Therapeutic Monitoring of Cyclosporine*†

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ABSTRACT

Therapeutic monitoring of immunosuppressive therapy with cyclosporine is a critical requirement because of intra- and interpatient variability of drug absorption, narrow therapeutic window and drug induced nephrotoxicity. The most widely used procedure involves measuring trough concentrations of cyclosporine (CsA) parent drug in whole blood using a specific monoclonal immunoassay. Trough concentrations generally correlate with adverse clinical events, e.g., episodes of rejection and nephrotoxicity. However, they do not correlate consistently with chronic events to allow prediction of adverse outcome. There is now increasing interest in using CsA pharmacokinetic profiling as a means of monitoring. This approach appears to offer a more valid index of drug exposure and hence a better prediction of clinical outcome. Nevertheless, multiple blood sample requirement and the complexity of pharmacokinetic profiling impose practical concern. The overall merit of either approach awaits further studies comparing clinical efficacy, technical requirements, and economic issues.

Introduction

Since the release of cyclosporine (CsA) for use in clinical transplantation in 1983, therapeutic monitoring of CsA has been the subject of numerous publications each year and the focus of much discussion at several national and international conferences. This underscores the incomplete understanding of CsA pharmacokinetic and the mechanism of CsA action even after a decade of use. From its inception, the objectives of CsA monitoring have been to optimize immunosuppression, discriminate rejection and drug-induced toxicity, and individualize dose regimen. Unfortunately, major difficulties exist with achieving these goals. They include variable CsA absorption and elimination, narrow therapeutic "window", large between-patient and within-patient variability, the presence of many metabolites with ill defined pharmacologic properties, and drug interaction. The availability of multiple

* Presented at the Frontiers in Clinical Science Meeting of the Association of Clinical Scientists, May 14-17, 1992, Syracuse, New York.
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methodologies for measuring CsA and its metabolites has further compounded the confusion.

In response to the need for improving and standardizing CsA monitoring, a task force for CsA therapeutic monitoring was established in 1987. A recent workshop on therapeutic CsA monitoring was recently held. The critical issues of CsA monitoring were discussed among participants comprising clinicians, laboratory scientists, pharmaceutical companies, and manufacturers of CsA kits. This meeting was closely followed by another consensus meeting on CsA monitoring which was held in response to the request of the Canadian Bureau of Drug Research Health Protection Branch. The charge of this Canadian group was to develop a “state-of-art” consensus paper on CsA monitoring. Inasmuch as most of the guidelines reached by the Canadian group were based on similar data and rationale as the Hawk’s Cay group, this Canadian consensus document also contains recommendations on certain controversial topics that were discussed but without reaching a consensus at the Hawk’s Cay meeting.

In this communication, some major considerations that most affect practical therapeutic CsA monitoring are reviewed. They include: (1) the selection of sample matrix, (2) the choice of analytical method to generate the most clinically useful results, and (3) the appropriate site for specimen collection to achieve the most optimal outcome, time of specimen collection and dosing intervals. Recommendations and guidelines on CsA monitoring included in this review are obtained from the most recent consensus reports published on the Hawk’s Cay and the Canadian meetings. Unresolved issues that were discussed at these meetings were also highlighted.

Background

Cyclosporine is a cyclic peptide consisting of 11 amino acid residues. Its structure was established by chemical degradation and x-ray crystallography and was confirmed by magnetic resonance spectroscopy. Absorption of CsA is through the gastrointestinal tract, with a peak concentration of 2.8 ± 1.1 hours and a mean bioavailability of 20 to 50 percent. Cyclosporine partitions between blood and plasma and binds to plasma proteins. The drug is metabolized extensively by the liver cytochrome P450 enzyme system. Thus far 24 metabolites have been found, and at least 12 have been identified structurally. The major metabolites are M17 and M1, then M21 and M8. In the past few years, intense investigation has focused on uncovering possible clinical relevance of metabolites to either immunosuppression or nephrotoxicity. Presently, there is no conclusive evidence to indicate that the metabolites cause side-effects or immunosuppression compared to CsA. Although Rosano et al reported about 10 percent immunosuppressive activity in metabolite M17, the authors themselves recommended caution in result interpretation because of the general incompatibility of animal models used in the studies compared to humans.

Biliary excretion is the main pathway of CsA elimination. Levels of CsA are affected when co-administered drugs act to induce or inhibit the common metabolic pathway. Inducers of the P450 system, e.g., barbiturates or phenytoin, tend to increase the elimination of CsA, which may lead to inadequate immunosuppression and acute rejection. Other drugs, such as erythromycin and verapamil,
inhibit metabolism of CsA, which may result in increased levels of CsA and predispose the patient to nephrotoxicity. Blood levels are also affected by gastrointestinal disorders, liver diseases, and food intake. Significantly decreased absorption of CsA caused by diarrhea has been reported both in bone marrow transplant recipients and in pediatric liver transplant recipients. Therefore in these patients, CsA should be administered intravenously to ensure adequate immunosuppression. While liver disease causes impairment in absorption of CsA on the one hand, it also decreases clearance of CsA which is primarily via hepatic metabolism. The extent of impairment in absorption of CsA appears to be related to the degree of cholestasis as measured by serum bilirubin assay. Available studies on the effect of lipids or food on absorption of CsA have been controversial and inconclusive. Keown et al observed marked influence of food on the bioavailability and pharmacokinetics of the drug in one study, but reported in a later study that food did not show a consistent effect. The discrepancy might be attributed to experimental designs that did not allow steady-state conditions to be maintained. The influence of time interval between drug administration and food intake was studied at our institution in kidney transplant recipients receiving CsA therapy. As shown in figure 1, absorption of CsA is significantly greater in patients who took the drug 1 hour after breakfast than in patients taking CsA with breakfast. Nephrotoxicity is the most frequent and clinically important complication associated with CsA therapy. In most cases, it is reversible by drug dosage reduction or complete withdrawal of therapy. However, the differentiation between nephrotoxic effects and rejection phenomena is a difficult determination, an experience that is shared by many transplant centers. Clinical features that are indicative of acute rejection, such as persistent fever, weight gain, oliguria, hypertension, and graft tenderness seem to be also symptoms associated with nephrotoxicity. Measurements of CsA do not always correlate with clinical events; other concomitant laboratory measurements, histopathologic evidence and renal biopsy are necessary to arrive at an unequivocal diagnosis. Other adverse effects of CsA include: hepatotoxicity, gingival hyperplasia, hirsutism, and the development of lymphoma.

**Type of Body Fluid Used in the Assay**

The choice of serum, plasma, or whole blood influences the concentrations of CsA because CsA and its metabolites partition between erythrocytes and plasma, and their distribution is reversible and temperature dependent. Decreasing temperature favors partitioning of CsA into the red blood cells. The advantages and limitations of measuring CsA in whole blood, plasma, or serum have been pre-
sented in detail elsewhere. Although plasma or serum concentration of CSA is more reflective of free drug concentration, use of either matrix requires that the temperature at which specimen separation must be rigidly specified and maintained to allow meaningful interpretation of day-to-day results. Furthermore, a changing hematocrit value, which frequently occurs during the first few months after transplant, will also affect CSA results. Whole blood is recommended over plasma or serum as the specimen of choice to avoid stringent sample separation protocol, to free interference from changing hematocrit, and to attain better assay performance with higher levels of CSA in whole blood.

Methodology Considerations

The measurement of CSA is complicated by its nonpolar nature, the presence of numerous metabolites, and its binding to proteins and hemoglobin. Many procedures for determination of CSA have been developed based on two primary techniques: high performance liquid chromatography (HPLC) and immunoassay.

Determination of CSA by HPLC

The determination of CSA by HPLC allows definitive separation and quantitation of the parent drug from its metabolites and is considered the reference procedure for the CSA parent compound. The procedure is technically demanding and time consuming, requiring lysis of red blood cell and extraction of CSA prior to chromatography. Despite its technical complexity, the value of determination of CSA by HPLC as an investigational pharmacokinetics tool and its ability to individually identify and measure metabolites of CSA cannot presently be replaced by immunoassay procedures.

Determination of CSA by Immunoassay

During the last few years, immunoassay technology for determination of CSA had undergone several important modifications with the result that different assay kits capable of measuring different CsAs are available on the market (table I). The first radioimmunoassay (RIA) kit for determination of CSA was developed by Sandoz* using tritium tracer and a polyclonal antiserum that cross reacts substantially with metabolites of CSA. Thus concentrations of CSA measured by RIA can be 30 to 100 percent higher than those obtained by HPLC, and the correlation between these two methods is poor. Nonetheless, this polyclonal RIA kit was used by many transplant centers because of its technical simplicity compared with HPLC and its adaptability for assaying large number of samples in batched mode.

One of the most important developments in the therapeutic monitoring of CSA has been the introduction of monoclonal antibody assays for the parent drug. This innovation was prompted by the general uncertainty of metabolite contribution to immunosuppression and nephrotoxicity. A specific RIA assay kit was developed by Sandoz in 1987 using tritium tracer. In vitro analysis of the monoclonal antibody demonstrated only limited cross-reactivity with the major CSA metabolites. Results from clinical samples correlated well between the Sandoz RIA and HPLC, and RIA/HPLC ratios of 0.96 to 1.2 have been reported.

A specific 125I-RIA kit for determination of CSA was subsequently developed† using the Sandoz antiserum. While these two RIAs correlate well with each other for samples from heart, kidney and liver

* Basel, Switzerland.
† Incstar, Stillwater, MN.
TABLE I

Available Immunoassays for Cyclosporine and Cyclosporine + Metabolites

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>Manufacturer</th>
<th>Assay Characteristics</th>
</tr>
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<tbody>
<tr>
<td>³H–RIA</td>
<td>Sandoz</td>
<td>Monoclonal antibody. Selective for parent drug.</td>
</tr>
<tr>
<td>¹²⁵I–RIA</td>
<td>Incstar</td>
<td>Monoclonal antibody. Selective for parent drug.</td>
</tr>
<tr>
<td>EMIT</td>
<td>DuPont</td>
<td>Monoclonal antibody. Specific for parent drug.</td>
</tr>
</tbody>
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RIA = Radioimmunoassay.
FPIA–P = Fluorescence polarization immunoassay using a polyclonal antiserum.
FPIA–M = Fluorescence polarization immunoassay using a monoclonal antiserum.
EMIT = Enzyme multiplied immunoassay technique.

transplants, the Incstar assay overestimated concentrations of CsA by at least 20 percent and up to 40 percent in another study. Despite the disparity, the Incstar ¹²⁵I–RIA kit has become an important assay for clinical monitoring, primarily because it shows consistent proportionality to HPLC even under stringent conditions, such as with heart and liver transplantations.

An automated fluorescence polarization immunoassay for CsA using polyclonal antiserum (FPIA–P) from Abbott Diagnostics was available in 1989. Being an automated procedure, this assay demonstrates good precision and sensitivity, provides more rapid turnaround time, and requires less technical expertise than all existing RIA or HPLC procedures. Because of its cross-reactivity with CsA metabolites, assay results are three to four times higher than those obtained with an HPLC procedure. It correlates poorly with either the HPLC or the Incstar RIA. One report found the FPIA–P results severely influenced by abnormal γ-glutamyl transferase and abnormal creatinine concentrations and raised the concern of its clinical utility in monitoring patients with coexisting liver and renal dysfunction.

In response to the increased use of monitoring of CsA and the current consensus of parent drug measurement, a monoclonal FPIA assay (FPIA–M) was recently developed by Abbott in an attempt to provide a rapid and specific means for routine monitoring of CsA. One study reported a positive bias of 6 to 8 percent compare with a specific RIA and 14 to 20 percent compared with an

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‡ Abbott Park, IL.
HPLC procedure. Another study comparing FPIA-M, HPLC and the Incstar RIA reported good correlations among these methods. However, results obtained from clinical samples differ significantly with FPIA-M results averaged 20 percent and 32 percent higher than those obtained by the Incstar RIA and by HPLC, respectively. This was attributed to antibody cross-reactivity with some CsA metabolites. Thus far, available data have shown this assay to be clinically useful for routine CsA monitoring of renal transplant recipients in whom ratio of metabolites/parent compound is relatively constant. In heart and liver transplant recipients with high concentration of metabolites especially during the immediate post transplant period and in patients with severe liver dysfunction, caution in the interpretation of CsA results obtained with the FPIA-M assay is recommended. Another specific assay for measuring CsA parent compound using the principle of enzyme-multiplied immunoassay has recently become available. Results from this assay appear to be compatible with those from HPLC and other immunoassay methods.

**Appropriate Time and Site for Monitoring of CsA**

**TROUGH LEVEL MONITORING**

Because of the large intra- and inter-subject differences in pharmacokinetics of CsA, measurements of single “peak” concentrations are not recommended because they do not correlate with drug toxicity and are difficult to interpret. The most widely used method for monitoring CsA is the determination of trough concentrations 24 h- (if receiving one dose/day) or 12 h- (if receiving 2 doses/day) after the dose of CsA. This allows the patient’s clinical condition and concentration of CsA to become stabilized and places the determination on a more reproducible part of the drug curve.

In general, trough concentrations show a certain degree of correlation with adverse events: low concentrations with transplant rejection and high concentrations with drug induced nephrotoxicity. Studies from several transplant centers have suggested that adjustment of dosage to maintain target concentrations in whole blood, as measured by HPLC or specific RIAs, within a narrow range reduce the risks for transplant rejection and nephrotoxicity. Target ranges for CsA with available assays are usually established based on studies of patient outcome versus drug level conducted at large transplant centers to guide dosage.

Unfortunately, the correlation between CsA trough concentrations and chronic events has not been optimally consistent. In fact, quiescent unaffected transplant patients on CsA therapy often display a wide spectrum of trough values some of which exceed the targeted therapeutic intervals. Failure of the trough measurement to reflect the total drug exposure during dosing interval is suggested to be an important cause. An example of such encounter in a CsA treated kidney transplant patient who was on verapamil is shown in figure 2. There is also the concern that with trough level monitoring, the therapeutic intervals may be difficult to standardize because of influence by age, type and time of transplant, co-administration of other drugs, and drug interaction.

**AREA UNDER THE CURVE MONITORING**

There is increasing interest in the use of pharmacokinetics profiling to individualize CsA therapy. The so called area-under-the-curve (AUC) monitoring requires collection and measurement of several timed blood specimens between dosing interval. The calculated average concentration, AUC/hour dosing interval,
appears to provide better correlation with the likelihood of subsequent rejection or drug-induced toxic events.

Monitoring of CsA by pharmacokinetics profiling reflects total drug exposure since it provides information on drug absorption, drug clearance, and elimination rate. It is suggested that AUC monitoring may improve the outcome of therapy and provide additional benefits of reduced routine visit, reduced dosing changes and eliminations of spurious trough values. However, the need for multiple blood sampling to perform pharmacokinetics profile is more costly, requires greater expertise and places added burden on the laboratory and added discomfort on the patient. Presently, attempts at reducing the number of blood samples for estimating average CsA concentrations are in progress. The practicality of routine AUC monitoring awaits the outcome of cost effective assessment of the abbreviated procedure against the patient's clinical events.

**Conclusion**

The following recommendations and guidelines have been formulated by both the Hawk's Cay participants and the Canadian Consensus Group who evaluated the therapeutic monitoring issues facing the transplant field:

1. After the efforts of over a decade, significant improvement in CsA assay methodology has been achieved. An automated procedure now enables the measurement of the parent drug with acceptable sensitivity, specificity, and ease of performance. It is recommended that strict intra- and interlaboratory quality assurance programs be instituted to ensure standardization of CsA assays.

2. Since whole blood trough concentrations of CsA by specific assays show diagnostic efficacy, the use of whole blood matrix and specific assay for CsA parent compound is recommended. Although wide ranges of trough values in quiescent patients have no prognostic significance, they can discriminate cause of deteriorating renal function with the help of clinical, laboratory, and histopathologic indices to differentiate rejection from drug-induced toxicity.

3. Quality Assurance programs are necessary for assays of CsA to provide ongoing feedbacks to participating laboratories regarding consistency of their performance and the interlaboratory comparison. A well-characterized CsA preparation should be available in whole blood for distribution to all laboratories for comparing standards of CsA in routine use.

4. A consensus therapeutic range for CsA should be established based on the recommended use of specific assay in a whole blood matrix for routine monitoring. Before adopting a therapeutic
range established elsewhere, a laboratory must ensure that the method of analysis and the criteria for diagnosis of clinical events be compatible with those used to establish the range.\textsuperscript{26}

5. The confusing array of metabolites of CsA with nonstandardized nomenclatures has prompted the recommendation of a more consistent coding system for metabolites to denote the molecular position of the metabolic biotransformation, \textit{i.e.}, according to the position of oxidation. It is now generally agreed that data existing thus far have failed to document a correlation of metabolite concentrations and clinical events. Routine monitoring of metabolites is not warranted in the majority of clinical situations.\textsuperscript{12}

6. Pharmacokinetics profiling to estimate total drug exposure shows promise for prediction of initial dosage and for adjustment of subsequent doses, if indicated.\textsuperscript{12}

7. There is a need for the measurement of biological activity of CsA to explain the apparent between-individual variability in immunologic sensitivity.\textsuperscript{12}

Several pertinent issues were discussed at the Hawks’ Cay meeting which still remain unresolved. The major concern appears to center on the clinical usefulness of measurement of CsA. Participants questioned if the concentrations of CsA or CsA plus metabolites are providing sufficiently sensitive and specific to predict adverse events in post-transplant patients to justify the cost of performing the assays? Would the availability of automated methodologies improve the validity of the measurement of CsA? Can tissue receptor assays differentiate between immunosuppressive and toxic action of CsA? If yes, what matrix would be suitable? Can rigid dosing and drug level control (\textit{e.g.}, by AUC monitoring) provide a useful framework to assess immunosuppressive activity and renal injury? Can this approach guide diagnostic interpretation of adverse post-transplant events?

References


