Abbreviated Kinetic Profiles in Area-under-the-Curve Monitoring of Cyclosporine Therapy

Joachim Grevel1,2 and Barry D. Kahan1

Abbreviated kinetic profiles can reduce the number of phlebotomies and drug assays, and thereby the cost of area-under-the-curve (AUC) monitoring. In the present investigation, we used two independent data sets: group 1, 101 AUC profiles from 77 stable renal-transplant patients, which included a 5-h sample in addition to the usual 0-, 2-, 4-, 6-, 10-, 14-, and 24-h samples; and group 2, 100 profiles from 50 stable renal-transplant patients before and after a change in their daily oral dose of cyclosporine. Group 1 demonstrated a fair correlation between cyclosporine trough concentrations and the AUC calculated from a complete set of seven concentrations ($r^2 = 0.820$ and 0.758 for the 24- and 0-h samples, respectively). Stepwise multiple linear-regression analysis revealed that the abbreviated set of three time points (2, 6, and 14 h) explained 96% of the variance in AUC values calculated from the full set of seven samples; additional time points increased the accuracy only slightly. For group 2, we examined the difference between the observed and the predicted concentrations by linear extrapolation; the error in the observed AUC value, compared with the predicted value calculated from seven time points (~$-13.2\%$ to $-1.2\%$), was similar to the error from just three time points (~$-11.5\%$ to $4.5\%$). Abbreviated AUC profiles involving three time points used with a model equation seem to provide a reliable alternative to full seven-point profiles.

Additional Keyphrases: renal transplantation · pharmacokinetics

Two approaches have been advocated for therapeutic drug monitoring of immunosuppressive therapy with cyclosporine (CsA): trough concentrations and complete pharmacokinetic profiles (area-under-the-curve, AUC) (1–8). Although monitoring trough concentrations of CsA may be useful in extreme cases of poor gastrointestinal absorption or rapid drug metabolism, such determinations are less useful for diagnosis or for prediction of adverse events. A better index of drug exposure is provided by the AUC, calculated by the trapezoidal method from results for CsA concentrations in seven timed blood samples obtained at 0, 2, 4, 6, 10, 14, and 24 h after drug administration. The average concentration (AUC/dosing interval in hours) at steady-state ($C_{\text{ss}}$) shows a better correlation with the probability of acute renal-transplant rejection than the trough concentration (9).

To reduce the number of samples for pharmacokinetic profiles, Johnston et al. (10) suggested that a single concentration at 5 h after dosing (twice daily) correlated fairly well ($r^2 = 0.89$) with the full AUC calculated from 13 data points. Using a model constructed from the pharmacokinetic data for 10 renal-transplant patients, they predicted the AUC values for a separate group of 10 heart-transplant patients in whom they measured only a single, 5-h concentration. Although the predicted AUCs were markedly different from the measured AUCs, they still were correlated fairly well ($r^2 = 0.867$). Interestingly, Cantarovich et al. (11) suggested that a single 6-h concentration of CsA within a 24-h dosing interval displayed a better correlation with clinical outcome than did trough concentrations. Here, we assess the benefit of individual concentrations and abbreviated pharmacokinetic profiles to predict absolute values of and dose-related changes in the AUC for a 127-patient cohort of renal-transplant patients.

Materials and Methods

Patients

Two consecutive cohorts of renal-transplant patients were treated with a standardized CsA–prednisone regimen of once-daily drug administration, as described previously (3). Group 1, 77 stable patients who were tested two weeks to five years post-transplantation after at least three days of unaltered once-daily dosing, included 10% repeat transplants, 19% diabetic recipients, and 42% nonwhite patients. The 101 pharmacokinetic profiles of group 1 patients at clinical steady-state were based on analyses of blood samples taken at 0, 2, 4, 5, 6, 10, 14, and 24 h after dosing. The CsA doses ranged from 125 to 1200 mg/24 h with a median value of 375 mg/24 h and a 95% confidence interval (all confidence intervals by signed-rank test) of 343–407 mg/24 h. We examined these profiles for correlation between full AUC values and the drug concentrations at each time point.

The group 2 cohort consisted of 50 different, stable patients, who were tested between six and 24 months post-transplantation. None of these subjects were included in group 1. Each patient underwent two full pharmacokinetic profiles: one before and one after a change in the oral CsA dose. The interval between the two pharmacokinetic profiles ranged from five to 156 days (median = 40; 95% confidence interval = 22–37 days). The initial doses ranged from 250 to 1050 mg/24 h (median = 425; 95% confidence interval = 366–484 mg/24 h). The doses after the change ranged from 125 to

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Divisions of 1 Immunology and Organ Transplantation, Department of Surgery, and 2 Clinical Pharmacology, Department of Pharmacology, The University of Texas Medical School at Houston, 6431 Fannin, MSB 8.252, Houston, TX 77030.

Presented in part at the 91st annual meeting of the American Society for Clinical Pharmacology and Therapeutics, March 1990.

Received April 18, 1991; accepted August 14, 1991.
900 mg/24 h (median = 400; 95% confidence interval = 354 to 446 mg/24 h). Ten doses were increased and 40 were reduced. The increases in dose ranged from 5% to 40% (median = 8%; 95% confidence interval = −1% to 17%), whereas the reductions ranged from −4% to −55% (median = −11%; 95% confidence interval = −13% to −8%).

CsA Assay

Each 3-mL venous blood sample was drawn into a tube containing EDTA, disodium salt, as an anticoagulant. The blood samples were stored at room temperature for <24 h before analysis by a specific monoclonal RIA with $^{3}$H tracer (Sandoz Pharmaceuticals, East Hanover, NJ), according to published methods (12, 13). All assays were performed between September 1989 and May 1990, and fulfilled a Medicare-approved control scheme.

Statistical Analysis

We assessed correlations between individual time-point concentrations of group 1 pharmacokinetic profiles and the AUC calculated by the trapezoidal rule from the seven concentrations in the full profile (0, 2, 4, 6, 10, 14, and 24 h) by linear-regression analysis combined with one-way analysis of variance (RS/1 release 4, 1989; BBN Software Products Corp., Cambridge, MA). We tested combinations of several individual concentrations for the ability to predict the result of the full AUC by multiple linear-regression analysis (RS/1). A complete model containing the standard set of seven concentrations plus the additional 5-h time point as independent variables was stepwise reduced by removing the concentration with the least significant contribution. Similarly, a minimal model containing only the concentration with the highest correlation was expanded stepwise. Both approaches led to the same optimal model.

Model for Development of Abbreviated Profiles

The average concentration derived from seven samples obtained at steady-state (C$_{\text{ssav}}$) was expressed as:

$$C_{\text{ssav}} = \frac{\text{AUC}(t)}{\tau}$$  \hspace{1cm} (1)

where AUC(7) is calculated from seven timed samples by using the trapezoidal rule, and $\tau$ is the dosing interval, i.e., 24 h.

A model that predicted the value of the seven time-point AUC from three concentrations at 2, 6, and 14 h [(CONC(2), CONC(6), and CONC(14)] was selected by regression analysis because it was the smallest model that explained more than 95% of the variability in the data set. Model-derived average concentrations at steady-state (C$_{\text{ssav-M}}$) were thus calculated as:

$$\langle C_{\text{ssav-M}} \rangle = \frac{\text{AUC}(M)}{\tau}$$  \hspace{1cm} (2)

where AUC(M) is the model-predicted AUC derived from the following equation:

$$\text{AUC}(M) = 2.91 \times \text{CONC}(2) + 5.95 \times \text{CONC}(6) + 11.68 \times \text{CONC}(14) + 153$$  \hspace{1cm} (3)

The 5-h time point was not included in the stepwise regression analysis because it offered no more information than did the 4- and 6-h values, and because it was included in group 1 only temporarily to test the findings of Johnston et al. (10).

With the pharmacokinetic profiles of group 2 patients, we assessed the proportionality between the oral drug dose and paired values of C$_{\text{ssav-M}}$ and C$_{\text{ssav}}$, as well as each of the seven individual CsA concentrations. The small variance of the error demonstrated that these concentrations were linearly related to the dosage adjustment, namely,

$$E\% = \frac{100 \left[ C_{\text{post}} - (C_{\text{pre}} + f \times C_{\text{pre}}) \right]}{(C_{\text{pre}} + f \times C_{\text{pre}})}$$  \hspace{1cm} (4)

where $E\%$ is the error or deviation from a proportionate relation of dose to CsA concentration; $C_{\text{post}}$ and $C_{\text{pre}}$ are the concentrations (e.g., $C_{\text{ssav}}$, $C_{\text{ssav-M}}$, or individual concentration) after and before the change in dosing, respectively; $f$ is the proportionality factor that describes the change in the oral dose rate from $D_1$ (pre) to $D_2$ (post). $f$ is zero when the dose is not changed:

$$f = \frac{(D_2 - D_1)}{D_1}$$  \hspace{1cm} (5)

The bias of the error from 0% was determined by estimating the 95% confidence intervals by parametric (Student’s $t$-test) and nonparametric (signed-rank test) hypotheses, which are based upon gaussian and log-gaussian distributions of data, respectively.

Results

Correlation between single time points and C$_{\text{ssav}}$ for a single pharmacokinetic profile. The addition of a 5-h sample to the standard seven time-point procedure failed to change the AUC value appreciably at clinical steady-state. We found a high degree of correlation between the 101 group 1 AUC values calculated from seven and eight concentrations ($r^2 = 0.998$). Table 1 shows that no single, individual concentration explained >82% of the variability described by the seven time-point AUC. A higher percentage was explained by using more than one concentration of the profile as an independent variable; three concentrations (2, 6, and 14 h) yielded a correlation ($r^2$ of 0.963 (Table 2). Including more than three concentrations improved these results only marginally. Indeed, the C$_{\text{ssav}}$ calculated from a seven time-point pharmacokinetic study correlated well with the value derived from the three-point 2-, 6-, and 14-h AUC (C$_{\text{ssav-M}}$) (Figure 1).

Linearity of individual time points vs full AUC profiles during CsA dose adjustment. Figure 2 addresses the proportionality between CsA concentrations and changes in oral drug doses. The error between predicted and measured CsA concentrations after dose adjustment
is depicted by lines showing 95% confidence intervals with reference to the ideal 0% error. Although $C_{\text{peak}}$ showed the smallest interval, it carried a negative bias by not reaching 0%. $C_{\text{peak-M}}$ showed a slightly higher confidence interval, which was not associated with a negative bias. Among the seven concentrations at individual time points, the one at 14 h was associated with the smallest error (95% confidence interval, -11.6% to 9.8%). Concentrations for samples corresponding to possible times of peak drug absorption (2, 4, 6, or 10 h) showed the poorest performance.

**Discussion**

Clinical CsA therapy remains fraught with uncertainty because of the pleiotropic array of toxic complications (14). Although trough blood concentrations may be useful, pharmacokinetic profiles based on CsA concentrations at seven time points over a 24-h dosing interval seem to offer a more valid index of drug exposure (3, 4, 9). Here, we show that no single concentration within the seven-point profile can predict satisfactorily the AUC value calculated from the entire study. Trough concentrations measured exactly 24 h after drug administration showed a better correlation than 0-h values ($r^2 = 0.820$ vs 0.758, respectively), probably attributable to the usual error in obtaining a properly timed baseline sample, whereas peak values showed poor correlations. However, for clinical application, the capacity of trough concentrations to explain only 82% of the variability in the AUC is an unreliable substitute. The study presented here shows that a model equation based on the three CsA concentrations obtained 2, 6, and 14 h after oral drug administration has a high predictive accuracy that is only marginally improved by using more than three time points.

These findings with CsA dosing once daily dispute the conclusion of a recent report (10) involving a data set of 10 profiles in 10 renal-transplant patients receiving CsA doses twice daily, which suggested that a single 5-h concentration showed a better correlation with the AUC value ($r^2 = 0.894$) than the 0- or 12-h trough concentrations ($r^2 = 0.68$ and 0.73, respectively). Because both studies used the same monoclonal antibody to measure CsA concentrations in whole blood, it is curious that they reached opposite conclusions about the importance of peak vs trough drug concentrations. Whether this is associated with some paradoxical relation of concentration to dosing interval, or more likely to the size of the underlying databases, should be elucidated by our ongoing studies in patients on a much-less-frequent twice-daily dosing regimen of CsA, a therapeutic program administered at our center. However, both studies show that a linear model equation derived from three concentrations provides an excellent substitute for complete AUC measurements. In the report of Johnston et al. (10), 3.5-, 8-, and 10-h concentrations within a 12-h profile predicted a 12-h AUC with $r^2 = 0.9888$; in the study we report, 2-, 6-, and 14-h concentrations provided a value with $r^2 = 0.963$.

The model-derived $C_{\text{peak-M}}$ was virtually identical to $C_{\text{peak}}$ over the entire dosage range used in the present study, and showed a tighter confidence interval than any individual concentration. Although the interval was wider than that for $C_{\text{peak}}$, it included the 0% value, and was therefore not as markedly biased as the $C_{\text{peak}}$.

The error estimates presented here were based on the assumption of a linear relation between concentration change and the oral dose rate. This assumption was deemed reasonable because of the limited dose changes, namely, from a median value of 425 to 400 mg/24 h, and because of the rather short time between the two pharmacokinetic profiles (median interval, 30 days). However, we have occasionally observed pharmacokinetic

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**Table 1. Correlation between Individual Concentrations and Corresponding AUCs Calculated from Seven Concentrations**

<table>
<thead>
<tr>
<th>Time of concn, h after dose</th>
<th>Slope,* h</th>
<th>Intercept,* ng \cdot h/mL</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>41.7</td>
<td>2583</td>
<td>0.820</td>
</tr>
<tr>
<td>0</td>
<td>47.6</td>
<td>1952</td>
<td>0.758</td>
</tr>
<tr>
<td>6</td>
<td>14.9</td>
<td>90</td>
<td>0.726</td>
</tr>
<tr>
<td>10</td>
<td>22.2</td>
<td>1400</td>
<td>0.684</td>
</tr>
<tr>
<td>14</td>
<td>30.3</td>
<td>1636</td>
<td>0.678</td>
</tr>
<tr>
<td>5</td>
<td>15.4</td>
<td>-1415</td>
<td>0.600</td>
</tr>
<tr>
<td>4</td>
<td>16.4</td>
<td>-3410</td>
<td>0.439</td>
</tr>
<tr>
<td>2</td>
<td>15.9</td>
<td>-4667</td>
<td>0.350</td>
</tr>
</tbody>
</table>

* AUC calculated by the trapezoidal rule from seven concentrations = slope \times concentration at specific time + intercept.

* Correlations between individual concentrations and full AUC values based on 101 data sets ($P = 0.0001$ by analysis of variance).

**Table 2. Multiple Linear-Regression Analysis to Predict the AUC Calculated from Seven Concentrations**

<table>
<thead>
<tr>
<th>No. of concns included in equation</th>
<th>Time points, h after dose</th>
<th>Model equations*: $\text{AUC(M)} =$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6, 14</td>
<td>7.15 \cdot \text{CONC(6)} + 12.28 \cdot \text{CONC(14)} + 1795</td>
<td>0.880</td>
</tr>
<tr>
<td>3</td>
<td>2, 6, 14</td>
<td>2.91 \cdot \text{CONC(2)} + 5.96 \cdot \text{CONC(6)} + 11.68 \cdot \text{CONC(14)} + 153</td>
<td>0.963</td>
</tr>
<tr>
<td>4</td>
<td>2, 4, 6, 14</td>
<td>2.47 \cdot \text{CONC(2)} + 1.81 \cdot \text{CONC(4)} + 4.68 \cdot \text{CONC(6)} + 12.18 \cdot \text{CONC(14)} + 213</td>
<td>0.995</td>
</tr>
<tr>
<td>5</td>
<td>2, 4, 6, 10, 14</td>
<td>2.39 \cdot \text{CONC(2)} + 2.05 \cdot \text{CONC(4)} + 3.45 \cdot \text{CONC(6)} + 4.15 \cdot \text{CONC(10)} + 9.21 \cdot \text{CONC(14) + 298}</td>
<td>0.995</td>
</tr>
<tr>
<td>6</td>
<td>2, 4, 6, 10, 14, 24</td>
<td>2.04 \cdot \text{CONC(2)} + 2.02 \cdot \text{CONC(4)} + 2.93 \cdot \text{CONC(6)} + 4.03 \cdot \text{CONC(10)} + 7.00 \cdot \text{CONC(14)} + 5.89 \cdot \text{CONC(24)} + 5.74</td>
<td>0.999</td>
</tr>
</tbody>
</table>

* AUC(M), AUC predicted by a model; \text{CONC}(i), individual concentration at the $i$th time point. All models were highly significant ($P < 0.001$ by analysis of variance). The analysis was based on 101 profiles in 77 stable transplant patients.
nonlinearity in the early, postrenal-transplant period when the oral dose rate is changed drastically (15).

These studies suggest that $C_{\text{mean}}$-M offers a reliable substitute for $C_{\text{mean}}$ particularly in situations where reduced blood sampling may be appropriate—e.g., severely anemic patients, Jehovah's Witnesses, or small pediatric transplant recipients—or where time or financial constraints demand abbreviated pharmacokinetic profiles. Abbreviated three-sample AUC studies reduce both the length of observation (from 24 to 14 h) and the assay costs (by 57%). Because this method seems to reduce the length of initial hospitalization as well as the number of outpatient visits and CsA dose changes (B. D. Kahan, unpublished data), the further advantage of a reduced observation time for blood sampling from 24 to 14 h places the AUC method within the capability of most transplant center outpatient facilities. In ongoing studies, we are comparing the utility of this approach with monitoring that of trough concentrations, not only for diagnosis, but also for prediction of adverse clinical events.

We gratefully acknowledge the skills of Ms. M. Welsh in supervising data collection, of Ms. P. Hartman for computer analysis, and of Ms. C. Albers for editorial assistance. This work was supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases, Grant no. DK38016.

References