Original Article

Clinical outcomes and C2 cyclosporin monitoring in maintenance renal transplant recipients: 1 year follow-up study

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Abstract

Background. Cyclosporin A (CsA) concentration monitoring with 2 h post-dosing levels (C2) correlates with the incidence of rejection and graft outcome in de novo renal transplant patients. The advantages of this policy beyond the first 12 months remain a matter of debate. The purpose of the present work was to evaluate the C2 target ranges on CsA monitoring after the first year in stable kidney transplant patients.

Methods. We studied 142 patients, 94 on CsA–steroids and 48 on triple therapy (CsA–azathioprin–steroids), transplanted for 104±42 months and with a serum creatinine of 1.53±0.52 mg/dl. C2 and C0 measurements were performed at baseline and at least twice more during the year of follow-up.

Results. The mean annual C2 blood levels in double therapy patients showed C2 in 23 (24.5%) of <600 ng/ml; in 53 (56.4%) of between 600 and 850 ng/ml; and in 18 patients (19.1%) of >850 ng/ml. In the triple therapy group, C2 in 12 (25%) was <500 ng/ml, in 24 (50%) between 500 and 700 ng/ml and in 12 patients (25%) >700 ng/ml. In both groups, higher C2 levels were associated with a better absorption of the drug measured by the ratio C2/C0 and C2/dose. There were no differences in incidence of infections, need for hospitalization and the presence of hypertension, hyperuricaemia, hypercholesterolaemia or diabetes between patients with low and high C2 blood levels. However, serum creatinine was higher in triple therapy patients with lower C0 levels (P = 0.004). In 135 patients (90 on double and 45 on triple therapy), renal function remained stable during follow-up and 120 of them (89%) had C2 values under the recommended ranges.

Conclusions. C2 monitoring in maintenance patients enabled us to identify overexposure to CsA. Target levels of C2 should be adjusted according to the immunosuppressive regime. C2 levels between 600 and 800 ng/ml in double therapy patients and between 500 and 700 ng/ml in triple therapy patients are sufficient to give an adequate immunosuppression. The superiority of C2 with respect to C0 levels could not be demonstrated.

Keywords: C2 monitoring; cyclosporin A; kidney transplant

Introduction

Cyclosporin A (CsA) has been the mainstay immunosuppresser in renal transplant patients for the last 20 years. Its introduction resulted in a reduction in the incidence and severity of acute graft rejection episodes. It is well known that the drug has a low therapeutic index and requires monitoring of blood concentration. Currently, pre-dose ‘trough’ concentration (C0) is being used, but there is sufficient evidence that this correlates with clinical outcome or with drug exposure [1–4]. Recent research has shown that CsA exposure measured by the area under the curve (AUC0–12 or AUC0–4) is a good predictor for outcome in de novo transplant patients [5,6]. As the correlation of trough levels of CsA with AUC0–12 or AUC0–4 is limited, alternative single time point predictors for AUC0–12 or AUC0–4 have been evaluated. The CsA blood level at 2 h post-dose has been found to be the most sensitive marker for the AUC and it has been proposed as a more convenient method for pharmacokinetic monitoring than conventional C0 [2,3]. Preliminary data have shown that monitoring of the CsA concentration with 2 h post-dosing levels (C2) correlated with the incidence of rejection and graft outcome in de novo renal transplant patients [2]. More recently, the MO2ART study has confirmed the previous findings. CsA-microemulsion (ME) C2
monitoring resulted in a low incidence of allograft rejection, and the incidence of impaired renal function and other adverse events was similar to that of patients monitored by CsA trough levels [7]. Another advantage of C2 monitoring is that the target therapeutic ranges are independent of the assay system used [8].

As in the case of de novo renal transplant patients, maintenance transplant patients might also benefit from more precise CsA monitoring and, for this purpose, C2 target levels of 0.8–1.3 μg/ml beyond the first 12 months from transplant were established [9,10]. At present, there are only limited data on the utility of C2 levels in renal allograft recipients in stable renal transplant patients after the first months post-transplantation, and C2 monitoring revealed undetected overexposure to CsA in a variable number of cases [11–13]. Hence, dose reduction of CsA and a target range for C2 levels lower than that previously recommended has been indicated, but a correlation among C2 blood levels and clinical outcomes has not been demonstrated. The conclusions of some of these clinical studies [11,13] are based on only one C2 measurement and the patients included were on different immunosuppressive drug combinations and no distinction was made when analysing the CsA blood levels. The purpose of the present work was to evaluate the C2 concentrations of CsA in long-term renal transplant patients, to investigate the possible association of these levels with the clinical outcome, to identify the most adequate C2 range in maintenance patients in two immunosuppressive regimes and to show the possible advantages with respect to C0 monitoring.

Patients and methods

A total of 161 renal allograft recipients engrafted for >12 months on treatment with CsA, with stable renal function and with no rejection episodes or increases in serum creatinine during the previous 3 months, were recruited for this study. We did not include any patients with medications that may interfere with CsA drug metabolism. Seven poorly complying patients who took CsA at variable times or missed some doses and 12 with only two check-ups during the year of follow-up were excluded from the analysis. Thus, 142 patients were included definitively in the study. There were 76 males and 66 females, the mean age at the time of starting the study was 50.4 ± 14.1 years (range 22–74 years) and the mean follow-up after transplantation was 104 ± 42 months. As well as receiving CsA-ME as maintenance immunosuppression, 94 were also receiving steroids and 48 patients were on triple therapy. The trough blood levels of cyclosporin were maintained within a range of 150–250 ng/ml and were measured using a specific monoclonal antibody (monoclonal antibody with TDx system; Abbot Laboratories, Diagnostic Division, North Chicago, IL). The previous change of Neoral dose had been made 28.9 ± 22.9 months before starting the study (range 1–84 months); in 46 patients within the previous 12 months, in 44 patients between 12 and 36 months, and in 52 patients at >36 months.

All patients were followed for at least 12 months; they were reviewed at least every 4 months or more frequently depending on the clinical situation. The monitoring of CsA was performed by determination on the same day of trough drug levels (C0) and levels obtained 2 h after the morning dose (C2). For C0, blood was drawn immediately before the morning intake of CsA, between 8 and 9.30 a.m., and 2 h later for C2, between 10 and 11.30 p.m. For C2 levels, a sampling time of 2 h ± 15 min after the dose was considered adequate. During the follow-up period, C0 and C2 were determined at each visit to the transplant clinic at least three times (mean 4.5 ± 1.1 times per year) and CsA doses were adjusted when two consecutive C0 values were above the recommended range. Renal function, measured by serum creatinine and by creatinine clearance according to the Cockcroft–Gault equation, CsA dose changes and occurrence of relevant clinical events were recorded. Graft function deterioration was defined as an increase of serum creatinine of at least 0.3 mg/dl or 20% of the initial level. The CsA absorption was expressed as dose-adjusted C2 levels (C2/dose) and the ratio C2/C0 [14].

All results are reported as mean ± SD. The χ² and Fisher’s exact tests were used to compare categorical data. Continuous variables were compared using the two-tailed unpaired and paired t-test and Mann–Whitney U-test as appropriate. In addition, we used one-way analysis of variance (ANOVA) for comparing numerical data in more than two groups. The comparison among the groups was done by the Neuman–Keuls test. The relationship between continuous variables was assessed by linear regression analysis. The reproducibility of CsA concentration measurements in an individual was determined as the coefficient of variation (%CV = SD × 100/mean) using all values obtained during the follow-up period. We performed a receiver operating characteristic (ROC) curve analysis to evaluate C0 and C2 levels as predictors of clinical events: infection, hospitalization and renal function. Patients on cyclosporin and prednisone and patients on triple therapy were analysed separately.

Results

At the time of the study, the mean baseline creatinine was 1.53 ± 0.52 mg/dl (range 0.7–3.5). The mean CsA-ME dose was 205 ± 61 mg/day (range 75–400) or 3.0 ± 0.9 mg/kg/day (range 1.3–6.4). The mean C0 was 177 ± 43.4 ng/ml (range 85–362) and the mean C2 was 679 ± 201 ng/ml (range 143–1265). The quotient C2/C0 was 3.92 ± 1.19 (range 1.40–7.85) and the dose-adjusted C2 concentration 234 ± 81 had a range from 86 to 536 (ng/ml)/(mg/kg). Linear regression studies showed a positive weak relationship between basal C0 and C2
(r = 0.38, P = 0.000), as well as between C2 and Neoral dose (r = 0.41, P = 0.000) and there was a weak inverse correlation between serum creatinine and C0 (r = −0.35, P = 0.000).

The patients were followed for 12 months. At the end of the study, all the patients were alive and had functioning grafts. No acute rejection episodes were observed during the follow-up. Serum creatinine remained stable in 135 patients and increased in seven patients. Three patients presented chronic allograft nephropathy in renal biopsies performed between 6 and 12 months before the study. The mean serum creatinine of the whole group did not change, 1.53 ± 0.52 mg/dl at baseline and 1.58 ± 0.51 mg/dl at 1 year. C0 and C2 were determined periodically during the follow-up. Altogether 639 determinations of C0 and C2 were performed. The CV in the patients without changing the dose throughout the study for C0 was 14.21 ± 6.53% (range 4–34%) and for C2 was 17.73 ± 9.00% (range 1–52%). The distribution of the CVs for C0 and C2 is shown in Figure 1. There was a good correlation between C0 at baseline and the annual mean of C0 (r = 0.82, P < 0.001) and between C2 at baseline and the annual mean of C2 (r = 0.72, P < 0.001).

When we divided the patients according to the immunosuppressive therapy, there were no differences in age, body mass index (BMI), time on dialysis, incidence of rejection and prednisone dose between CsA-ME–steroids and triple therapy (CsA-ME–azathioprin–steroids) groups. However, males predominated in the group on triple therapy (44.2% vs 67.3%; P = 0.014) and serum creatinine was higher (1.41 ± 0.45 vs 1.71 ± 0.46 mg/dl; P = 0.000). However, CsA dose (3.15 ± 0.93 vs 2.79 ± 0.79 mg/kg/day; P = 0.019), C0 (188 ± 44 vs 156 ± 33 ng/ml; P = 0.000) and C2 blood levels (727 ± 206 vs 587 ± 154 ng/ml; P = 0.000) were lower in the triple therapy group than in the group on CsA-ME–steroids. Due to the differences in CsA doses and C0 and C2 blood levels between the two groups, we analysed them separately. In the triple therapy group, the third drug was introduced at 30.7 ± 6.9 months, in eight cases because of severe acute rejection (OKT3 treated), in 14 cases because of an increase in serum creatinine (four patients underwent biopsy and they all had chronic allograft nephropathy), in 13 cases because of serum creatinine >2 mg/dl after transplantation, in six cases because of non-renal CsA toxicity and in seven cases by protocol.

**Patients on CsA-ME and steroids**

To evaluate the relationship between the CsA blood levels and clinical events, the patients were divided into groups according to the percentiles of C2 values. Three groups were established: group 1 which included patients in the 25th percentile with C2 blood levels below 600 ng/ml, group 2 formed by patients between the 25th and 75th percentiles with C2 blood levels between 600 and 850 ng/ml, and group 3 consisting of patients in the 75th percentile. At baseline, 31 patients (33.0%) had C2 blood levels below 600 ng/ml; 53 patients (56.4%) had C2 levels between 600 and 850 ng/ml and 18 patients (19.1%) had C2 >850 ng/ml (Table 1). There were no differences in age, gender (data are not shown) and time of follow-up among the three groups. Serum creatinine showed a tendency to lower values when C2 increased, but the differences were not statistically significant. CsA dose at baseline was higher in the groups of patients with higher C2, as well as C0 and obviously C2 blood levels. Furthermore, patients with higher levels of C2 had higher C2/C0 and C2/dose quotients, indicating that these patients had a better absorption of the drug. We did not find differences in serum cholesterol, serum triglycerides, serum urate, systolic and diastolic blood pressure and the incidence of infection and hospitalization according to C2 CsA blood levels. Hypertension was nearly associated with higher serum creatinine (1.47 ± 0.44 vs 1.30 ± 0.35 mg/dl; P = 0.062), hyperuricaemia was
associated with younger age (38 ± 15.1 vs 46.6 ± 13.4 years; P = 0.003) and higher serum creatinine (1.61 ± 0.40 vs 1.23 ± 0.37 mg/dl; P = 0.000), and diabetes was associated with older age (50.1 ± 9.1 vs 40.5 ± 15.1 years; P = 0.001). A total of 29 patients presented bacterial or viral infections and 10 patients required hospitalization. The annual means of C0 and C2 blood levels as a predictor of infection, hospitalization or high serum creatinine at 12 months (>1.5 mg/dl) were assessed by ROC analysis. The area under the ROC for C0 and each of the events was: 0.433, 0.575 and 0.381, respectively. For C2, it was 0.433 for infection, 0.357 for hospitalization and 0.466 for high serum creatinine. No discriminative power of C0 and C2 was detected for any of the three events studied. Serum creatinine remained stable in 90 patients, 74 of them with mean C2 levels lower than 850 ng/ml. Graft function deterioration was observed in four patients. Baseline C2 blood levels were >850 ng/ml in two patients, between 600 and 850 ng/ml in one patient and <600 ng/ml in one patient.

During the follow-up, the CsA dose was reduced in 17 patients from 3.7 ± 1.01 to 3.09 ± 0.921 mg/kg/day (P = 0.000). Fourteen of these 17 patients had C0 blood levels below 250 ng/ml before CsA dose reduction. Serum creatinine remained unchanged (P = 0.328), C0 levels decreased but the differences did not reach statistical significance (P = 0.362), and C2 levels significantly decreased (P = 0.003). These findings could be explained by the relationship between CsA dose and C2 that was not observed with C0. Blood pressure, serum cholesterol, serum triglycerides, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol did not change with respect to baseline (Table 2).

Patients on triple therapy

At baseline, no patient had C2 levels higher than 800 ng/ml. As in the patients on CsA-ME, we also divided the triple therapy patients into three groups according to the C2 blood levels: group 1 which included patients in the 25th percentile with C2 blood levels <500 ng/ml; group 2 formed by patients between the the 25th and 75th percentiles with C2 blood levels between 500 and 700 ng/ml; and group 3 consisting of patients in the 75th percentile with C2 blood levels >700 ng/ml. According to the annual mean of C2,
12 patients (25\%) had C2 < 500 ng/ml and in four of them it was < 400 ng/ml, 24 patients (50\%) had C2 between 500 and 700 ng/ml, and 12 patients (25\%) had C2 > 700 ng/ml. The characteristics of the groups are shown in Table 3 and Figure 2. Patients with the lowest C2 levels (<500 ng/ml) had higher serum creatinine and lower creatinine clearance than patients with C2 above 500 ng/ml. As in the group on CsA-ME–steroids, there were no differences in the prevalence of hypertension, hyperuricaemia, hypercholesterolaemia, diabetes, infections or necessity of hospitalization. The CsA-ME dose was reduced in nine patients, three from each group, and it was increased in five. Three patients experienced graft function deterioration, two with C2 >700 ng/dl and one with C2 <500 ng/ml.

### C2 levels and graft function

The patients were divided according to serum creatinine levels at 12 months into three groups (Table 4). In the group on CsA-ME steroids, there were 34 patients (36.2\%) with serum creatinine <1.25 mg/dl, 50 patients (53.2\%) with serum creatinine between 1.25 and 2.0 mg/dl and 10 patients (10.6\%) with serum creatinine >2.0 mg/dl. Patients with the highest serum...
Table 4. Serum creatinine at 12 months and C0 and C2 blood levels

<table>
<thead>
<tr>
<th>Patients on CsA-ME–steroids</th>
<th>SCr &lt;1.25</th>
<th>SCr 1.25–2</th>
<th>SCr &gt;2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>24</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Time of follow-up (months)</td>
<td>112±35</td>
<td>95±39</td>
<td>103±38</td>
<td>NS</td>
</tr>
<tr>
<td>C0 at baseline (ng/ml)</td>
<td>175±28</td>
<td>159±31</td>
<td>146±36</td>
<td>NS</td>
</tr>
<tr>
<td>C0 annual mean (ng/ml)</td>
<td>179±29</td>
<td>157±24</td>
<td>138±35</td>
<td>0.016</td>
</tr>
<tr>
<td>C2 at baseline (ng/ml)</td>
<td>572±107</td>
<td>645±113</td>
<td>523±197</td>
<td>0.026</td>
</tr>
<tr>
<td>C2 annual mean (ng/ml)</td>
<td>628±125</td>
<td>648±129</td>
<td>523±149</td>
<td>0.057</td>
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</table>

<table>
<thead>
<tr>
<th>Patients on triple therapy</th>
<th>n</th>
<th>34</th>
<th>50</th>
<th>10</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of follow-up (months)</td>
<td>104±41</td>
<td>110±44</td>
<td>84±52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C0 at baseline (ng/ml)</td>
<td>195±44</td>
<td>190±43</td>
<td>153±43</td>
<td>0.028</td>
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<tr>
<td>C0 annual mean (ng/ml)</td>
<td>192±31</td>
<td>168±31</td>
<td>157±55</td>
<td>0.020</td>
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<tr>
<td>C2 at baseline (ng/ml)</td>
<td>704±214</td>
<td>766±193</td>
<td>641±219</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>C2 annual mean (ng/ml)</td>
<td>699±149</td>
<td>725±151</td>
<td>608±219</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs SCr <1.25 mg/dl; **P<0.01 vs SCr <1.25 mg/dl; †P<0.05 vs SCr 1.25–2 mg/dl; NS = non-significant.

Discussion

Therapeutic monitoring of CsA has traditionally been performed using trough concentrations of whole blood taken before the morning oral dose (C0), but these levels correlate poorly with the exposure to the drug and with clinical outcomes in de novo renal transplant patients [1–4]. The introduction of C2 monitoring in the de novo renal transplant recipients seems to be a more sensitive tool for monitoring CsA exposure when compared with C0 monitoring [2]. The use of C2 monitoring in maintenance renal transplant recipients could result in a more precise control of CsA exposure and perhaps in other clinical benefits. We did not perform 12 h pharmacokinetic measurements of CsA and because of that we could not investigate the superiority of C2 with respect to C0 in the assessment of CsA exposure. Einecke et al. [13] found a better relationship of C2 with AUC0–4 and AUC0–12 than C0, but they concluded that the correlation of C0 with AUC is sufficient guide therapy in patients on maintenance immunosuppression and their conclusion is supported by the patients' clinical outcome. Our data showed a correlation between CsA dose and C2 that was not observed with C0, but it was too weak to support the advantage of C2 monitoring in the measurement of CsA absorption in maintenance renal transplant recipients. Moreover, C2 monitoring did not show a better reproducibility of C2 concentrations with respect to C0. In some reports, the CV of C2 based on two consecutive determinations in a small number of cases was found to be lower than that of C0 and, for this reason, C2 was considered a more accurate and reliable measure of drug exposure in individual patients [14,15]. Our results based on ~700 determinations did not agree with these findings, and the CV obtained from at least three different determinations from each patient was slightly better for C0 than for C2.

In previous works, the adoption of C2 monitoring in maintenance renal transplant recipients has documented a high percentage with CsA overexposure which was not identified by C0 levels. At baseline, only 18.3% (26 patients) from the whole group had C2 cyclosporin levels >800 ng/dl, the recommended C2 target level. These figures are far from the 30–69% in other series [11,12,16,17]. The higher C2 levels could be due to a higher absorption of the drug, as indicated by the C2/C0 and the C2/dose quotients, and to a lesser extent to a higher CsA dose. The lower prevalence of overexposure with respect to other series has been attributed in part to the lower dose of cyclosporin that the patients were taking at the beginning of the study, perhaps as a result of a more precise adjustment of the CsA dose to the clinical situation. Moreover, the dose of CsA was decreased in 17 patients and we only observed improvement of renal function in two patients without modifications in other parameters such as blood pressure or lipid levels. These results are different from those reported by other authors. Cole et al. [12] observed that CsA dose reduction and adjustment to lower C2 target levels resulted in improved renal function in a considerable number of patients. The lack of improvement in our patients after CsA dose reduction could be explained by the good renal function before reducing the CsA dose, by the cause of the renal dysfunction not being due to CsA toxicity or by the irreversibility of the renal injury because of the length of overexposure to the drug.

To investigate the effect of CsA-ME exposure on graft function and the clinical situation of the patients, we divided the patients into groups according to the annual mean of C2 and also according to serum creatinine at 12 months. In the patients on
CsA-ME–steroids, we found no differences between the renal function at baseline, at the end of the study, or in the C2 levels, although there was a tendency to have lower graft function with the lowest C2 levels. In triple therapy patients, serum creatinine decreased when the C2 levels increased. That could be the expression of the presence of chronic allograft nephropathy due to inadequate immunosuppression or, alternatively, to the reduction of CsA dose and/or the introduction of a third drug to control graft function deterioration or other undesirable side effects of CsA. Some researchers have found that the development of chronic rejection was related to low CsA exposure measured by AUC or C2 [18,19], but their data showed neither whether low CsA exposure was before or after the development of graft dysfunction nor whether some therapeutic measure was taken such as CsA dose reduction. Others found no relationship between C2 levels and chronic allograft nephropathy [20]. When we divided the patients according to the serum creatinine, C0 levels were higher in those patients with lower serum creatinine, perhaps as an expression of the inverse correlation between the two variables. The relationship between C0 and serum creatinine level indicates that C0 is also important in the monitoring of graft function [11].

One of the most important findings of the present work is that the vast majority of our patients (135 patients) had a stable clinical situation during the follow-up and many months before C2 monitoring. As most of them had C2 levels <800 ng/ml, in patients on CsA-ME–steroids, a C2 between 600 and 800 ng/ml, or even lower if the situation of the patients is stable, is sufficient to give adequate immunosuppression. Moreover, C2 levels between 500 and 700 ng/ml are sufficient for patients on triple therapy. This target range is similar to that recommended by Einecke et al. [15] in patients on different immunosuppressive combinations. As new and more potent immunosuppressive drugs, such as sirolimus, everolimus or mycophenolate mofetil, are used in combination with CsA, the dose should be adjusted to maintain lower C2 levels than those reported here in the long-term follow-up in order to prevent nephrotoxicity.

We realize that our study has some limitations that might have biased the results. First of all, the patients included in the analysis have different follow-up times. We did not observe any difference in the length of follow-up when we divided the patients according to C2 levels or to serum creatinine levels. We consider that its influence on our findings is probably negligible. Secondly, graft function measured by serum creatinine varies between 0.7 and 3.5 mg/dl. The influence of graft function on CsA monitoring is analysed in Table 3. Lower C0 levels in the groups with worse graft function was the only difference found that could indicate a tendency to maintain low CsA levels in those patients with graft function deterioration. Alternatively, poorer graft function could be the consequence of inadequate immunosuppression.

In conclusion, monitoring of CsA dose by C2 is feasible and its measurement allows the identification of a variable number of overexposed patients. We could not demonstrate its superiority with respect to C0 in long-term renal transplant recipients in the management of graft dysfunction, or other complications such as hypertension, hypercholesterolaemia, hyperuricaemia or diabetes. In the majority of stable patients, C2 levels were lower than those previously recommended, and the target C2 levels should be defined according to the immunosuppressive regime. As the number of cases is limited, more and longer studies are necessary to find the adequate levels of C2 in stable renal transplant patients.

Acknowledgements. We thank Mary Harper for her assistance in preparing the English version of this article.

Conflict of interest statement. None declared.

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Received for publication: 12.7.04
Accepted in revised form: 1.12.04