Limited Sampling Models for Amonafide (NSC 308847) Pharmacokinetics

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ABSTRACT

The limited sampling model (LSM) offers a means of estimating the area under the concentration-time curve (AUC) from only two timed plasma concentrations. In this study, pharmacokinetic profiles were simulated for 23 patients treated with amonafide, using each patient's individual pharmacokinetic parameters. Data were simulated for a dose of 250 mg/m² administered over 1 h. The initial 15 patients formed the training data set. Based on the training data set, five different LSMs were generated, with the multiple r ranging from 0.92 to 0.98. A single model was selected as optimal:

\[
\text{AUC} (\mu g \text{ min/ml}) = 292.9 \text{ (min) } C_{45} (\mu g/ml) + 3626 \text{ (min) } C_{\text{inf}} (\mu g/ml) + 21.8 (\mu g \text{ min/ml}) \text{ dose (mg/m²) } / 250 \text{ mg/m²}
\]

where \( C_{45} \) = 45-min plasma concentration and \( C_{\text{inf}} \) = 24-h plasma concentration. This model was revalidated on a second test data set of seven patients actually treated with a 1-h infusion. The relative root mean square predictive error was 15.8%, acceptable for most clinical uses. We conclude that the LSM is a powerful tool for estimation of the AUC in a large patient population. The LSM may facilitate population pharmacodynamic studies in conjunction with Phase II trials.

INTRODUCTION

Understanding the relationships between pharmacokinetic parameters, such as steady-state plasma concentration, and therapeutic or toxic outcomes has proven to be of clinical benefit in many fields of medicine. Until recently, few studies have been undertaken to examine the clinical pharmacodynamics of antineoplastic agents. Several recent studies have demonstrated that the AUC, a measure of total drug exposure, can be correlated with the extent of myelosuppression (1-4). Furthermore, Rodman and coworkers have demonstrated that the AUC after teniposide administration is related to antitumor response in children with acute lymphocytic leukemia (5). These relationships appear to be especially relevant for relatively non-phase-specific agents, since the effects of these drugs are generally schedule independent (6-8). Although the AUC is a useful pharmacokinetic parameter, its exact quantitation is inconvenient and costly, usually requiring the measurement of the plasma drug concentration at 10 to 20 time points.

One method of circumventing these problems is the LSM, recently described by Ratain and Vogelzang, and applied to vinblastine (9). This study demonstrated that the AUC for a small dose of vinblastine could be accurately estimated from only two serum concentrations obtained at 10 and 36 h after treatment. In the current study, we sought to develop a similar model for amonafide (10), a new intercalating agent (11) which recently completed Phase I testing (12-14). Amonafide was selected for this study primarily because of its similarity to menogaril (2) in both mechanism of action and dose-limiting toxicity, myelosuppression (13, 14). Furthermore, its linear pharmacokinetics (12) allowed development of a LSM based on data from patients treated at a wide range of doses and infusion durations.

MATERIALS AND METHODS

Patients. The 30 patients studied were part of two Phase I/pharmacokinetic trials of amonafide conducted at the Ohio State University Cancer Center (12). All patients had histological proof of malignant disease refractory to standard therapy, and acceptable end-organ function (serum bilirubin ≤ 1.5 mg/dl and serum creatinine ≤ 1.5 mg/dl). Written informed consent was obtained in accordance with federal and institutional guidelines.

Treatment Plan. Patients were treated with either a single monthly dose of amonafide \((n = 28)\) or on a daily \(\times 5\) (repeated monthly) schedule \((n = 2)\). In the single dose study, the patients received 92.0-697.8 mg/m² over 10 to 70 min. Both patients treated on the daily \(\times 5\) schedule received 121.6 mg/m²/day.

Pharmacokinetic Studies. For the pharmacokinetic analysis, approximately 20 timed plasma samples were collected for up to 72 h following each dose of amonafide, and the plasma concentrations were determined by high-performance liquid chromatography (15). The detection limit of the assay used was 0.5 ng/ml. The timed plasma concentrations were fit to a linear three-compartment model using NONLIN (16). The AUC (extrapolated to infinity) and plasma clearance were calculated by standard compartmental methods (17).

LSM Development. Initially, complete pharmacokinetic data were available for 23 patients. The first 15 patients formed the training data set for development of the LSM, and the remaining eight patients formed the test data set, for initial model validation.

During the course of the Phase I studies, a wide range of dose and infusion durations were employed. A dose of 250 mg/m² by 1-h infusion \((\times 5\) days) had been tentatively selected for Phase II studies. Thus, plasma concentration profiles for this dose were simulated for the first 23 patients based on their fitted parameters using standard pharmacokinetic transformations (17), since amonafide had previously been demonstrated to have linear pharmacokinetics (12). Plasma concentrations were simulated at 19 time points ranging from completion of the infusion to 24 h later. In addition, the previously determined AUC was also normalized for a dose of 250 mg/m².

Using the training data set (first 15 patients), a LSM was developed as previously described (9). Briefly, separate univariate analyses were performed for each time point versus the AUC, and a LSM was developed by stepwise forward multiple regression (RS/1; Bolt Beranek and Newman, Cambridge, MA). As described in “Results,” multiple LSMS could be developed by initiating the stepwise regression software program with variables other than the “best” univariate time point.

LSM Validation. Each of the LSMS generated from the training data set was validated on the first test data set of eight patients (also simulated data). For initial model validation, the estimated AUC was correlated with the actual AUC. In addition, the MPE and RMSE were also calculated as a measure of bias and precision, respectively (18). One model was then selected, as “optimal,” for future evaluation. This

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2 To whom requests for reprints should be addressed, at Division of Hematology/Oncology, Michael Reese Hospital and Medical Center, Lake Shore Drive at 31st Street, Chicago, IL 60616.

3 The abbreviations used are: AUC, area under the plasma concentration-time curve; LSM, limited sampling model; MPE, mean predictive error; RMSE, root mean squared predictive error.
A detailed analysis of the pharmacokinetics and metabolism of amonafide will be reported separately. There was significant interpatient pharmacokinetic variability in the simulated plasma amonafide concentrations for the initial 23 patients (training set and first test set) (Fig. 1). The coefficient of variation for each individual time point ranged from 24 to 62%, continuously increasing with time. In comparison, the coefficient of variation for the AUC was 28% (271 ± 76 µg min/ml). The mean AUC for the training data set was slightly higher than the mean AUC for the first test data set (290 ± 79 versus 234 ± 55 µg min/ml, P = 0.09).

LSM Development. The amonafide concentrations at each of the 19 time points were correlated with the total AUC, using the training data set. The correlation coefficient increased from 0.74 at 10 min and 15 min to 0.93 at 8 h, and then decreased to 0.76 at 24 h.

The simulated plasma concentrations at all 19 time points were subsequently utilized in a multiple regression analysis. As with the prior LSM developed for vinblastine (9), we restricted all LSMs to two or fewer time points. However, a distinctly optimal LSM could not be developed from the two best single time points, 8 h and 10 h, because of the high partial correlation coefficient between these two variables (r = 0.98). Using a standard stepwise forward multiple regression program, the initial model selected included the plasma concentrations at the end of the infusion (models A, C, and D, respectively, of Table 1). A fifth model was obtained using the concentrations at the end of the infusion and 24 h later, since their partial correlation coefficient was only 0.39 (model E of Table 1). The multiple r for these five LSMs ranged from 0.92 to 0.98. There was virtually no bias in any of the five models (MPE ranged from −1.7 × 10⁻⁷ to 5.7 × 10⁻⁷ µg min/ml), and a high degree of precision (RMSE ranged from 14.0 to 30.6 µg min/ml) was also demonstrated.

LSM Validation. At the time of initial model validation, the pharmacokinetic data were only available on the initial 23 patients. The five LSMs were each validated on the first test data set of eight patients. The correlation coefficient between the AUC estimated from the LSM and actual AUC (dose normalized) ranged from 0.94 to 0.98. More importantly, there was little bias, except for model B, and excellent precision with the RMSE ranging from 13.5 to 19.6 µg min/ml (Table 1).

Since the aim of this analysis was to develop a LSM which could easily be incorporated into an outpatient daily × 5 treatment schedule, model C was selected for future development (Fig. 2). This LSM requires sampling only at 45 min and 24 h after the end of the first day's infusion. Thus, the second sample could be obtained just prior to the second dose. In addition, model C had the lowest RMSE (equivalent to 5.7% of the mean AUC) and an acceptable degree of bias (2.6%). Model E also provides the advantages of convenient sampling, but has a higher RMSE (8.4%).

Model C was then validated on a second test data set of seven patients actually treated with a planned infusion duration of 1 h.

RESULTS

Interpatient Pharmacokinetic Variability. A detailed analysis of the pharmacokinetics and metabolism of amonafide will be reported separately. There was significant interpatient pharmacokinetic variability in the simulated plasma amonafide concentrations for the initial 23 patients (training set and first test set) (Fig. 1). The coefficient of variation for each individual time point ranged from 24 to 62%, continuously increasing with time. In comparison, the coefficient of variation for the AUC was 28% (271 ± 76 µg min/ml). The mean AUC for the training data set was slightly higher than the mean AUC for the first test data set (290 ± 79 versus 234 ± 55 µg min/ml, P = 0.09).

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Additional models could then be generated by varying the choice of the initial independent variable entered into the multiple-regression program. Three additional models were ob-tained using initial variables of 6 h, 24 h, and at the end of infusion (models B, C, and D, respectively, of Table 1). A fifth model was obtained using the concentrations at the end of the infusion and 24 h later, since their partial correlation coefficient was only 0.39 (model E of Table 1). The multiple r for these five LSMs ranged from 0.92 to 0.98. There was virtually no bias in any of the five models (MPE ranged from −1.7 × 10⁻⁷ to 5.7 × 10⁻⁷ µg min/ml), and a high degree of precision (RMSE ranged from 14.0 to 30.6 µg min/ml) was also demonstrated.

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Model C was then validated on a second test data set of seven patients actually treated with a planned infusion duration of 1 h (Table 2). There was significant interpatient pharmacokinetic variability in this second test data set, as the actual AUC ranged from 419.7 to 1149.4 µg min/ml and the total body clearance

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Table 1 Limited sampling models for estimation of the amonafide AUC

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (h)</th>
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<td>T₂</td>
<td>K₁</td>
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</tr>
<tr>
<td>D</td>
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<td>6</td>
<td>82.4</td>
<td>1099</td>
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<tr>
<td>E</td>
<td>0</td>
<td>24</td>
<td>137.7</td>
<td>2826</td>
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</table>

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Fig. 1. Summary of simulated pharmacokinetic data for first 23 patients. Points, mean simulated plasma concentration at the indicated time from the end of a 1-h infusion of 250 mg/m². Error bars, one standard deviation.

LSM was then validated on a second test data set of seven patients actually treated with a planned infusion duration of 1 h.

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The estimation of the AUC, but would obviously be less convenient. Unlike the prior LSM developed for vinblastine (9), the LSM for amonafide was developed using simulated data, based on previously calculated individual pharmacokinetic parameters. This was only possible because amonafide’s pharmacokinetics are linear (12). However, despite this potential flaw in the LSM, the model could subsequently be validated on a second test data set of actual data.

Although relatively slight errors in infusion duration or sampling time did not markedly affect the precision of this model (Table 2), large deviations from the protocol may invalidate its usefulness, particularly if the infusion is interrupted or significantly delayed. In contrast, the 24-h plasma level is relatively insensitive to errors in sampling time, as a 1-h error in sampling would result in approximately a 6% change in the plasma level, based on a mean terminal half-life of 11.3 h (12). The use of this methodology is subject to the caution that the precision of this model may be significantly less precise in those patients who have markedly abnormal pharmacokinetics.

Like most drugs, amonafide and many other anticancer agents exhibit marked interpatient pharmacokinetic variability. However, oncologists are limited in their ability to individualize dosing primarily due to a lack of data regarding the optimal plasma concentrations, or AUC. It is hoped that the development of LSMs for amonafide and other anticancer drugs will allow us to improve our knowledge of anticancer pharmacodynamics. As an example, companion pharmacodynamic studies are planned in conjunction with Phase II trials of amonafide in breast cancer, lymphoma, and pancreatic cancer. Two plasma samples will be collected after the first dose of each cycle and the estimated AUC will be used for further pharmacodynamic modeling. These studies will be based on the assumption that the total AUC is approximately equivalent to five times the AUC following the first dose. This is a reasonable assumption for amonafide, as its pharmacokinetics are known to be linear (12), but must be considered as a potential source of error in the use of this particular model, or in the development of LSMs for other drugs. It is hoped that this approach will eventually allow oncologists to better individualize the dosing of these highly toxic drugs.

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**REFERENCES**

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