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A limited sampling model for the pharmacokinetics of etoposide given orally

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Abstract. A limited sampling model of etoposide after oral administration to estimate the area under the plasma concentration-time curve from 0 to 24 h (AUC) by determination of the drug plasma levels at only two time points was developed by a multiple regression analysis on a training data set of 15 patients receiving oral doses ranging from 54 to 90 mg/m^2. The equation describing the model is AUC (μg ml^{-1} h) = 5.183 (μg ml^{-1} h) + 1.193 (h) × C_{1h} (μg/ml) + 8.439 (h) × C_{4h} (μg/ml) (R^2 = 0.93, P = 0.0001), where C_{1h} and C_{4h} represent the plasma etoposide concentrations at 1 and 4 h, respectively. The model was validated prospectively on a test data set of 13 patients receiving oral doses ranging from 52 to 87 mg/m^2 and, additionally, on a data set of 7 patients receiving oral doses ranging between 176 and 200 mg/m^2, investigated in a previous study. Validation on both test data sets gave a relative mean predictive error of 0.1% and a relative root mean square error of 15.8% and 16.7%, respectively. The present study shows that it is possible to obtain a good estimate of the plasma AUC after oral administration of etoposide using a two-time-point sampling model. The model can be used to monitor the etoposide AUC in patients receiving chronic oral treatment.

Introduction

Etoposide is a very active anticancer agent with a broad spectrum of anticancer activity [12]. Preclinical and clinical studies in sensitive tumour types have previously demonstrated that the efficacy of etoposide is highly schedule-dependent, the therapeutic index being higher after repeated administration than after a single administration of the same total dose [2, 18]. Clinical studies have been performed to evaluate whether etoposide can be given orally daily for 2–3 weeks without producing excessive toxicity [4, 10]. Although preliminary, the results obtained by daily prolonged oral treatment in sensitive tumour types appear to be at least comparable with those obtained using the classic i.v. schedule of etoposide (i.e. 100–150 mg/m^2 given for 3–5 days). An important drawback of the p.o. administration of etoposide is that the bioavailability of the drug varies among patients [6, 8]. This means that the same oral dose could cause severe toxicity in patients absorbing a high fraction of the dose or, conversely, could be ineffective in patients absorbing a low fraction [3]. Etoposide plasma-level monitoring might therefore be helpful in the conduct of clinical studies and in the evaluation of their results.

Monitoring of the area under the plasma concentration-time curve (AUC) for etoposide requires blood sampling at different times during the first 24 h after the treatment and can be carried out only in in-patients, involving significant expense and inconvenience. A possible alternative would be the development of limited sampling strategies that could enable an accurate and precise estimate of AUC values to be made by determining plasma drug levels only at specific intervals, increasing the feasibility and lowering the costs. The limited sampling model (LSM) has proved to be applicable to a variety of anticancer agents, including vinblastine, amonafide and doxorubicin [13–15]. Initial data published in abstract form suggest the possibility that the LSM can be applied for the prediction of etoposide AUC values after i.v. [11] and p.o. administration [9]. The aims of the present study were to develop an LSM for prediction of the plasma etoposide AUC after oral administration using one or fewer concentration time points, bearing in mind the statistical criterion and the clinical feasibility, and to validate its precision and accuracy in different sets of patients.

Patients and methods

The patients entered two separate studies on oral etoposide. The first group consisted of 28 patients who participated in a phase II study of daily oral etoposide. They had histological/cytological diagnoses of small-cell (n=14) or non-small-cell lung cancer (n=14), a median age of 64 years (range, 47–78 years), normal renal/liver functions (serum

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creatinine, ≤124 μmol/l; transaminases, ≤2 times the upper normal values; bilirubin, ≤17 μmol/l), and no concomitant pathology that would interfere with oral absorption. The serum albumin concentration was below the normal value in only one patient (serum albumin concentration, 28 g/l); this patient had normal liver function but had been diagnosed as having small-cell lung cancer with extensive disease and her performance status worsened rapidly. In all other patients the serum albumin concentration was in the normal range.

Etoposide was given as a single daily dose of 100 mg (corresponding to a range of 52–90 mg/m^2) for 21 consecutive days every 4 weeks. The first 15 patients (as chronologically entered) were used for model development (training data set) and the subsequent 13, for the initial validation (validation data set A). The second group (validation data set B), which was used for a second validation of the model, consisted of 7 patients who entered a study, the results of which have previously been reported [6]. These patients were suffering from gestational choriocarcinoma relapsing from previous chemotherapy or metastatic involvement of distant organs. Oral etoposide was given at a daily dose of 176–200 mg/m^2 for 5 consecutive days.

Pharmacokinetics studies. In all patients the experimental AUC (AUC_{exp}) was evaluated on day 1 of the first cycle of the oral therapy. Plasma samples were taken at the following 12 time points: 0 (before drug consumption), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h. Etoposide concentrations were determined in plasma by high-performance liquid chromatography (HPLC) as previously described by Evans et al. [7]. In the first group of 15 patients we also investigated the pharmacokinetics after i. v. administration of 100 mg etoposide given as a 30-min infusion 48 h before the beginning of the oral therapy so as to obtain a complete drug wash-out before the first oral dose. The sampling times after the i. v. administration were the same as those listed above, with the addition of a sample at the end of the infusion.

The AUC_{exp} was calculated by a trapezoidal method from 0 to 24 h. The half-life ($t_{1/2}$) and the bioavailability (F) were calculated according to the following formulas:

$$t_{1/2} = \ln 2/\beta \text{ and}$$

$$F = \frac{AUC_{p.o.}}{AUC_{i.v.}} \times 100,$$

where β is the slope of the terminal phase of the plasma concentration-time curve calculated using a general non-linear program [16].

Development of the model. The LSM was developed using the training data set. Initially, the correlation between the etoposide concentration at each time point (independent variable) versus the AUC_{exp} (dependent variable) was studied by univariate analysis on the training set. Then, by linear regression, we found subsets with all possible combinations of etoposide concentrations best predicting the AUC. The adjusted R^2 statistic (R^2_{adj}) and the Akaike information criterion (AIC) [1] were used to select a subset; we arbitrarily considered the model with $R^2_{adj}>0.90$ to be sufficiently accurate. The R^2_{adj} and the AIC were considered to be more appropriate than R^2 since they take into consideration the degree of freedom of the model and, differently from R^2, they do not increase monotonically with the increasing number of parameters. All analyses were performed using release 6.7 of the SAS package.

Validation of the model. For initial model validation, the predicted AUC (AUC_{pr}) was correlated with the actual AUC_{exp} on the first test data set (validation data set A). A second validation was then performed on the second test data set (validation data set B). The bias and the precision of the model were measured, respectively, by calculating the mean predictive error (MPE) and its percentage (MPE%) as well as the root mean square prediction error (RMSE) and its percentage (RMSE%) [17] according to the following formulas:

$$MPE = \frac{\Sigma (AUC_{pr} - AUC_{exp})}{n},$$

$$MSE = \frac{\Sigma (AUC_{pr} - AUC_{exp})^2}{n}, \text{ and}$$

$$RMSE = \sqrt{MSE}.$$

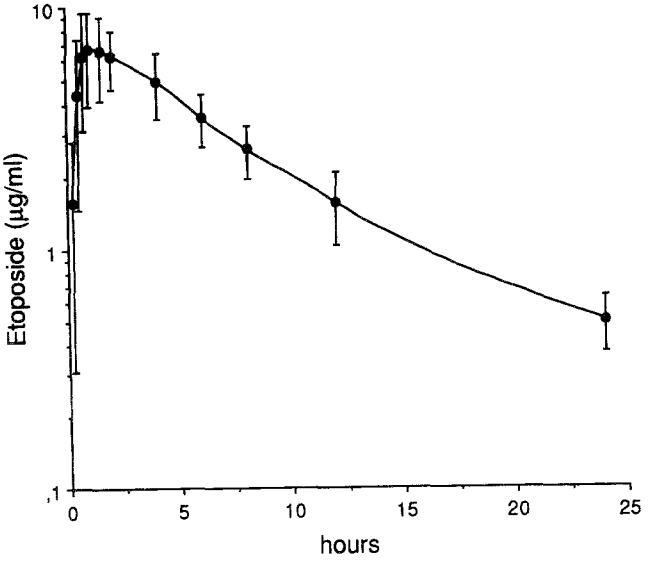

Fig. 1. Etoposide plasma levels (mean ± SD) after oral administration of 100 mg in the training set (n=15)

Table 1. Summary of the pharmacokinetic parameters of etoposide obtained from the full experimental data for the training data set and the first validation data set

	Training set (n = 15)			Test set A (n = 13)	
	AUC[a] (μg ml^{-1} h)	$t_{1/2}$ (h)	F%	AUC[a] (μg ml^{-1} h)	$t_{1/2}$ (h)
Mean	58.4	5.4	63.2	53.7	6.2
Range	32–98	3.7–7.6	33–114	30.0–88.9	4.1–9.8
CV	37.8%	18.7%	33.0%	38.8%	26.4%
SE	5.7	0.3	5.4	5.8	0.5

[a] Normalized to the dose of 100 mg/m^2
CV, Coefficient of variation; SE, standard error

Results

Interpatient variability

A summary of the pharmacokinetic parameters obtained from the full experimental data of the training data set and test data set A is reported in Table 1. Both the elimination half-life and the AUC values found for etoposide were in agreement with the pharmacokinetic profile previously reported [5, 6, 8, 19]. The bioavailability of etoposide (F%), determined in the patients of the training set, was also comparable with that previously described in other studies [5, 6, 8, 9]. Figure 1 shows the etoposide plasma decay curve (mean ± SD) in the 15 patients of the training data set. Similar pharmacokinetic profiles were found for both of the validation data sets.

Model development

Table 2 shows the correlation between the etoposide concentration at each time point and the AUC_{exp}, showing the

Table 2. Correlation between the etoposide concentration at each time point and the AUC_{exp} at 24 h for the training data set ($n = 15$), showing the adjusted R^2, the AIC and the RMSE

C (μg/ml)	R^2_{adj}	AIC	RMSE
$C_{0.25h}$	0.007	82.48	13.7
$C_{0.5h}$	0.009	82.52	13.7
$C_{0.75h}$	0.225	78.55	12.0
C_{1h}	0.277	77.51	11.6
$C_{1.5h}$	0.436	73.78	10.2
C_{2h}	0.791	58.94	6.2
C_{4h}	0.893	48.85	4.5
C_{6h}	0.794	58.67	6.2
C_{8h}	0.877	50.95	4.8
C_{12h}	0.641	67.00	8.2
C_{24h}	0.225	78.55	12.0

Table 3. Correlation between the 10 couples of time points and the AUC_{exp} at 24 h showing the highest adjusted R^2 and the lowest AIC; also listed is the RMSE value for each of the 10 couples

1st C (μg/ml)	2nd C (μg/ml)	R^2_{adj}	AIC	RMSE
$C_{0.75h}$	C_{4h}	0.936	41.90	3.44
C_{1h}	C_{4h}	0.933	42.53	3.52
C_{1h}	C_{12h}	0.933	42.74	3.54
$C_{1.5h}$	C_{12h}	0.928	43.79	3.66
$C_{0.5h}$	C_{8h}	0.925	44.31	3.73
C_{4h}	C_{8h}	0.923	44.71	3.78
$C_{0.25h}$	C_{8h}	0.921	45.17	3.84
$C_{0.5h}$	C_{4h}	0.920	45.23	3.84
C_{4h}	C_{6h}	0.919	45.41	3.87
$C_{1.5h}$	C_{4h}	0.916	46.10	3.97

adjusted R^2, the AIC coefficient and the RMSE. The best univariate correlation was found for the drug concentrations at 4 h (C_{4h}; $R^2_{adj} = 0.89$, $P = 0.0001$) and 8 h (C_{8h}; $R^2_{adj} = 0.87$). The worst correlations were observed at $C_{0.25h}$ and $C_{0.5h}$ during drug absorption. The same time points were utilized for the multiple regression analysis. Table 3 reports the ten couples of time points showing the highest adjusted R^2 as well as the lowest AIC coefficient and the RMSE. The best two couples were $C_{0.75h}$, C_{4h} and C_{1h}, C_{4h}. We selected C_{1h} and C_{4h} to develop the model since its R^2_{adj} ($R^2_{adj} = 0.93$, $P = 0.0001$) value was almost equivalent to that of $C_{0.75h}$, C_{4h} but was more feasible for clinical management. The accuracy of the model is well denoted by Fig. 2, which shows the residual values (i.e. AUC_{pr}–AUC_{exp}) for each patient. The range of residual percentage was from 1.7% to 11.9%.

The equation describing the model is:

$$AUC_{pr}\ (\mu g\ ml^{-1}\ h) = 5.183\ (\mu g\ ml^{-1}\ h) + 1.193\ (h) \times C_{1h}\ (\mu g/ml) + 8.439\ (h) \times C_{4h}\ (\mu g/ml). \quad (1)$$

An additional model with three time points was developed, the best equation being:

$$AUC_{pr}\ (\mu g\ ml^{-1}\ h) = 0.267\ (\mu g\ ml^{-1}\ h) + 1.776\ (h) \times C_{1h}\ (\mu g/ml) + 6.113\ (h) \times C_{6h}\ (\mu g/ml) + 15.956 \times C_{12h}\ (\mu g/ml), \quad (2)$$

with $R^2_{adj} = 0.98$ and $P = 0.0001$.

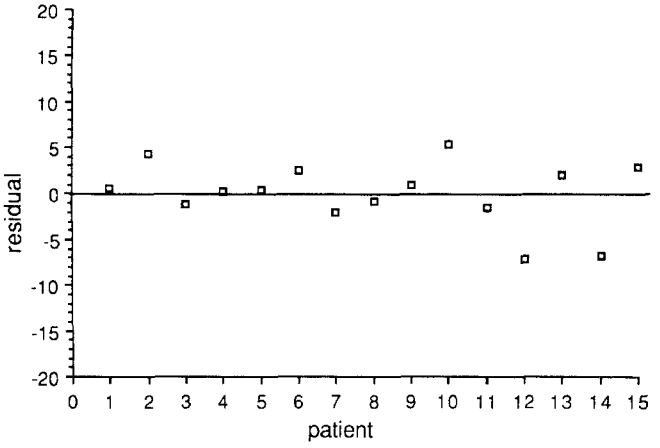

Fig. 2. Residual for each patient of the training set, calculated as (AUC_{pr}–AUC_{exp}). The patients are ordered according to increasing AUC_{exp}

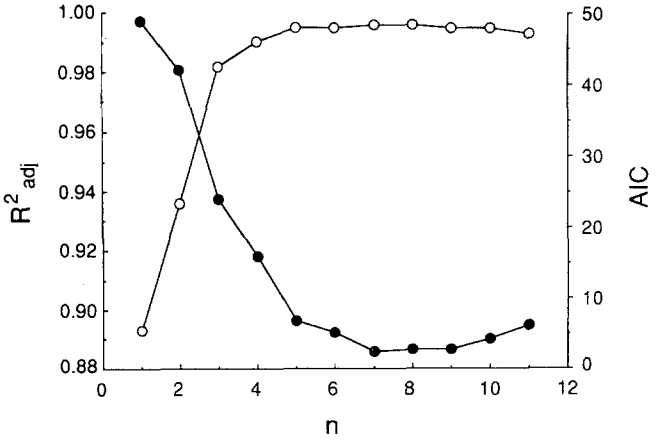

Fig. 3. Adjusted R^2 (○) and AIC (●) versus the number of time points (n)

As was to be expected, the addition of a second and a third variable improved the model, as can be seen by comparing the R^2_{adj} values for the models with 1, 2 and 3 variables (respectively, $R^2_{adj} = 0.89$, $P = 0.0001$; $R^2_{adj} = 0.93$, $P = 0.0001$; and $R^2_{adj} = 0.98$, $P = 0.0001$). Figure 3 shows the curves for adjusted R^2 and AIC versus the number of time points; it is evident that increasing the number of points up to 7 enhances the prediction efficiency. However, we chose to validate the model with only two time points because that makes it sufficiently accurate (R^2_{adj}>0.90) and more feasible clinically.

Validation of the model

Equation 1 was used to calculate the AUC_{pr} in the two test sets. Figure 4 shows the correlation between the AUC_{exp} and the AUC_{pr} for the validation test data sets A ($R = 0.91$, $P = 0.0001$) and B ($R = 0.93$, $P = 0.0028$) and the comparison with the training data set.

Table 4 summarizes the bias (MPE, MPE%) and the precision (RMSE, RMSE%) of the model. The relative RMSE value (RMSE%, 15.8 for test A and 16.7 for test B)

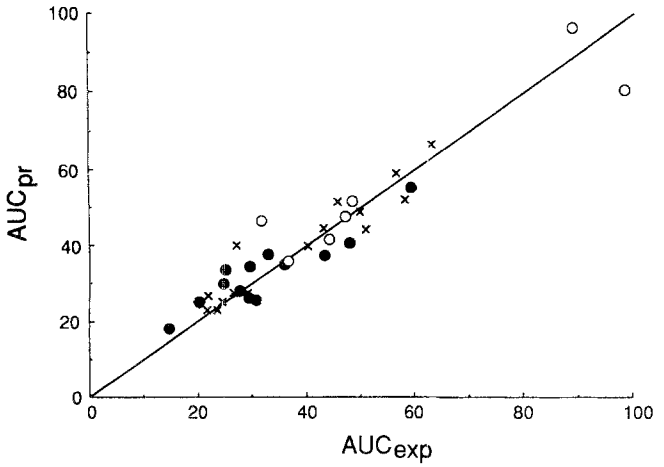

Fig. 4. Validation of the model on training set (x), the first set A (●), and the second (○) test data set. The *solid line* is the line of identity. *Horizontal axis*, Experimental AUC; *vertical axis*, AUC predicted from the model

Table 4. Validation of the limited sampling model

Data set	R^a	MPE±tSE[b]	MPE%	RMSE	RMSE%
Training	0.97	0.00±1.94	0.0	3.38	8.9
Test A	0.91	0.03±5.32	0.1	4.99	15.8
Test B	0.93	0.06±9.29	0.1	9.30	16.7

[a] Correlation coefficient between AUC_{pr} and AUC_{exp}
[b] t was chosen for a confidence interval of 95%

was acceptable as compared with the coefficient of variation (CV) of the AUC_{exp} (CV, 38.8% for test A and 43.5% for test B). The table also shows the 95% confidence interval for MPE as calculated according to Sheiner and Beal [17]. The accuracy and the precision of the three-time-point model were tested by Eq. 2. The RMSE% values were 14 for test A and 13 for test B.

All computations were made without normalizing the dose. In fact, normalization does not appear to be of practical relevance, considering that for oral etoposide therapy a precise dose per square meter of body surface area cannot be given, as the only tablets available contain 50 or 100 mg. However, since most of the previously published LSMs for the prediction of the AUC of antitumour agents given i.v. were developed by normalizing the dose (i.e. normalizing the values of AUC_{exp} and drug concentrations in the training data set and subsequently adjusting the constant for the dose), we compared the efficiency of the LSMs developed with and without normalization. The results were very similar, with an RMSE% of 16.2 vs 15.8 and an MPE% of 2.3 vs 0.1 being obtained. Therefore, only the model developed without normalization has been presented in detail herein.

Discussion

In the present report we describe the development of an LSM for estimation of the AUC of etoposide after oral administration. We demonstrated that it is possible to estimate the plasma etoposide AUC by determining plasma drug concentrations at only two time points (1 and 4 h) with a high degree of accuracy and precision. The use of a single time point such as 4 h gave a highly significant R^2_{adj} value of 0.89 ($P = 0.0001$), but the use of two time points (C_{1h} and C_{4h}) markedly improved the performance of the model. The LSM was built up by performing multiple regression analysis on the pharmacokinetic data obtained in a training data set of 15 patients and was then validated in two test data sets. The MPE% value, which gives an estimate of the bias, was 0.1 in both of the validation data sets. The RMSE%, which is an indicator of the precision of the model, was found to be 15.8 and 16.7 in the two data sets, respectively. These values appear to be acceptable, considering that the CV values for the AUC_{exp}, normalized for the dose, were 38.8% and 43.5% in the same data sets, i.e. they were much higher than the error of precision of the model.

It is noteworthy that the model was successfully validated not only in those patients who entered the study consecutively after the 15 patients of the training data set but also in a historical set of data we published in 1982 [6], obtained in a different clinical setting and by using a different analytical method. This was in fact the first study of the bioavailability of etoposide performed by an HPLC method employing extraction procedures and HPLC conditions different from those currently used. In addition, that study was carried out in a gynaecological hospital, that is, in a different environment and with a different personnel staff. That the present model was also verified on these historical data indicates that it too is applicable to different clinical and laboratory environments, provided that the analytical procedures and the sampling times are correct. We also had to consider that the doses in the two sets were markedly different (range, 54–87 mg/m^2 in set A and 176–200 mg/m^2 in set B), suggesting that it is possible to use this model in a dose range of 50–200 mg/m^2 with good precision and a low bias. We cannot ensure the same accuracy and precision for higher doses, particularly considering that for doses of more than 200 mg/m^2 a reduced bioavailability of etoposide has been reported [8].

By using a three-time-point model we found a very high correlation with AUC_{exp} ($R^2_{adj} = 0.98$). However, the increase in precision was limited in comparison with that observed for the two-time-point model (RMSE%, approximately 14 vs 15) and was counterbalanced by a much greater inconvenience for the patients, who had to be hospitalized for blood sampling at 1, 6 and 12 h.

Joel et al. [9] have recently reported the development of an LSM to predict the etoposide AUC (0–12 h) after oral administration of 50 mg twice a day. Although there are differences between our model and theirs, possibly because the AUC was evaluated in Joel et al.'s study up to 12 h and in our study up to 24 h, both studies show that a two-time-point model provides a good estimate of the plasma AUC and is feasible in practical terms.

One application of the LSM described herein would be for evaluating whether there are marked differences in drug absorption between the beginning of therapy and different other times during chronic treatment. We cannot in fact

exclude that the absorption of etoposide might change after repeated administration and during subsequent courses. A second application of this LSM would be for evaluating whether a correlation exists between the AUC of etoposide (on different days and during different courses of oral treatment) and its pharmacological effects. Should a correlation be found, dose adjustments to obtain similar etoposide AUC values in all patients would be recommended. A further potential application of our LSM would be for evaluating the influence of diet (including a malnourished status) and concomitant treatment with antitumour and non-antitumour drugs on the pharmacokinetics of oral etoposide. Despite their obvious potential relevance, we in fact have no information about either of these issues. The LSM described herein makes it possible to answer these questions by analysis of etoposide plasma concentrations in a limited number of samples.

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