Large Within-Day Variation in Cyclosporine Absorption: Circadian Variation or Food Effect?

John J Curtis,* Patsy Jones,* and Ralph Barbeito†

*Department of Medicine, University of Alabama Medical Center, Birmingham, Alabama; and †Novartis Pharmaceuticals, East Hanover, New Jersey

With the recent focus of monitoring cyclosporine (CsA) therapy using measures of CsA absorption, it is important to understand published reports of diurnal variation in CsA exposure. In 10 renal transplant patients, CsA concentrations were measured 0, 1, 2, 3, and 4 h after both the morning and the evening doses and in a repeat session at least 1 wk later. Both area under the curve for the final 4 h after cyclosporine dose and cyclosporine concentrate 2 h after the cyclosporine dose were more than two-fold higher after the morning dose in both sessions. Because the morning levels were collected in a fasted condition and the evening ones in a fed condition, the study was extended to collect evening levels after fasting. The area under the curve for the final 4 h after cyclosporine dose and cyclosporine concentrate 2 h after the cyclosporine dose values observed now were comparable to the morning fasted values. That the large diurnal variation was due to variation in food consumption, as opposed to a biologic circadian rhythm affecting CsA absorption, has significant implications for therapeutic drug monitoring. Clin J Am Soc Nephrol 17: 462–466, 2006. doi: 10.2215/CJN.01531005

The clinical management of calcineurin inhibitors so as to maintain long-term function of allografts has required continual refinement. Recently, difficulty in using these agents has prompted protocols to eliminate them as a means of avoiding associated toxicity, yet with this approach, the immunologic consequences for some patients (acute or subacute rejection) are worse than the toxicity (1). Others have suggested that optimal dosing of a calcineurin inhibitor, cyclosporin A (CsA; Neoral; Novartis, Basel, Switzerland), preserves the benefits while minimizing the toxicity (2). However, ideal therapeutic drug monitoring has yet to be defined. It has been suggested that trough level monitoring to guide optimal dosing be replaced with monitoring of more direct measures of absorption [e.g., CsA cyclosporine concentrate 2 h after the cyclosporine dose (C2), area under the curve for the final 4 h after cyclosporine dose (AUC0-4)] (3–5). Empirical support for the potential importance of such absorption profiling of CsA monitoring, e.g., using C2, is being established (6–10).

Clinical experience with such monitoring, however, has been met with an apparently high variability of exposure that complicates effective management. One of the sources of variability that has been reported in the literature has been generally attributed to “diurnal” or “circadian” effects. For example, Baraldo et al. (11) and Milianian et al. (12) reported that CsA C2 levels were higher in the morning than in the evening. Other studies have shown that food can alter CsA absorption (13). Both of these effects could accentuate the variability of monitored drug levels, especially if exposure in the early postdosing period is being monitored. Such effects also have been reported for tacrolimus; Min et al. (14) showed that tacrolimus exposure is greater in the morning than in the evening, and others (15,16) have shown that food affects the absorption of tacrolimus. Other studies, however, did not report finding such within-day variations (17,18).

The literature on this topic is difficult to integrate fully, however, because the definitions of terms, e.g., fasting versus fed, are not consistent and study methods differ. Furthermore, methodologic aspects that are crucial for interpreting results in terms of possible food or circadian effects are not always presented.

We examined within day-variation of the metrics of therapeutic drug monitoring by evaluating morning and evening AUC0-4, C2, and C0 (trough) levels in maintenance kidney transplant patients who were taking CsA in their usual manner. We also controlled for the effects of food versus fasting on these measures by providing standardized meals at standardized times in a restricted setting of a General Clinical Research Center (GCRC).

Materials and Methods

Patients

From a population of 100 maintenance renal transplant recipients who were at the University of Alabama at Birmingham and for whom C2 and C0 (trough) levels were known, we selected 10 patients with C2 values that were representative of the larger group of 100. The study was approved by the Institutional Review Board for Human Use, and all participants provided written informed consent.

Study Design

The study was conducted in the GCRC in the University of Alabama Hospital. Venous blood samples were drawn immediately before the CsA dose (C0) and then 1, 2, 3, and 4 h later. CsA concentrations were measured using the standard assay in the University of Alabama Hospital Laboratory, TDx. For these pharmacokinetic (PK) determinations, patients were admitted to the GCRC the night before or the morning of the PK assessments. The study progressed, as depicted in Figure 1, in the follow-
ing manner. After a fast of at least 8 h, blood for the morning C0 level was drawn just before administration of the morning CsA dose at 7 a.m. Blood then was drawn hourly until 11:00 a.m. A standardized breakfast (15 g of fat) was served at 9:00 a.m. immediately after blood for the C2 level was drawn. A standardized lunch (25 g of fat) was served at noon, and a standardized dinner (25 g of fat) was served at 5:00 p.m. At 7:00 p.m., blood for the evening C0 level was drawn, followed by administration of the evening dose of CsA and the subsequent hourly blood draws.

Each participant consumed equivalent standardized meals during the study. The choices of food varied by personal preference, but the content of protein and fat was identical. No other food was consumed during the study. Water was permitted ad libitum. All other maintenance medications were provided as usual for the outpatient schedules. For establishing the reproducibility of the PK values, this protocol was repeated during a second hospitalization in the GCRC not less than 1 wk later.

Preliminary analysis of the results suggested the importance of conducting a third session with a “reverse” fasting paradigm (Figure 1). Six of the 10 participants were able to return for this study extension. For this session, participants were given the same standardized meals as in the two previous sessions. However, after lunch, patients fasted, i.e., did not consume dinner at 5:00 p.m. At 7:00 p.m., blood for the evening C0 level was drawn. Immediately thereafter, the evening dose of CsA was administered, and the C1 and C2 CsA levels were measured 1 and 2 h later. The standardized dinner was provided after blood had been drawn for the C2 level, and the C3 and C4 levels were measured accordingly. Therefore, during this third hospitalization, the evening C0, C1, and C2 CsA blood levels were measured during a “fasted” condition that was otherwise, as practically as possible, at the same time of day as the evening “fed” condition of the two earlier sessions, thereby reversing the fasting-fed cycle of the earlier sessions (Figure 1).

![Figure 1. Timing of Neoral dosing, pharmacokinetic sampling, and meals on study (session) days.](image)

| Table 1. Patient characteristics and maintenance medications |
|------------------|-------------|-------------|-------------|
| Patient | Age (yr) | Gender | Race | BMI | Months since Transplantation | Daily Medications |
| 1 | 64 | M | AA | 24.4 | 20 | N 100; MMF 1000, 1000; P 10, atenolol |
| 2 | 73 | F | C | 18.3 | 147 | N 75; A 50; P 5; propranolol |
| 3 | 45 | M | C | 57.8 | 40 | N 125; MMF 250, 250; P 10; captopril |
| 4<sup>b</sup> | 35 | F | C | 47.7 | 26 | N 150; MMF 500, 750, 750; P 5; insulin; alprazolam |
| 5<sup>b</sup> | 54 | M | AA | 32.9 | 134 | N 175; MMF 1000, 1000; P 10; atenolol, metformin; clonidine |
| 6<sup>b</sup> | 56 | M | C | 24.5 | 69 | N 125; MMF 1000, 1000; P 5; glyburide; clonidine |
| 7<sup>b</sup> | 62 | M | C | 28.4 | 13 | N 100; MMF 1000, 1000; P 10; metoprolol; rosiglitazone |
| 8 | 68 | M | C | 22.2 | 88 | N 50; MMF 1000, 1000; P 10; lisinopril |
| 9 | 56 | M | AA | 25.0 | 89 | N 100; MMF 1000, 1000; P 10; doxazosin; enalapril |
| 10<sup>b</sup> | 41 | F | AA | 30.0 | 60 | N 100; MMF 1000, 1000; P 10; insulin; ezetimibe; atenolol |

<sup>a</sup>N, cyclosporine twice-daily regimen, in mg; MMF, mycophenolate mofetil regimen, in mg; P, prednisone, in mg; A, azathioprine, in mg.

<sup>b</sup>Patient has diabetes.
We analyzed the C0, C2, and AUC0-4 measures of CsA exposure. AUC0-4 was determined using the linear trapezoidal rule. Data were summarized and evaluated using standard descriptive statistics and dependent t test, as appropriate.

Results

Details of the 10 participants are provided in Table 1. Their mean (SD) age was 55 (±12) years, four participants were black, and three participants were female. The mean serum creatinine was 1.5 (±0.4) mg/dl, and the mean CsA dose was 110 (±36) mg given twice daily. The mean interval after transplantation was 68 (±46.5) mo.

The primary comparison was between the morning and evening metrics of CsA exposure. We found that whereas CsA exposure was consistent across sessions (Table 2), there were large differences between the morning and evening measurements. Values in the morning fasting condition were, on average, at least twice those of the evening fed condition. The same pattern was evident but to a lesser degree for the C0 levels. The a.m.–p.m. differences all were statistically significant, with P values ranging from ≤0.001 to ≤0.0022.

These differences also are apparent in the concentration versus time curves (Figure 2). These plots show a clear separation between the morning fasted and evening fed CsA levels, and this finding was consistent between the two study sessions.

On an individual patient basis, all 10 showed a greater CsA absorption, AUC0-4, after the morning dose than after the evening dose in both sessions 1 and 2, whereas for C2 this was true for nine of 10 patients. All patients also showed the difference for C0.

Examination of the individual PK profiles revealed an unusual result in one patient relative to the other nine. This patient showed a morning PK profile in session 1 that was similar to her two evening profiles. Investigation determined that this patient had type 1 diabetes and because of a marked hypoglycemia at the start of session 1 broke the required fast by eating breakfast immediately before the morning dose of CsA. However, this relatively poor absorption in the morning of session 1 was not due to diabetes per se (e.g., gastroparesis) because during session 2, the fasting protocol was completed and the CsA concentration versus time profile was comparable to that of the other patients. This observation along with the overall study data suggested the possibility of a “food effect” and prompted us to repeat a portion of the original study protocol, with a fasting period before administration of the evening dose of CsA.

Circadian Effect or Food Effect?

When the evening PK data were collected under a fasting condition, the PK profiles no longer looked like the evening profiles of sessions 1 and 2. Indeed, the evening fasted AUC0-4 profiles now were very similar to those of the morning fasting profiles of the two earlier PK assessments (Figure 3). Fasting for the evening PK profiles resulted in an increase in the average

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th></th>
<th>Session 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a.m.</td>
<td>p.m.</td>
<td>Ratioa</td>
</tr>
<tr>
<td>AUC0-4</td>
<td>2196 (787)</td>
<td>1052 (424)</td>
<td>2.14</td>
</tr>
<tr>
<td>C2</td>
<td>744 (258)</td>
<td>338 (151)</td>
<td>2.32</td>
</tr>
<tr>
<td>C0</td>
<td>135 (63)</td>
<td>116 (55)</td>
<td>1.18</td>
</tr>
</tbody>
</table>

aAUC0-4 = (ng*h/ml), C2 and C0 are ng/ml. All a.m. versus p.m. differences are statistically different, with P values ranging from ≤0.001 to ≤0.0022.

Figure 2. (A) Mean (±SE) concentration versus time plots for session 1. (B) Mean (±SE) concentration versus time plots for session 2.
AUC0-4 of 67% and C2 of 65%. The fasting evening AUC0-4 and C2 values, on average, fell short of the morning fasted values, by 15% for AUC0-4 and by 18% for C2: This shortfall might be due to “incomplete” fasting compared with the overnight fasting of the earlier morning sessions or perhaps to random variation. Little effect on the C0 values was evident.

**Discussion**

We found notable differences in CsA levels between the morning fasted and evening fed PK evaluations in a testing situation that was typical of a clinical setting. These differences were very large, with morning AUC0-4 and C2 being at least twice that of the evening measures. Furthermore, we could remove this large morning–evening difference by changing the meal schedule so that the evening PK data were collected under fasting conditions. The morning–evening difference also was found, to a much less degree, for the trough levels C0.

It is difficult to interpret the relevant literature in toto because there seems to be little agreement on the definition of some terms or of their potential importance to the method of the study reported. For example, the usage of the terms “diurnal” and “circadian” is not standardized, and there is no consistent definition of fasting versus fed. Our data suggest that it is important to know when the drug is taken relative to food to assess adequately such effects. We speculate that many of the seemingly inconsistent findings in the literature regarding diurnal or circadian effects of calcineurin inhibitors may be understood in terms of differences in the study method concerning the timing of food consumption relative to drug. Reports of morning versus evening differences may be explained by a fasting versus fed difference, per se, and failure to find a difference may be explained if both the morning and the evening PK evaluations were collected under similar fed or fasted conditions. Finally, across-study inconsistencies in the magnitude of any observed morning versus evening difference may be explained by the extent of a fasting versus fed difference. Although “diurnal” and “circadian” effects have been used (19) in connection to the mechanism underpinning this morning–evening difference, our “reverse fasting” results clearly suggest that the morning versus evening differences that we saw under the study protocol are largely if not wholly attributed to a food effect and not to a “circadian” or “diurnal” effect per se. Applying Occam’s razor, there seems to be no reason to hypothesize any mechanism beyond that associated with food effects to explain a.m.–p.m. differences in exposure to CsA.

![Figure 3](https://example.com/f3.png)

*Figure 3.* Mean (±SE) concentration versus time plots with p.m. fasting shown in both panels compared with sessions 1 and 2 mean profiles taken under p.m. (fed; A) and a.m. (fasting; B) conditions.
Our results are consistent with some reports in the literature for tacrolimus as well (14,16,20,21). Min et al. (14) found morning–evening differences in absorption of tacrolimus that were comparable to those of our study; however, they did not investigate the effect of food per se. Christiaans et al. (16) did investigate the food effect in tacrolimus by comparing AUC that were determined when breakfast was taken with the medication (fed) versus 1 h later (fasting) and found similar differences to ours for tacrolimus exposure. It seems that our results and conclusions also apply to tacrolimus.

Our results have significant implications for therapeutic drug monitoring, especially with the recent emphasis on the importance of monitoring levels that assess CsA absorption, e.g., C2 or AUC0-4 more directly than C0. The largest influence of food for calcineurin inhibitors seems to be in the first few hours after dose administration with fasting levels being meaningfully higher than levels that were determined after food intake. The usual CsA therapeutic drug monitoring measure, C0, while also showing a morning–evening difference was far less sensitive to the effect such that it might not be considered clinically relevant. However, for patients who need close monitoring as a result of a need for a more delicate balance between immunosuppression and toxicity, a better realization of the influence of food could prove beneficial for optimizing dosing and thereby extending graft function. Our results might lead to improved therapeutic drug monitoring by providing an understanding of “unexplained” variation in observed levels and so provide for the establishment of more consistent exposure through more meaningful dose adjustments.

Acknowledgments
This study was supported in part by a investigator-initiated grant from Novartis Pharmaceuticals.

We thank Joseph N. Young for data analysis and graphics.

References