinuria.

Statistical analysis

Maintenance of renal function was defined as a mean decrease of ≤25% from baseline cGFR, as estimated by the Nankivell formula [14]. The null hypothesis stated that the ratio of week 48 cGFR to baseline cGFR was ≤75%. The null hypothesis would be rejected if the confidence intervals (CI) for the mean ratio of week 48 to baseline cGFR fell completely to the right of 75%. No corrections for multiple testing were made. Summary statistics are presented as frequency and percentage, mean and standard deviation (SD) or median and interquartile range [IQR: 25th percentile (Q1), 75th percentile (Q3)], as appropriate. All tests of significance were performed at the 0.05 level.

Study conduct

Written informed consent was obtained from all enrolled patients, and the study was performed in accordance with the Declaration of Helsinki and the US Food and Drug Administration guidelines for Good Clinical Practice.

Results

In total, 36 patients (mean 60.1 ± 43.7 months post-transplant), were enrolled and met the criteria for inclusion in the intent-to-treat (ITT) and safety populations. Three patients discontinued the study because of withdrawal of consent, such that 33 completed the ITT 48-week study. Of these 33 patients, 11 discontinued study drug before attending the week 48 visit, because of adverse events ($n = 9$) and withdrawal of consent ($n = 2$). Patient and donor characteristics are presented in Table 1.

Immunosuppression

Everolimus dose changed only slightly from baseline (week 1), to week 48 (Table 2), with a mean decrease of 0.015 ± 0.497 mg/day (1.0%) from baseline. Over the same period, there was a mean decrease of 2.46 ng/ml in

Table 1. Patient and donor characteristics (ITT population, n = 36).

Recipient age, years (mean ± SD)	57.2 ± 8.6
Male recipient, *n* (%)	33 (91.7)
White recipient, *n* (%)	34 (94.4)
Cause of end-stage disease, *n* (%)	
Coronary artery disease	15 (41.7)
Cardiomyopathy	12 (33.3)
Congenital heart disease	2 (5.6)
Valvular heart disease	3 (8.3)
Other	4 (11.1)
Time post-transplant at study entry, months (mean ± SD) (range)	60.1 ± 43.7 (13.6–154.8)
Donor age, years (mean ± SD)	33.6 ± 11.7
Cold ischemia time, hours (mean ± SD)	2.6 ± 1.6

Table 2. Everolimus and cyclosporine dose and exposure (ITT population, n = 36).

Everolimus	Baseline*	Week 48
Dose (mg/day)	1.49 ± 0.02	1.4 ± 0.50
C_0 (ng/ml)	9.0 ± 4.9	6.2 ± 1.9
C_2 (ng/ml)	16.0 ± 6.2	12.0 ± 5.1
Cyclosporine	**Baseline†**	**Week 48**
Dose (mg/kg/day)	2.2 ± 0.9	1.2 ± 0.6
C_0 (ng/ml)	124 ± 64	58 ± 42
C_2 (ng/ml)	509 ± 168	296 ± 169

*Week 1.
†Day 1.

everolimus C_0 and 3.75 ng/ml in everolimus C_2 (27.0% and 23.4%, respectively), probably caused by concurrent reduction in CsA exposure. Mean everolimus C_0 remained within the target range (≥5 and ≤10 ng/ml) throughout the study. Mean CsA dose at baseline, week 1, week 12, week 24 and week 48 was 2.2 ± 0.9, 1.7 ± 0.9, 1.6 ± 0.7, 1.4 ± 0.5 and 1.2 ± 0.6 mg/kg/day, respectively. From baseline to week 48, there was a mean decrease in CsA dose of 44.5%. While the greatest reduction in CsA dose was observed during week 1 as a result of the mandatory 25% dose decrease at the time of everolimus initiation, further reductions occurred, particularly after week 24. The decrease in CsA dose was paralleled by mean reductions in CsA C_0 and C_2 of 50.9% and 44.6%, respectively, from baseline 1 to week 48.

The *a priori* definition of maintenance of renal function was met, i.e. there was a ≤25% decrease in cGFR between baseline and week 48 [the 95% CI interval for the mean ratio of week 48 cGFR to baseline cGFR (0.864, 0.980) was completely to the right of 75%]. However, mean cGFR was 68.9 ± 14.5 ml/min at baseline and 61.6 ± 11.5 ml/min at week 48 (median values 67 and 63 ml/min, respectively), a difference that was statistically

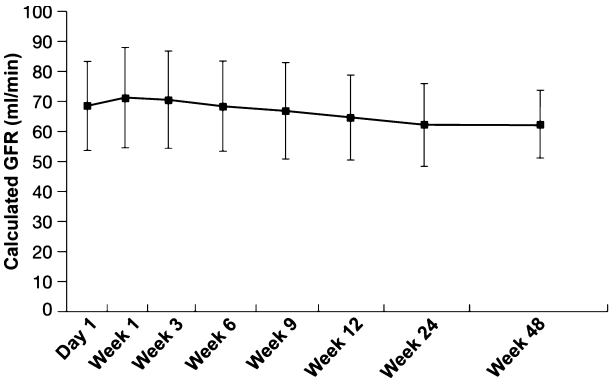

Figure 2 Calculated glomerular filtration rate to week 48 (cGFR, Nankivell formula) (ITT population). The change from day 1 to week 48 was significant (P = 0.018). Values are shown as mean ± SD.

significant (P = 0.018) (Fig. 2). Serum creatinine was 133 ± 30 μmol/l at baseline and 154 ± 34 μmol/l at week 48 (P = 0.014), with the greatest change having occurred by week 12 (146 ± 39 μmol/l).

Analyses of renal function were repeated for patients who completed the 48-week trial while still receiving study drug, and without major protocol violations (n = 18). In this population, the 95% CI interval for the mean ratio of week 48 cGFR to baseline cGFR again fell to the right of 95% (0.909, 1.024). Mean cGFR was 68.2 ± 12.6 ml/min at baseline in these patients, and 65.4 ± 9.5 ml/min at week 48 (median values 66 and 65 ml/min, respectively); serum creatinine was 139 ± 25 μmol/l at baseline and 148 ± 27 μmol/l at week 48.

Two patients experienced BPAR Grade 3A (5.6%, days 82 and 208 postconversion) and four further patients (11.1%) experienced milder acute rejection (1 Grade 1A, 2 Grade 1B and 1 Grade 2, on days 20, 81 and 271, and 133 postconversion, respectively). One of the patients with rejection Grade 1B experienced hemodynamic compromise and was given antilymphocyte therapy.

All 36 patients experienced at least one adverse event, of which the most frequent were peripheral edema (33.3%), diarrhea (22.2%), acne (16.7%), headache (16.7%), nasopharyngitis (16.7%) and rash (16.7%). Eleven patients (30.6%) experienced at least one serious adverse event; in five of these patients (13.9%) the events were suspected to be study drug related (tongue edema, rejection, pneumonia, mediastinitis and aphthous ulcers). Nine patients discontinued study medication because of one or more adverse events, which consisted of recurrence of worsening renal insufficiency (n = 2), worsening proteinuria/periorbital edema, anemia, lingual edema/neck swelling/bronchitis/face swelling, headache, skin eruptions and aphthous ulcers (n = 2).

Table 3. Laboratory values at baseline (day 1) and week 48 (safety population).

	Baseline	Week 48	*P*-value*
Fasting total cholesterol (mmol/l)	4.1 ± 0.8	4.9 ± 1.4	<0.001
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.1 ± 0.3	0.757
LDL-cholesterol (mmol/l)	2.2 ± 0.7	2.5 ± 0.7	0.026
Fasting triglycerides (mmol/l)	1.7 ± 0.8	2.8 ± 2.1	<0.001
Hemoglobin (g/l)	134 ± 13	129 ± 18	0.146
Hematocrit (%)	39 ± 5.7	39 ± 5.2	0.138
White blood cells (10^9/l)	6.6 ± 1.7	6.9 ± 2.6	0.378
Platelets (10^9/l)	224 ± 58	207 ± 62	0.179
Serum creatinine (μmol/l)	133 ± 30	154 ± 34	0.014
Proteinuria (g/l)	0.27 ± 0.29	0.41 ± 0.42	0.018

**t*-Test comparing baseline to week 48. Values shown are mean ± SD.

Lipid and hematological values are summarized in Table 3. Mean levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides increased significantly between baseline and week 12, but thereafter remained stable from week 12 to week 48. However, all values except HDL-cholesterol were still significantly higher at week 48 than at baseline (Table 3). All except one patient received lipid-lowering therapy (35/36, 97.2%). Hemoglobin decreased from 134 g/l at baseline to 121 g/l at week 12, but increased again to 129 g/l by week 48; hematocrit also showed a decrease between baseline and week 12 ($P < 0.001$) before returning to the baseline value of 39% by week 48. There was a significant increase in proteinuria from a mean baseline value of 0.27 ± 0.29 g/l to a mean value of 0.49 ± 0.60 g/l at week 12 ($P = 0.011$); after week 12 there was a slight decrease to 0.41 ± 0.49 g/l at week 48 but the difference from baseline remained significant ($P = 0.018$). Median proteinuria was unchanged from baseline (0.2 g/l, IQR 0.1–0.3 g/l) to week 48 (0.2 g/l, IQR 0.1–0.5 g/l). A minor increase from baseline to week 48 was observed in mean fasting blood glucose (6.3 ± 1.4 to 6.8 ± 3.2 μmol/l, $P = 0.173$) and in liver enzymes, where the differences reached significance, however levels remained within the normal range (ALT: 22.1 ± 10.0 to 26.9 ± 11.5 U/l, $P < 0.001$; AST: 22.3 ± 6.8 to 28.7 ± 8.3 U/l, $P < 0.001$). Total bilirubin decreased slightly from baseline (17.6 ± 8.3 μmol/l) to week 48 (13.5 ± 3.8 μmol/l, $P = 0.072$).

Discussion

This 1-year, multicenter pilot study is the first prospective trial to evaluate the degree of CsA reduction required to maintain renal function after introduction of everolimus therapy in stable maintenance heart transplant recipients. Results showed that in the presence of everolimus, a reduction of ca. 40% in CsA dose was associated with maintenance of renal function, as assessed by a ≤25% change in cGFR from baseline to week 48. Despite an increase in serum creatinine and a decrease in cGFR during the course of the study, the change in cGFR (≤25%) met the *a priori* definition for maintenance of renal function.

This study addressed maintenance of renal function and not renal sparing, and thus CsA dose was only decreased beyond 25% if required to maintain renal function. An increase in cholesterol levels was observed following introduction of everolimus, which in the presence of protocol-specified lipid-lowering therapy, stabilized with little change between weeks 12 and 48 although significant differences from baseline persisted. Detrimental changes in hematological profile occurred, again improving after week 12. The increase in the incidence of proteinuria during the study also warrants attention, although as seen with the changes in lipid and hematological values, this stabilized after week 12. These findings underscore the importance of regular safety monitoring and administration of lipid-lowering therapy in everolimus-treated heart transplant patients. They also give rise to the question of whether such changes might be ameliorated, and discontinuations due to adverse events avoided, if CsA dose and exposure had been reduced more extensively.

The only other published study describing initiation of everolimus with CsA reduction in this setting is a single-center, retrospective analysis of 37 heart transplant recipients by Schweiger *et al.* [16]. Maintenance patients were converted from MMF to everolimus with CsA dose reduced according to predefined CsA trough levels, and followed for 8 months. Taking into account the shorter time post-conversion, the mean reduction in CsA achieved in the study by Schweiger *et al.* (25%) was consistent with that seen in our population, as was the mean reduction in CsA C_0 (37%). As in the current trial, Schweiger *et al.* found renal function to be stable, and the rate of BPAR postconversion was similar compared to a control group who continued to receive standard-dose CsA with MMF, but again an increase in cholesterol and triglyceride levels was observed postconversion, albeit a nonsignificant change.

Two episodes of acute rejection of Grade ≥3A occurred, and one episode of hemodynamic compromising rejection (1B) suggesting the possibility of rejection risk with CsA reduction. In a recent study [17] comparing CNI withdrawal and initiation of sirolimus to CNI reduction with continued MMF, four of 30 patients in the CNI reduction arm and two of 30 patients in the CNI-free arm experienced rejection ≥1B, an overall rejection rate comparable to that seen in the current study and elsewhere [18]. While rejection can be avoided following CNI reduction or elimination in low-risk heart transplant patients [19] there is a need for heightened surveillance and strong consideration for mandatory follow-up biopsy after conversion to everolimus in maintenance patients.

In kidney transplant patients, a cross-study comparison has indicated that larger reductions in CsA exposure (57%) may be feasible in everolimus-treated patients [20]. The recent EVEREST study employed very low CsA targets in *de novo* kidney transplant patients with higher everolimus trough level targets and showed good efficacy [21]. It would be highly relevant in future studies of maintenance heart transplant recipients to explore the extent to which CsA exposure could be reduced without compromising efficacy, particularly in view of data suggesting that low CsA concentration has little impact on risk of BPAR in everolimus-treated patients [22]. Higher targets for everolimus exposure could also be considered given the significant inverse correlation between everolimus trough and risk of BPAR [22,23] and the success of very low CsA exposure with raised everolimus targets in renal transplantation [21], but this would require close attention to proteinuria and other potential side effects.

The limitations of this pilot study – notably, the absence of a control arm, small patient population and a significant drop-out rate – must be taken into account. The 33% drop-out rate is concerning and higher than that seen in the RAD001 B253 study in *de novo* heart transplant recipients (15.8%) [6], but less than that seen with sirolimus (44%) [24] or MMF (40.1%) [25].

Patients with marked renal dysfunction (GFR < 40 ml/min) were excluded. In their retrospective study, Schweiger *et al.* reported that conversion from everolimus to MMF with CsA dose reduction was associated with stable serum creatinine levels in heart transplant patients with normal renal function but that serum creatinine levels increased in those with renal impairment at baseline [16], highlighting that the results presented here may not be applicable to recipients with poor renal function.

In conclusion, 1-year results from this pilot study in stable heart transplant recipients suggest that CsA dose must be reduced by at least 40% after introduction of everolimus to maintain cGFR within 25% of baseline. Larger, controlled trials are required to identify the optimal strategy for CsA dose reduction in everolimus-treated maintenance heart transplant patients to preserve immunosuppressive potency while minimizing CNI-related renal damage and other complications.

Authorship

HR: contributed to design of study at Canadian Cardiac Transplant Network meetings, performed the study and collected data, wrote the paper as primary author in collaboration with all other authors, and took the decision to submit the paper for publication. PP, HH, MC, MW, AI, JH, RD and JRB: contributed to design of study at Canadian Cardiac Transplant Network meetings, performed the study and collected data, contributed to the manuscript and approved the final version. MV: analyzed data.

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