Effect of assay methodology on pharmacokinetic differences between cyclosporine Neoral® and Sandimmune® formulations

Launa J. Aspeslet, Donald F. LeGatt, Gerard Murphy, and Randall W. Yatscoff

The new oral formulation of cyclosporine (CsA), Neoral® (CsA-N), results in increased area under the curve (AUC) and decreased intra- and interindividual variation in blood concentrations and other pharmacokinetic (PK) parameters when compared with the current Sandimmune® (CsA-S) formulation. The present study examines the effect of assay methodology on variability in blood concentrations and PK parameters for renal transplant patients receiving CsA-N and CsA-S and whether this variation is reduced with CsA-N. The results show that interindividual variations in PK parameters for patients receiving CsA-N were less than those for patients receiving CsA-S. Both blood concentrations and dose of CsA better correlated with abbreviated (4-h) AUC after administration of CsA-N. For both CsA-S and CsA-N, blood concentrations at 4 h postdose exhibited the best correlation with AUC. All samples were analyzed by three common procedures: HPLC, RIA, and fluorescence polarization immunoassay (FPIA). There were no significant differences observed in blood concentrations or PK parameters obtained from FPIA and RIA. HPLC results, however, were lower because of specificity of this method for the parent drug. The assay methodology did not have an effect on interindividual variability, indicating that the cross-reactivity of metabolites in commonly used immunoassays for CsA does not contribute to the PK variability observed in renal transplant patients.

INDEXING TERMS: pharmacokinetics • therapeutic drug monitoring • metabolite cross-reactivity • renal transplants • immunosuppression • radioimmunoassay • high-performance liquid chromatography • fluorescence polarization immunoassay

Therapeutic drug monitoring (TDM) of cyclosporine (CsA) is required for dosing of the drug to optimize immunosuppressive efficacy, while minimizing its side effects. The majority of experience with the therapeutic monitoring and pharmacokinetics (PK) of CsA has been when CsA is administered orally with olive or corn oil vehicles, more commonly referred to as the Sandimmune® (CsA-S) preparation. These formulations must be digested by pancreatic enzymes and emulsified by bile into hydrophilic particles before absorption. A new oral formulation of CsA, Neoral® (CsA-N), incorporates the drug into a microemulsion, which readily emulsifies upon contact with aqueous fluids without the requisite action of bile, enzymes, or small intestinal secretions.

Recent reports have shown that the CsA-N formulation results in increased bioavailability, more rapid absorption, and decreased intra- and interindividual variation in blood concentrations and PK parameters. The data also indicate an improved correlation between trough concentrations and area under the curve (AUC), as compared with the CsA-S formulation. CsA-N is expected to improve initial immunosuppression with CsA, since there is a good correlation between higher CsA AUC and freedom from rejection in the critical posttransplant period.

TDM of CsA is most commonly performed by one of the following three procedures: HPLC, RIA, and fluorescence polarization immunoassay (FPIA). These assays differ in their precision and specificity for the parent drug. The FPIA, in general, exhibits better precision than HPLC and RIA, whereas both the RIA and FPIA procedures

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3 Nonstandard abbreviations: TDM, therapeutic drug monitoring; CsA-S, -N, cyclosporine Sandimmune, Neoral; PK, pharmacokinetics; AUC, area under the curve; FPIA, fluorescence polarization immunoassay; and SDI, standard deviation intervals.
exhibit cross-reactivity with CsA metabolites [9–13]. Both of these assay methodology issues may influence the intra- and interpatient variation for CsA-N. In this study, we report on the effect of assay methodology on variability in blood concentrations and PK parameters for patients receiving CsA-N and CsA-S.

**Methods**

**Patient Selection and Specimen Collection**

Fifty stable renal transplant patients participating in a multicenter trial on CsA-N were randomly selected for the study. All patients were receiving triple immunosuppressive therapy consisting of CsA (CsA-S), azathioprine, and prednisone. Specimens were collected before conversion to CsA-N (month 0) and 3 months after conversion. At these intervals, whole-blood specimens (EDTA anticoagulant) were collected at the following times: trough, 1, 2, and 4 h postdose. Specimens were transported to the central laboratory site in Edmonton where they were stored at 4 °C until analysis. Use of human subjects received approval from each participating institution’s Ethics Committee on use of human subjects in research.

**Method of Analysis and Data Calculation**

All specimens were analyzed by HPLC [13], RIA (CycloTrac; IncStar, Stillwater, MN), and FPIA (Monoclonal Whole Blood Assay, Abbott TDx; Abbott Diagnostics, Abbott Park, IL). The two immunoassays were performed according to the manufacturers’ instructions. The interassay CVs at 100 and 300 μg/L were: HPLC, 12% and 8%; RIA, 11% and 7%; FPIA, 6% and 4%, respectively.

Noncompartmental PK parameters were calculated by using PC NONLIN 4.2 (Scientific Consulting, Apex, NC). AUC were calculated for the complete 12-h dosing interval by extrapolation of the 4-h PK profile to a 12-h profile by using the t = 0 as the t = 12 time point. These are referred to as abbreviated AUC. For dose-normalized calculations, all parameters were normalized to a CsA dosage of 100 mg/day. We, as well as others [14], have previously shown that this provides an appropriate standardization. Standard linear regression analyses to determine correlation coefficients (r) were performed for comparison of time points postdose with AUC.

**Analytical Validation**

To ensure that blood concentration results for parent CsA as monitored by HPLC were comparable with those generated by FPIA and RIA, the calibrators from both assays were analyzed by HPLC. All assays provide similar results for the parent drug (data not shown) and one can therefore assume that any difference among assays will be due to cross-reactivity with metabolites.

**Effect of Assay and Formulation on CSA PK Parameters**

A comparison of PK parameters for the 50 patients receiving the CsA-S and CsA-N formulations as determined from HPLC, RIA, and FPIA blood concentration data is shown in Table 1. For all three assays, significant (P ≤ 0.01) increases in 1- and 2-h postdose concentrations, C_max concentrations, C_max/trough ratio, and abbreviated AUC were observed for patients receiving CsA-N vs CsA-S. No significant differences in trough (t = 0) or 4-h postdose concentrations were observed. Similar results were obtained when the concentrations were normalized for dose (data not shown). The averaged HPLC concentration vs time profiles (0–4 h) for CsA-N and CsA-S with both raw and dose-normalized concentrations are shown in Fig. 1.

For each formulation, there was no significant difference (P > 0.05) in the blood concentrations or the PK parameters obtained with FPIA and RIA. In contrast, the concentrations obtained by FPIA and RIA were significantly (P ≤ 0.01) higher than those obtained by HPLC. For CsA-N, RIA, and FPIA, mean concentrations were 10–41% higher than those obtained by HPLC, whereas for CsA-S, mean concentrations were 12–51% higher. Values for abbreviated AUC calculated from RIA and FPIA data were 27–32% higher than those derived from HPLC data for CsA-N and 28–36% higher than HPLC-derived values for CsA-S. The PK profiles for CsA-N constructed from concentrations generated by the three assays are shown in Fig. 2.

The variations in the PK parameters were determined by comparison of the standard deviation intervals (SDI = SD/√x) obtained from each analytical method for both the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FPIA</th>
<th>RIA</th>
<th>HPLC</th>
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<tbody>
<tr>
<td>Trough, μg/L</td>
<td>213 ± 82</td>
<td>237 ± 98</td>
<td>NS</td>
</tr>
<tr>
<td>1 h, μg/L</td>
<td>1084 ± 628</td>
<td>485 ± 386</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2 h, μg/L</td>
<td>971 ± 379</td>
<td>598 ± 336</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4 h, μg/L</td>
<td>526 ± 198</td>
<td>484 ± 296</td>
<td>NS</td>
</tr>
<tr>
<td>AUC, μg h/L</td>
<td>6084 ± 2244</td>
<td>4868 ± 2242</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C_{max}, μg/L</td>
<td>1200 ± 569</td>
<td>733 ± 401</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C_{max}/trough</td>
<td>6.0 ± 2.5</td>
<td>3.3 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NS, not significant.
CsA-S and CsA-N formulations. Fig. 3A and B demonstrate that the variation in PK parameters was less for CsA-N as compared with CsA-S for all assays. This was true when both raw and dose-normalized data were considered. Variations in concentrations and PK parameters (SDI) among assays for a given formulation were similar (Fig. 3A and B).

**CORRELATION OF TIME POINTS WITH BIOAVAILABILITY (AUC)**
Regression analyses of abbreviated AUC vs [CsA] at the specified time points (trough, 1, 2, and 4 h postdose) for each assay (HPLC, FPIA, RIA) were performed for both CsA-N and CsA-S. Two representative analyses are depicted in Fig. 4 (A, B). The correlation coefficients are presented in Fig. 5. For both the CsA-N and CsA-S formulations, the 4-h postdose concentration provided the best correlation with abbreviated AUC, regardless of the analytical assay used ($r = 0.92–0.95$), followed by, in order of decreasing $r$ value: trough = 2 h > 1 h. For a given time point and analytical method, CsA-N generally gave more favorable correlations to abbreviated AUC than CsA-S. When the linear regression analyses were reanalyzed, based on dose-normalized data, 71% of the $r$ values averaged 16% less than the corresponding original $r$ values derived from nonnormalized data (data not shown), while no change was observed for the remaining values.

**CORRELATION OF DOSE WITH BIOAVAILABILITY (AUC)**
Correlations of abbreviated AUC vs CsA doses were performed with data from the three assays for both CsA-N and CsA-S (Fig. 6). Regardless of assay, correlations of AUC with CsA-N dose were significantly ($P < 0.01$) greater than those for CsA-S dose. There was negligible difference in the correlations between assays for both CsA-S and CsA-N.

**Discussion**
The current formulation of CsA, CsA-S, exhibits high intra- and interindividual variations in absorption, distribution, metabolism, and elimination, which complicates its use [1, 15]. A new microemulsion formulation of CsA, CsA-N, has shown increased bioavailability [4] and a less variable PK profile [5]. This more consistent absorption profile has led to an improved correlation between the trough concentrations and AUC as compared with CsA-S [6]. This is important since the systemic exposure to CsA in patients is typically predicted on the basis of the trough CsA blood concentration [16, 17]. The increase in drug exposure or bioavailability observed with CsA-N may
also lead to improved immunosuppression with CsA, since there is good correlation between higher CsA AUC and freedom from rejection in the critical posttransplant period [7].

The present study confirms previous findings that CsA-N exhibits an increased bioavailability over that found with CsA-S [6, 18, 19]. The abbreviated AUCs with CsA-N were significantly higher ($P < 0.01$) than with CsA-S, both with and without dose normalization of the data. The dose normalization was not expected to have a great effect on the PK parameters, since CsA follows first-order kinetics and the CsA clearance and $V_d$ are independent of dosage [11]. Also consistent with previous findings is the significant increase in the $C_{max}$ with CsA-N and no significant difference in trough concentrations between the two formulations [5, 6, 18, 19]. Also, the concentrations of CsA were higher with CsA-N than with CsA-S at 1 and 2 h postdose, but after 4 h the concentrations were similar. The interindividual variations in the PK parameters in this study were less with CsA-N than with CsA-S. This allows for better predictability of PK profiles and thus makes blood concentration monitoring of more value when CsA-N is prescribed. The study design did not allow evaluation of the diurnal variation of the PK of CsA with the various formulations. All trough specimens were collected in the morning, with the PK studies being performed during the afternoon.

In the present study, better correlations of blood concentrations with AUC at most time points after administration of CsA-N compared with CsA-S demonstrates the enhanced clinical usefulness of TDM for the former formulation. Furthermore, we found that the blood concentrations best correlated to abbreviated AUC at 4 h postdose for both CsA-N and CsA-S. The 4-h time point may therefore provide a more accurate prediction of total drug exposure; however, it may not be as practical or realistic to obtain as a trough concentration in the clinical situation [20]. TDM of CsA was further justified by poor correlations ($P < 0.50$) between CsA dose and abbreviated AUC for both formulations.

For both formulations of CsA, there were no significant differences in the observed blood concentrations or PK parameters obtained from either the FPIA or RIA methods. This finding has previously been reported by other investigators [21]. Also, in good agreement with previous reports, values obtained with the HPLC method were lower than those obtained by either FPIA or RIA methods [22].

The discrepancy between the immunoassays and the
HPLC method is due to the specificity of the HPLC method for the parent drug. Both the FPIA and RIA methods exhibit some cross-reactivity with numerous CsA metabolites [9–12]. However, these two assays do not exhibit the same cross-reactivity patterns, which has led to some concern over the interpretation of the results obtained by the immunoassay procedures. There is some suggestion that this cross-reactivity with metabolites could result in misleading and variable estimates of parent drug because of the interindividual variation in rates of metabolite formation and clearance [23].

The primary focus of the present study was to examine the effect that assay methodology has on variability of CsA blood concentrations and calculated PK parameters. However, we found that the interindividual variability in the blood concentrations and the PK parameters was comparable between all three methods. This suggests that the metabolites do not have a strong influence on the interindividual variability observed in patients receiving CsA and, in turn, the cross-reactivity seen with both the FPIA and RIA methods does not negate the validity of the results obtained by these assays.

One should take into account, however, that all the results reported here are from renal transplant patients. For heart and liver transplant patients, an increase is seen in the variability in absorption and clearance, which leads to an increase in variability in metabolite concentrations [24]. In these situations, assays such as FPIA and RIA, which cross-react with CsA metabolites, may show greater variability in CsA concentrations and PK parameters as compared with the more parent-drug-specific HPLC method.

In conclusion, the data presented here suggest that the new formulation of CsA, CsA-N, offers PK advantages over CsA-S. Variations in CsA concentration and PK parameters observed for CsA-N and CsA-S are equivalent among the assays studied, confirming the clinical validity of immunoassay procedures for the routine TDM of CsA in the clinical setting.

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References