Limited Sampling Model for Area under the Concentration Time Curve of Total Topotecan

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ABSTRACT

Antitumor activity of topotecan, a new derivative of camptothecin, has been documented in many tumors. The active lactone form of topotecan is in equilibrium with the inactive hydroxyacid form. However, dose-limiting toxicity, neutropenia, is correlated to the area under the concentration time curve (AUC) of not only the lactone form but also total (lactone + hydroxyacid) topotecan. Because the determination of the total topotecan plasma concentration is technically much easier than the lactone, we sought to establish a limited sampling model for the AUC of total topotecan. Thirty-four pharmacokinetic profiles were obtained in 19 patients in a Phase I study of topotecan, which was infused over 30 min for 5 days. Multiple regression models predicting the AUC were developed using 17 profiles and validated using the rest of the data. The best model was:

\[ \text{AUC}_{\text{pred}} (\text{ng} \times \text{h/ml}) = 1.75 \times C_{15\text{m}} (\text{ng/ml}) + 11.2 \times C_{6\text{h}} (\text{ng/ml}) + 7.90 \times \text{dose (mg/m}^2) \]

where AUCpred was the AUC predicted by the model, and C_{15\text{m}} and C_{6\text{h}} were the measured concentrations of total topotecan at 15 min and 6 h after the end of infusion, respectively. When this model was validated, it was unbiased (percentage of mean predicted error $\pm$ SE, $-1.0 \pm 3.3\%$) and precise (percentage of root mean square error, 11%). Because this model requires only two concentrations of total topotecan to estimate the AUC, it will be useful for further pharmacodynamic evaluation of topotecan in multi-institutional studies.

INTRODUCTION

Topotecan is a water-soluble semisynthetic analogue of camptothecin. Its impressive antitumor activity has been demonstrated in many preclinical tumor models (1). In Phase II studies, clinical activity has been documented in small cell (2, 3) and non-small cell lung cancer (4), head and neck cancer (5), ovarian cancer (6), malignant glioma, and soft tissue sarcoma (7). Clinical studies of combination chemotherapy with other anticancer agents are now being conducted (8-11). Daily administration for a prolonged time is associated with an increased antitumor effect in a xenograft system (12). Although different dosing schedules have been used in clinical Phase I studies (13-19), the most convincing evidence of antitumor activity has occurred with a daily (13-15) or continuous infusion (19) schedule.

In solution at physiological pH, topotecan exists in an equilibrium between the closed-ring lactone form and the open-ring hydroxyacid form. Although the closed lactone ring is believed to be necessary for the activity of topotecan, the correlation of the AUC or steady state concentration of total (lactone + hydroxyacid) topotecan to neutropenia was comparable with (16, 20) or even better than (21, 22) that of the lactone form. The lactone ring opens to form the hydroxyacid, achieving equilibrium rapidly, with a half-life of 18 min in plasma (23). Therefore, accurate measurement of lactone levels depends on immediate extraction of a blood sample at the bedside within 5 min of blood drawing (24), which creates an additional source of error. Because the interconversion between lactone and hydroxyacid is reversible and dependent primarily on pH, the correlation of the true lactone and total concentrations should be high, and observed interpatient variability in the ratio may be simply due to technical difficulties in the assay. Furthermore, antitumor activity of the open form has been observed in preclinical tumor systems (15, 22). Therefore, pharmacological evaluation of topotecan using the total concentration is theoretically appropriate. In addition, other investigators have found significant pharmacodynamic relationships between total concentrations of topotecan (and other camptothecin analogues) and toxicity (16, 20-22, 25).

A previous study using the same data demonstrated that the AUC of the lactone and hydroxyacid could be estimated by sampling at 2 h (26). However, this requires the measurement of lactone and hydroxyacid concentrations separately. In this study, we established a limited sampling model to estimate the AUC of total topotecan infused over 30 min for use in population pharmacodynamic studies in conjunction with Phase II clinical trials.

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The abbreviations used are: AUC, area under the concentration time curve; %MPE, percentage of mean predictive error; SE, standard error; %RMSE, percentage of root mean square error; AUC_{\text{pred}}, AUC predicted by a limited sampling model; C_{15\text{m}}, concentration of total topotecan at 15 min after the end of infusion; C_{6\text{h}}, concentration of total topotecan at 6 h after the end of infusion.
Limited Sampling Model for AUC of Total Topotecan was performed on day 1 in 19 patients and on day 4 or 5 in 15 individuals. Intrapatient dose escalation was not performed.

**Pharmacokinetic Studies.** Pharmacokinetic sampling was performed on day 1 in 19 patients and on day 4 or 5 in 15 patients. On each sampling day, 11 timed plasma samples were collected during and after the infusion. The closed and open forms of topotecan were quantitated by high-performance liquid chromatography (24), and the total concentration was calculated as the sum. The AUC of total topotecan was calculated using the trapezoidal method from zero to the last point of quantifiable concentration and from there to infinite time by exponential extrapolation using the elimination constant. Calculation of pharmacokinetic parameters was performed by the computer program PCNONLIN (SCI, Lexington, KY).

**Limited Sampling Model.** Thirty-four complete concentration time curves of total topotecan were obtained from 19 patients. After we confirmed that there was no correlation between the AUC on day 1 and the AUC on day 4 or 5 when they were normalized for the dose (mg/m²), we divided all data randomly into two groups: a training and a test data set of 17 profiles each. Using the training data set, dose-normalized concentrations at each time point were correlated to the dose-normalized AUC by linear regression analysis, and models with r ≥ 0.9 were selected for validation as a one-variable model. For models using two time points, every combination of two time points was tested by multiple linear regression analysis. The criteria for choosing a model for validation were a Pearson's r of the model > 0.95 and r < 0.3 between the two concentrations (independent variables). Because a satisfactory model was developed in this way, and because the sampling of three or more concentrations at each specific time ranged from 28 to 72%.

Three one-variable models were selected based on the correlation between the AUC on day 1 and the AUC on day 4 or 5 (r = 0.17; P = 0.54). Therefore, we used all data to develop the model. The coefficient of variation of the dose-normalized AUC was 36%. The coefficient of variation of the dose-normalized concentrations at each specific time ranged from 28 to 72%.

<table>
<thead>
<tr>
<th>Training data set</th>
<th>Validation data set</th>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>5.89</td>
</tr>
<tr>
<td>2</td>
<td>3.89</td>
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<tr>
<td>3</td>
<td>6.67</td>
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</tbody>
</table>

\( ^a \) C_t, concentration of total topotecan at time after the end of infusion.

\( ^b \) r, correlation coefficient between AUC_{\text{pred}} and actual AUC.

**RESULTS**

After normalization for dose, there was no correlation between the AUC on day 1 and the AUC on day 4 or 5 (r = 0.17; \( P = 0.54 \)). Therefore, we used all data to develop the model. The coefficient of variation of the dose-normalized AUC was 36%. The coefficient of variation of the dose-normalized concentrations at each specific time ranged from 28 to 72%.

Three one-variable models were selected based on the correlation coefficient. Each model used concentrations at 1, 2, and 3 h after the end of infusion (Table 1). When the %MPE and %RMSE were compared, the model with a concentration at 3 h after the infusion was the best. However, it still seemed to be biased and imprecise, considering that its %MPE and %RMSE were -6.6 and 16.7%, respectively.

After all possible combinations of two dose-normalized concentrations of total topotecan were tested for the prediction of dose-normalized AUC by multiple regression analysis, three pairs were selected: 10 min after the initiation of infusion plus 3 h after the end of infusion; 15 min after the initiation plus 3 h after the end of infusion; and 15 min and 6 h after the end of infusion (Table 2). When these models were validated and compared, the best one was the model that used \( C_{15 \text{min}} \) and \( C_{6 \text{h}} \) after the infusion (Table 2): AUC_{\text{pred}} (ng × h/ml) = 1.75 × \( C_{15 \text{min}} \) (ng/ml) + 11.2 × \( C_{6 \text{h}} \) (ng/ml) + 7.90 × dose (mg/m²).

We tested the correlation of \( C_{15 \text{min}} \) or \( C_{6 \text{h}} \) between the 2 sampling days, there was no correlation for \( C_{15 \text{min}} \) (r = 0.10; \( P = 0.79 \)) and a weak correlation for \( C_{6 \text{h}} \) (r = 0.52; \( P = 0.07 \)). We checked the validity of this model further by using eight sets of data and the validation data set separately; %MPE and %RMSE were 3.7 ± 3.9% and 10.7%, respectively, in day 1 data and 8.9 ± 4.2% and 12.2%, respectively, in day 4 or 5 data. Fig. 1 depicts a scatter plot of this model for both the training and test data sets.

**DISCUSSION**

The limited sampling model developed by multiple regression analysis in this study requires only two concentrations of total topotecan for the estimation of AUC. Developing a limited sampling model in this way is an established method to estimate...
Table 2  Limited sampling models for the AUC of total topotecan using two concentrations: \( \text{AUC}_{\text{pred}} \) (ng × h/mL) = \( A \times C_{t1} \) (ng/ml) + \( B \times C_{t2} \) (ng/ml) + \( C \times \text{dose (mg/m}^2) \)

| \( t \) (min) | \( t_2 \) (h) | A | B | C | \( n \) | \( r^b \) | \( \%\text{MPE} \pm \text{SE} \) (%) | \( \%\text{RMSE} \) (%) | Training data set | Validation data set | \( n \) | \( r^b \) | \( \%\text{MPE} \pm \text{SE} \) (%) | \( \%\text{RMSE} \) (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---||
| -20\(^c\) | 3\(^d\) | 0.44 | 6.74 | 15.0 | 16 | 0.99 | -0.1 ± 1.9 | 7.3 | 12 | 0.95 | -9.0 ± 4.0 | 16.1 |
| -15\(^c\) | 3\(^d\) | 0.36 | 6.50 | 16.5 | 17 | 0.99 | 0.1 ± 1.9 | 7.8 | 14 | 0.96 | -7.7 ± 4.1 | 16.7 |
| 15\(^c\) | 6\(^d\) | 1.75 | 11.2 | 7.90 | 16 | 0.99 | -1.0 ± 2.0 | 7.8 | 13 | 0.97 | -1.0 ± 3.3 | 11.4 |

\(^a\) \( C_{t1} \) and \( C_{t2} \), concentrations of total topotecan at times \( t_1 \) and \( t_2 \), respectively. 
\(^b\) \( r \), correlation coefficient between \( \text{AUC}_{\text{pred}} \) and actual AUC. 
\(^c\) -20 and -15, 20 and 15 min before the end of infusion, respectively. 
\(^d\) 15, 3, and 6, 15 min, 3 h, and 6 h after the end of infusion, respectively.

Fig. 1  Relationships between the AUC of total topotecan predicted by the limited sampling model and the actual AUC. The solid line represents the line of identity. The model uses \( C_{15m} \) and \( C_{6h} \) after the end of a 30-min infusion. \( \text{AUC}_{\text{pred}} \) (ng × h/mL) = \( 1.75 \times C_{15m} \) (ng/ml) + 11.2 \( \times C_{6h} \) (ng/ml) + 7.90 \( \times \text{dose (mg/m}^2) \). \( \triangle \), training data set; ■, validation data set.

AUC with the minimum number of blood samples for the purpose of pharmacological evaluation in a large, multiinstitutional study. Many models have been developed for anticancer drugs (28–31). Previously reported models for the AUC of lactone and hydroxyacid forms of topotecan (26) will be useful in the circumstances in which rapid extraction of plasma is possible. The model of this study requires only total topotecan concentrations. They can be measured by transforming the hydroxyacid to lactone by acidifying a plasma sample (15, 22), and immediate extraction is not necessary after plasma separation, which will facilitate the participation of community hospitals in pharmacological studies of this drug. To determine whether plasma must be separated immediately after blood drawing, further study of the stability of topotecan in whole blood will be necessary. By using the data of two points and by exploring models more extensively, we could increase the predictability compared with the previous model (26). Considering the convenience of measuring only total levels and the goodness of the model developed in this study, the evaluation of topotecan using this model in multi-institutional studies is favored.

A potential concern is that we used data for both day 1 and day 4 or 5 in 15 patients. Ideally, this model should be validated further using an independent data set. However, there was no correlation of AUC or \( C_{15m} \), only a weak correlation for \( C_{6h} \) between the 2 sampling days, and no collinearity between the two variables used in this model. Furthermore, this model was validated separately in the data of day 1 and in the data of day 4 or 5. Therefore, we believe that we can use this model as a robust method for estimating the AUC of total topotecan on either day 1 or day 4 or 5 of the 5-day dosing schedule.

The recommended model uses two concentrations at 15 min and 6 h after the end of a 30-min infusion. This model is preferable to the other two models (Table 2), because it avoids relying on sampling during infusion (which would be affected greatly by minor changes in infusion rate) and also has the lowest \( \%\text{MPE} \) and \( \%\text{RMSE} \) in the validation data set. We allowed 10% error in the sampling time while developing and validating models. Therefore, when this model is applied in future studies, it is necessary that the actual sampling times be within 10% of those defined in the model for the accurate estimation of AUC. The model also assumes that the topotecan is administered continuously over a 30-min period. We plan to use our model in a multi-institutional study of combination chemotherapy with topotecan and paclitaxel in patients with metastatic breast cancer.

REFERENCES

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