Influence of Sampling-Time Error on Cyclosporine Measurements Nominally at 2 Hours After Administration, Frank Saint-Marcoux,1 Annick Rousseau,1,2 Yann Le Meur,3 Marc Estenne,4 Christiane Knoop,4 Jean Debord,1 and Pierre Marquet1 (Departments of 1Pharmacology and Toxicology and 2Nephrology, University Hospital, 87042 Limoges cedex, France; 3Laboratory of Biophysics, Faculty of Pharmacy, 87025 Limoges cedex, France; 4Departments of Chest Medicine and Clinical Chemistry, Erasme University Hospital, 1070 Brussels, Belgium; *address correspondence to this author at: Service de Pharmacologie et Toxicologie, CHU Dupuytren, 87042 Limoges cedex, France; fax 33-55-05-61-62, e-mail marquet@unilim.fr)

Cyclosporine (CsA) blood concentrations measured 2 h after Neoral® administration (c2) are a sensitive predictor of clinical outcome in organ transplantation, as suggested by a recent prospective clinical trial in liver transplant patients (1). c2 is now recommended as the target exposure index for the therapeutic drug monitoring (TDM) of CsA (2–8). The aim of this study was to investigate, for different types of grafts, the concentration–time relationships around c2 to evaluate the concentration error as a function of the sampling-time error and to identify the sampling-time range compatible with acceptable performance of this c2 TDM strategy.

Data obtained from three different clinical trials were studied retrospectively. Each patient gave written informed consent, and each trial was approved by a local ethics committee (9–11). The three populations were as follows:

- Twenty stable adult renal transplant patients (grafted for more than 3 months and with stable renal function), from whom samples were collected immediately before a morning dose (t0) and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, and 9 h post dose. These 20 full profiles were used for this study.
- Sixteen heart transplant patients for whom three full pharmacokinetic profiles (12 samples) were obtained, at 1 week (W1), 12 weeks (M3), and 52 weeks (Y1) after transplantation. Samples were collected immediately before and at 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 9, and 12 h after a morning dose. Sixteen profiles were available at W1, and 29 were available for M3 and Y1 combined. We previously found no statistical difference between these two time periods with respect to pharmacokinetic parameters, whereas there were significant differences between W1 and either M3 or Y1. For this reason the last two periods were pooled (11).
- Twenty stable adult lung transplant patients with (n = 10) and without (n = 10) cystic fibrosis. Three full pharmacokinetic profiles were collected within 1 week for each patient at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 12 h after a morning dose. A total of 29 profiles were obtained from cystic fibrosis transplant patients, and 30 from non-cystic fibrosis patients.

All the patients from these three clinical trials were dosed twice daily with microemulsified CsA (Neoral). The data are summarized in Table 1.

For both kidney and lung transplant patients, CsA whole-blood concentrations were measured using an enzyme-multiplied immunoassay technique (Emit; Dade-Behring Diagnostics). This method has an upper limit of quantification of 500 µg/L. In the clinical trial conducted with cardiac transplant recipients, whole-blood CsA was measured with a fluorescence polarization immunoassay (FPIA; Abbott TDx). This method has an upper limit of quantification of 1500 µg/L. For both assays, samples with CsA concentrations greater than the upper assay range were diluted 1:4 with human blank whole blood (100 µL of sample + 300 µL of blank whole blood) and then reanalyzed.

Individual pharmacokinetic profiles were fitted using

<table>
<thead>
<tr>
<th>Table 1. Mean CsA exposure indices in the populations studied.</th>
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<tr>
<td>Type of graft</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>Stable (&gt;3 months)</td>
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<tr>
<td>Lung</td>
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<td>Cystic fibrosis</td>
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<td>Non-cystic fibrosis</td>
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<td>Heart</td>
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<tr>
<td>De novo (week 1)</td>
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<td>Stable (month 3 and year 1)</td>
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nonlinear regression (NLR) to a two-compartment pharmacokinetic model where the absorption phase is described by a $\gamma$ distribution \cite{12}. This pharmacokinetic model was specifically designed previously to deal with oral CsA profiles and was validated in the patient data sets analyzed here \cite{9,11,13}.

The consistency between observed and calculated concentrations within the first 4 h post dose was studied within each population. Seven or eight time points (i.e., 0.33, 0.66, 1, 1.5, 2, 3, and 4 h for all plus 2.5 h in heart transplant patients) were taken into account, representing a total of 140, 360, 203, and 210 concentrations for renal, heart, and lung cystic and noncystic transplant recipients, respectively. The relative differences between observed and predicted concentrations at all time points between 0 and 4 h post dose and the root mean squared error \cite{14} were calculated using Excel (Microsoft). Regression and correlation analyses were performed using Statview (Abacus Concept).

For each patient and each profile, concentration values within ±15 min around 120 min post dose (i.e., at 105, 110, 115, 125, 130, and 135 min) were estimated by NLR using the same pharmacokinetic model.

The relative concentration error (RCE) with respect to the concentration actually measured at 120 min ($c_{120}$) post dose was then computed as follows:

$$\text{RCE} (%) = \frac{(c_{\text{Calc}} - c_{120})}{c_{120}}$$

where $c_{\text{Calc}}$ is the calculated concentration at sampling times $t = 105, 110, 115, 125, 130$, and 135 min post dose using NLR; and $c_{120}$ ("true") is the measured concentration at $t = 120$ min. The mean RCE values were calculated for each virtual sampling time and each type of graft separately.

A total of 124 full blood CsA concentration profiles over 12 h were analyzed for the present study. We found excellent correlation over the first 4 h after dosing between observed concentrations and concentration values calculated using NLR. Correlation coefficients ($r^2$) varied from 0.972 for cystic fibrosis transplant patients to 0.985 for renal recipients. Intercepts and slopes were not significantly different from 0 and 1, respectively. The good predictive performance resulted from the very small and nonsignificant differences between measured and calcu-
lated concentrations [mean (SD) differences, 0.6 (4.5)% 0.7 (3.9)%, 0.2 (13)% and 0.8 (9.6)% for renal, heart, lung cystic fibrosis, and lung non-cystic fibrosis transplant patients, respectively] and good precision (root mean squared error, 1.1–5.7%).

The calculated RCE values at each studied sampling time for each profile for the five different populations are shown in Fig. 1. For each transplant patient, whatever the type of graft, the RCE value was within ± 20% in a sampling-time interval from 110 to 130 min. A sampling-time error of ± 15 min produced a RCE >20% (up to 30%) in a few heart and lung transplant patients. As can be inferred from Fig. 1, in many patients $t_{\text{max}}$ was later than $c_2$, which could be designated as “delayed absorption”. The present data set included patients with a $t_{\text{max}}$ up to 5 h. In renal transplant patients, overestimation of $c_2$ was observed for $t < 120$ min and underestimation for $t > 120$ min. In lung and heart transplant patients, over-as well as underestimation of $c_2$ could be observed regardless of the time error, depending on the patient.

On the basis of data obtained in renal, heart, and lung transplant patients and using a validated pharmacokinetic method, the present study shows that when the sampling-time error around 2 h post dose increases, the relative concentration error and its interindividual variability also increase significantly. Although the impact of sampling-time error differed with the type of graft, an acceptable (± 20%) estimation of the true $c_2$ value was obtained within a time-error range of ± 10 min for all 124 profiles. Interestingly, for renal transplant patients, the mean RCE was always <10% within this time range.

The $c_2$ target values defined for CsA TDM in renal and de novo liver transplant patients have been proposed with a range of ± 20% (e.g., 1.7 mg/L ± 20% for the 0–1 month post-transplantation period in renal transplantation) (4). We compared this range with the interlaboratory CV values of the International Cyclosporin Proficiency Testing Scheme, taken as estimates of the analytical error (15). For concentrations <500 µg/L (n = 12; results for the year 2002), the mean CVs were 10% for the Emit and 6.0% for the FPIA. The interlaboratory CV obtained with a whole blood sample to which 2000 µg/L cyclosporin had been added was 7.9% for the Emit (n = 38) and 7.2% for the FPIA (n = 30).

In summary, numerous studies have promoted a Neoral monitoring strategy using CsA blood concentrations measured 2 h after drug administration, called $c_2$, to improve the clinical benefits for transplant patients. Guidelines for $c_2$ interpretations propose target values with a range of ± 20%. The present study shows that the accuracy of $c_2$ monitoring is highly dependent on the correct sampling time and that a substantial difference (up to 30%) from the 2-h values (which are themselves subject to analytical inaccuracy and imprecision) can occur with a sampling-time error of 15 min. Consequently, such time errors could lead to inappropriate dose adjustment and to inadequate immunosuppression or increased risk of adverse effects. Timing errors of ± 10 min seem to be the acceptable limit for use of $c_2$ and subsequent dose adjustment of CsA.

References


Denaturing HPLC Procedure for Factor IX Gene Scanning, Giuseppe Castaldo,1,2 Paolo Nardello,3 Fabiana Bellitti,1 Angiola Rocino,1 Antonio Coppola,4 Giovanni di Minno,4 and Francesco Salvatore1 (1 Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli “Federico II” and CEINGE-Biotechnologie Avanzate, I-80131 Napoli, Italy; 2 Facoltà di Scienze, Università del Molise, 86100 Isernia, Italy; 3 Centro Emofilia e Trombosi, Ospedale S.G. Bosco, 80141 Napoli, Italy; 4 Centro di Coordinamento Regionale Emocoagulopatie, Dipartimento di Medicina Clinica e Sperimentale, Università di Napoli “Federico II”, Napoli, Italy; * address correspondence to this author at: Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli “Federico II”, via S. Pansini 5, I-80131 Naples, Italy; fax 39-081-746-3650, e-mail salvator@unina.it)

Hemophilia B (HB) is an X-linked recessive bleeding disorder caused by mutations that produce factor IX (FIX)