Optimization of pediatric haematopoietic stem cell transplant outcomes through the application of pharmacokinetics and supportive care
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Determination of Area Under the Whole Blood Concentration versus Time Curve after First Intravenous Cyclosporine Dose in Children Undergoing Hematopoietic Stem Cell Transplant: Limited Sampling Strategies

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Abstract: Achievement of target trough cyclosporine whole blood concentrations after hematopoietic stem cell transplant (HSCT) reduces the risk of acute graft versus host disease (aGvHD). In solid organ transplant, prevention of acute graft rejection correlates with achievement of target area under the whole blood concentration versus time curve during the 12-hour dosing interval (AUC-12) after oral administration. This study describes a limited sampling strategy for determination of cyclosporine AUC-12 after administration of the first intravenous (IV) dose in children undergoing HSCT and explores the relationships between individual whole blood concentrations during the dosing interval and the AUC.

Children undergoing HSCT and receiving cyclosporine prophylaxis were eligible to participate. The first cyclosporine dose was given as a 2 hour infusion, and eight cyclosporine concentrations were determined at 2 (end of the infusion), 2.5, 3, 4, 6, 8, 10, and 12 hours after the start of the IV infusion. The relationship between AUC-12 and whole blood cyclosporine concentrations at individual time points after IV administration was assessed by the Spearman rho correlation coefficient. Limited sampling strategies were developed using three to six time points by way of multiple linear regression analysis. The agreement between the AUC-12 calculated using all eight data points and the limited sampling strategies was assessed by intraclass coefficient and Bland-Altman analysis.

Twenty-four children (0.5–16.9 yr) participated. The mean cyclosporine dose was 2793 ± 1165.6 μg/L-hr. Whole blood cyclosporine concentrations obtained within the first 4 hours from the start of the infusion correlated strongly with AUC-12 (Spearman rho coefficient, 0.717–0.868). Limited sampling strategies were developed to estimate AUC-12 with adjusted r² of 0.955 to 0.998, mean bias of 0% to 0.93%, and precision of 1.6% to 8.1%. The actual AUC-12 and predicted AUC-12 values agreed strongly (intraclass coefficient, 0.981–0.999).

Limited sampling strategies using three to six data points have been developed that will estimate cyclosporine AUC-12 after administration of the first IV dose given over 2 hours. Information regarding the possible association between aGvHD and cyclosporine AUC-12 is not available. The limited sampling strategies described here will facilitate the prospective evaluation of the clinical importance of cyclosporine AUC-12 in the prevention of aGvHD.

Key Words: cyclosporine, pharmacokinetics, hematopoietic stem cell transplant, pediatrics

INTRODUCTION

Cyclosporine, in conjunction with methotrexate or methylprednisolone, is commonly used to prevent acute graft versus host disease (aGvHD) in patients undergoing allogeneic hematopoietic stem cell transplant (HSCT).1,2 In the solid organ transplant arena, cyclosporine dose intensity as described by the area under the whole blood concentration versus time curve (AUC) has been shown to be important for effective prevention of acute graft rejection in both adult and pediatric solid organ transplant patients.3,4 The maximal immunosuppressant effect of cyclosporine is exerted in the first 4 hours after oral administration,5 and, in solid organ transplant patients, the AUC achieved during this period is highly predictive of graft rejection.6 For solid organ transplant patients, the AUC achieved during the first 4 hours after an oral cyclosporine dose is highly variable because of inter- and intra-patient differences in the pattern of absorption and the extent of bioavailability. After oral administration, the cyclosporine concentration taken 2 hours after the dose (C2) correlates well with the AUC achieved during the first 4 hours after the dose and with prevention of graft rejection.3,7 Conversely, the
through cyclosporine concentration (C0) achieved after an oral dose does not correlate well with either total AUC over the 12 hour dosing interval (AUC-12) or the AUC achieved during the first 4 hours after the dose (AUC-4). Thus, in adult renal and liver transplant patients, it is recommended that the oral cyclosporine (Neoral) dose be individualized to achieve a C2 of 600 to 2000 µg/L, depending on the type of organ transplanted and the time from the date of transplant.8,14

We and several other investigators have reported that the achievement of certain minimum C0 values before engraftment is associated with a reduced risk of developing aGvHD.8-11 Unlike solid organ transplant patients, patients undergoing HSCT often experience extensive mucositis and are most likely to receive intravenous (IV) rather than oral cyclosporine during the time period critical to the success of aGvHD prevention. Thus, extrapolation of the C2 monitoring recommendation of solid organ transplant to the HSCT setting may well be inappropriate, at least immediately after HSCT. Prospective trials are necessary to determine whether AUC after IV cyclosporine administration correlates with aGvHD outcomes in HSCT and which single sample time point best correlates with the target AUC during the critical period. The blood sampling schedule that is traditionally required for AUC determination is problematic in children because of concerns regarding the possible clinical consequences of frequent blood sampling and withdrawing large blood volumes.12 A limited sampling strategy that would reliably determine AUC after IV cyclosporine administration would facilitate future studies of the clinical significance of cyclosporine AUC in patients undergoing HSCT.

The goal of the current study was to describe a limited sampling strategy for determination of cyclosporine AUC-12 after administration of the first IV dose as a 2 hour infusion in children undergoing HSCT. In addition, the relationships between individual whole blood concentrations taken at various times during the dosing interval and the AUC-12 were explored.

METHODS

This study was approved by the Research Ethics Committee, The Hospital for Sick Children, and formed part of a larger, prospective study of cyclosporine pharmacokinetics after both IV and oral administration during HSCT. Methods and findings pertaining to the oral phase of this study have been published in full elsewhere.13 Children who were undergoing HSCT and who were scheduled to receive cyclosporine as aGvHD prophylaxis were eligible to participate. Patients with impaired liver function (defined as transaminase concentrations >3 times the upper limit of normal for age or total bilirubin concentrations >2 mg/dL [34 µmol/L]) were excluded, as were patients receiving drugs known to substantially alter cyclosporine disposition (eg, oral fluconazole, itraconazole, erythromycin, carbamazepine, clarithromycin, and rifampin). However, patients who received phenytoin for the purpose of prophylaxis of seizures secondary to busulfan during the week before IV cyclosporine were eligible. Consumption of grapefruit or grapefruit juice was not permitted.

The day of HSCT was designated as day 0. The first IV cyclosporine dose was given at 0500 hours on day 1 as a 2 hour infusion by way of a nonpolyvinyl chloride IV set through one lumen of a double-lumen, nonpolyvinyl chloride-containing, central venous catheter. Whole blood samples for cyclosporine measurement were obtained after this first IV cyclosporine dose at 2 (end of the infusion), 2.5, 3, 4, 6, 8, 10, and 12 hours after the start of the IV infusion by way of the lumen of the central venous catheter not used for cyclosporine administration. Whole blood cyclosporine concentrations were measured by means of specific high-pressure liquid chromatography.14 The limit of quantification of this assay was 5 µg/L, and reproducibility of sample quantification was demonstrated at concentrations as low as 13 µg/L. The observed within day coefficient of variation of the assay was 0.2% to 4.8%, whereas the observed between day coefficient of variation was less than 6%.

AUC-12 was determined for each patient using all eight data points and standard noncompartmental methods (WinNonLin version 5.0.1; Scientific Consulting, Inc., Mountain View, CA). Because AUC-12 was calculated after the first dose, the whole blood cyclosporine concentration was assumed to be 0 µg/L before dose administration (time 0).

The relationship between AUC-12 and whole blood cyclosporine concentrations at individual time points after IV administration was assessed by the Spearman rho correlation coefficient. Limited sampling strategies were developed using three to six time points by way of multiple linear regression analysis. The association between the AUC-12 calculated using all eight data points and the AUC-12 values predicted by each limited sampling strategy was described using the adjusted coefficient of determination (r²); values greater than 0.9 were considered acceptable. Bias and precision of the limited sampling strategies were measured using the mean prediction error and the root mean squared prediction error, respectively.15 Bias and precision values of less than 15% were considered to be acceptable.16

The agreement between the AUC-12 calculated using all eight data points and the limited sampling strategies was assessed by single measures, one-way random effect intraclass coefficient (ICC). Agreement was considered to be acceptable if the lower limit of the 95% confidence limit of the ICC was 0.8 or higher. Extent of agreement was also assessed by Bland-Altman analysis,17,18 although clinically acceptable limits of agreement could not be set because the target AUC-12 and the clinical significance of differences in AUC-12 relative to the development of aGvHD are unknown. The limits of agreement (mean % difference ± 2 SD; where % difference = 100 [(AUC-12 – AUC-LSS)/ mean of AUC-12 and AUC-LSS]) were calculated. Statistical analysis was performed using SPSS 14.0.1 (Chicago, IL). Mean data are reported ± standard deviation. A P value of 0.05 or less was considered to indicate statistical significance.

RESULTS

Demographic data relating to the 24 study patients have been published previously.13 Patients ranged in age from 0.5 to 16.9 (mean, 8.8 ± 4.81) years; 16 were boys. Most patients (18/24) underwent HSCT for leukemia. Ten patients had received phenytoin during the week before administration of the first IV cyclosporine dose.
The mean IV cyclosporine dose administered was 1.51 ± 0.05 mg/kg based on actual weight. Of note, two patients had whole blood concentrations below the limit of quantification of the assay 10 hours and 12 hours after initiation of the cyclosporine infusion. The whole blood cyclosporine concentrations for these patients were considered to be 0 at these time points for the purposes of AUC-12 calculation.

Whole blood cyclosporine concentrations drawn within the first 4 hours from the start of the infusion correlated strongly with AUC-12 (Table 1) (Spearman rho coefficient = 0.717–0.868). Significant, although moderate, correlations were observed at all other time points. Details regarding the limited sampling strategies that met the criteria for acceptability (ie, adjusted \( r^2 > 0.9 \), mean bias < 15% and precision <15%) and the extent of their agreement with the AUC-12 calculated using all eight data points are presented in Tables 2 and 3. The Bland-Altman plot for the three-point limited sampling strategy is presented in Figure 1. The mean actual AUC-12 and the values predicted by the limited sampling strategies are presented in Table 4.

**DISCUSSION**

We have developed several three- to six-point limited sampling strategies that can be used to estimate the AUC-12 in children receiving IV cyclosporine. In addition, we observed moderate to strong correlations between AUC-12 and whole blood cyclosporine concentrations at individual time points, including the C2 and the trough concentrations. This information will facilitate the evaluation of cyclosporine AUC-12 as a factor influencing the effectiveness of acute GvHD prophylaxis in children undergoing HSCT.

Monitoring of C2 after oral cyclosporine administration, a surrogate for AUC-12 monitoring, is the current standard of care in solid organ transplant recipients.\(^6\) This practice is supported by elegant prospective studies that clearly describe the correlation between improved patient outcomes and dose adjustment to achieve AUC-12 and C2 targets.\(^6,7,19\) Although HSCT clinicians can certainly learn from the experience of solid organ transplant clinicians, the practice of C2 monitoring cannot be applied directly to the HSCT setting given the lack of information regarding the relationship between AUC-12 and HSCT outcomes or between individual cyclosporine concentrations and AUC-12. Because HSCT patients are most often unable or unwilling to take oral medications early after transplant and because gut function may be impaired after transplant,\(^20\) most patients receive IV cyclosporine before engraftment, the period critical to its effectiveness.\(^11\) We have chosen to describe AUC determination after the first IV cyclosporine dose as a preliminary step toward subsequent investigations of the relationship between HSCT outcomes and the AUC achieved from administration of the first cyclosporine dose through to engraftment.

In solid organ transplant, the practice of C2 monitoring grew from the need to develop a practical technique of estimating AUC in patients receiving oral cyclosporine in an outpatient setting. The cyclosporine concentration at the end of a 2 hour IV infusion will depend heavily on the rate of infusion before the end of the infusion period. Because infusion rates may be increased or decreased during the intended infusion period, the concentration at the end of a 2 hour infusion (C2) may not be a reliable or consistent surrogate for AUC. In our patients, the whole blood cyclosporine concentration 3 hours after the start of a 2 hour cyclosporine infusion was most strongly correlated with AUC-12 (Spearman rho coefficient = 0.868; \( P < 0.0005 \)). Clearly, the relationship between whole blood cyclosporine concentrations at individual time points after IV administration and AUC-12 and the utility of dose adjustment to achieve a single target concentration other than the trough concentration require further investigation. The availability of a limited sampling strategy to estimate AUC-12 with confidence will facilitate such investigations in pediatrics in which minimization of blood sampling and blood sample volumes is especially important.

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**TABLE 2.** Limited Sampling Strategies for Intravenous Cyclosporine Determined by Multiple Linear Regression Analysis

<table>
<thead>
<tr>
<th>Sample Times (Hours from Start of 2 hr Infusion)</th>
<th>Limited Sampling Strategy (LSS) Equation</th>
<th>Adjusted ( r^2 )</th>
<th>Mean Bias, % (95% Confidence Interval)</th>
<th>Precision (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 6 and 8</td>
<td>LSS 3-point: 1.429C2 + 5.722C6 + 2.741C8 + 380.862</td>
<td>0.955</td>
<td>0.93 (−3.1 to 5)</td>
<td>8.1</td>
</tr>
<tr>
<td>2, 2.5, 6 and 8</td>
<td>LSS 4-point: 1.372C2 + 5.449C6 + 2.870C8 + 0.470C2.5 + 165.709</td>
<td>0.991</td>
<td>0 (−1.6 to 1.6)</td>
<td>3.5</td>
</tr>
<tr>
<td>2, 2.5, 4, 6 and 8</td>
<td>LSS 5-point: 1.229C2 + 5.242C6 + 0.740C8 + 0.514C2.5 + 2.7C4 + 23.740</td>
<td>0.997</td>
<td>0 (−0.8 to 0.8)</td>
<td>1.9</td>
</tr>
<tr>
<td>2, 2.5, 4, 6, 8 and 10</td>
<td>LSS 6-point: 1.253C2 + 3.798C8 + 1.171C6 + 0.519C2.5 + 2.514C4 + 1.476C10 + 14.590</td>
<td>0.998</td>
<td>0 (−0.6 to 0.6)</td>
<td>1.6</td>
</tr>
</tbody>
</table>
The limited sampling strategies presented in Tables 2 and 3 display excellent precision and minimal bias. On Bland-Altman analysis, the three-point strategy displays limits of agreement that are much wider than other strategies. Thus, despite the findings of adequate agreement on the basis of ICC and other analysis, the three-point strategy may not be as reliable a predictor of AUC-12 as other strategies. The selection of a particular limited sampling strategy from among the remaining strategies described may be based on practical considerations such as the availability of staff to obtain blood samples in individual HSCT centers. Certainly, it appears likely that cyclosporine AUC-12 may well be estimated with reasonable confidence using as few as four properly timed samples.

Prospective validation of these limited sampling strategies will determine the optimum strategy from among those identified in this study.

The limited strategies described here apply only to children undergoing HSCT and receiving the first IV cyclosporine dose as a 2 hour infusion. Other strategies must be developed and evaluated to determine AUC after IV cyclosporine doses other than the first. This study is furthermore limited by its small sample size. Thus, it is not possible to describe the possible impact of genetic polymorphisms, lipoprotein concentrations, or other factors that are known to alter cyclosporine disposition. Ideally, a Bland-Altman analysis that incorporates an a priori assessment of the clinical agreement

![FIGURE 1. Bland-Altman plot. Percent difference against mean for actual and predicted area under whole blood cyclosporine concentration versus time curve during 12 hour dosing interval (AUC-12) generated by 4-point limited sampling strategy (equation provided in Table 2). Percent difference = 100 multiplied by (AUC-12 calculated using all 8 data points minus AUC-12 calculated using the limited sampling strategy) divided by mean of actual AUC and predicted AUC.](image)

| TABLE 3. Extent of Agreement Between AUC-12 Values Predicted by Limited Sampling Strategies and AUC-12 Values Calculated Using Eight Data Points |
|-----------------|-------------|----------------|-------------|
| Limited Sampling Strategy (LSS) | ICC (95% CI) | Limits of Agreement* (%) |
| LSS 3-point | 0.981 (0.957–0.992) | −21.5 to 22.3 |
| LSS 4-point | 0.997 (0.992–0.998) | −8.0 to 8.0 |
| LSS 5-point | 0.999 (0.998–1.0) | −4.0 to 4.0 |
| LSS 6-point | 0.999 (0.998–1.0) | −3.2 to 3.2 |

Equations for each limited sampling strategy are provided in Table 2. AUC-12, area under whole blood cyclosporine concentration versus time curve during 12 hour dosing interval; ICC, intraclass coefficient; CI, confidence interval.

*As per Bland-Altman analysis.

| TABLE 4. Mean Actual and Predicted AUC-12 Values Generated by Limited Sampling Strategies in 24 Children Receiving Intravenous Cyclosporine as 2 Hour Infusion |
|-----------------|-------------|-------------|
| Mean AUC-12 (±SD) (µg/L.h) | 95% CI |
| Actual AUC | 2793 ± 1165.6 | 2301 to 3285 |
| LSS 3-point | 2792 ± 1142.1 | 2310 to 3275 |
| LSS 4-point | 2793 ± 1161.5 | 2303 to 3284 |
| LSS 5-point | 2793 ± 1164.4 | 2301 to 3285 |
| LSS 6-point | 2792 ± 1164.5 | 2301 to 3284 |

Equations for each limited sampling strategy are provided in Table 2. AUC-12, area under whole blood cyclosporine concentration versus time curve during 12 hour dosing interval; CI, confidence interval; LSS, limited sampling strategy.
between AUC-12 values would be applied to these data to evaluate the clinical agreement between the actual AUC-12 values and those predicted by the limited sampling strategies. This was not attempted because the target AUC and the clinical significance of differences in AUC relative to the development of aGvHD are unknown.

CONCLUSION

Several limited sampling strategies for the determination of AUC-12 after administration of the first IV cyclosporine as a 2 hour infusion to children undergoing HSCT have been described. These limited sampling strategies will be helpful in future investigations of the relationship between AUC-12 after the first IV cyclosporine dose and the development of aGvHD and other HSCT outcomes. Individual whole blood cyclosporine concentration values within the first 4 hours after the initiation of the 2 hour cyclosporine infusion correlate most strongly with AUC-12. The clinical utility and relevance of AUC-12 after the first and subsequent IV cyclosporine doses, as well as whole blood cyclosporine concentrations other than the trough concentration, require prospective investigation.

REFERENCES