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Original Paper

Limited Sampling Models for Reliable Estimation of Etoposide Area Under the Curve

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Limited sampling models are able to estimate the area under the concentration–time curve (AUC) from plasma concentrations measured at only a few time points. The purpose of this study was to establish a model estimating etoposide AUC independently of specific chemotherapy protocols, underlying malignancies, concomitant diseases and age. Pharmacokinetic parameters were measured in 30 patients treated with polychemotherapy including etoposide (80–150 mg/m^2). Etoposide analysis was performed by thin layer chromatography and consecutive quantitative sample detection by ^{252}Cf-plasma desorption mass spectrometry. Data from the first 15 patients formed the training set. Based on the training data, five different models were generated, with the multiple regression coefficient r ranging from 0.91 to 0.96. The following model was selected as "most accurate":

$$\text{AUC} = 343\ (\text{min})\ C_{4h}(\mu\text{g/ml}) + 650\ (\text{min})\ C_{8h}\ (\mu\text{g/ml}) + 1252\ (\text{min}\ \mu\text{g/mol}),$$

where C_{4h} is the plasma concentration of etoposide at 4 h after the end of infusion and C_{8h} at 8 h. This model was validated on the test set, comprising the data of the remaining 15 patients. The mean predictive error (MPE) was −0.2% and the root mean square predictive error (RMSE) was 4.7%. When used for a large number of patients, this practicable and simple model is an instrument for use in prospective studies, to measure a correlation between drug dosage and efficacy or toxicity of the drug.

Key words: etoposide, pharmacokinetics, limited sampling model, mass spectrometry
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INTRODUCTION

A CENTRAL QUESTION in pharmacological studies is the relationship between pharmacokinetic parameters, for example, area under the concentration–time curve (AUC), and therapeutic or toxic effects. Only a few studies have examined the clinical pharmacodynamics of epipodophyllotoxines [1, 2]. Several studies have demonstrated that the AUC as a measure of total drug exposure can be correlated with the extent of myelosuppression [3–5]. Rodman and coworkers have demonstrated that the AUC after teniposide (VM 26) administration is related to antitumour response in children with acute lymphocytic leukaemia [6]. The AUC is the best pharmacokinetic parameter for predicting anticancer pharmacodynamic effects, although its exact quantification is inconvenient and costly, usually requiring the measurement of the plasma drug concentrations at 8–12 time points. To apply pharmacokinetic and pharmacodynamic studies to a large number of patients, practicable and simple tools to determine pharmacokinetic parameters, such as the AUC or the clearance of a drug, are needed. A suitable limited sampling model could be one of these tools. This study demonstrates that the AUC of etoposide can be accurately estimated from only two plasma concentrations obtained at 4 and 8 h after the end of drug infusion, in patients receiving etoposide as part of different polychemotherapy protocols.

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PATIENTS AND METHODS

Patients and treatment

After informed consent as approved by the Human Subject Committee of the Philipps-University Marburg, 30 patients, 2 females and 28 males, were included in the study. Age ranged from 22 to 66 years with a median of 48 years. All patients had normal liver and kidney function. Cancer types included: acute leukaemia, $n = 1$ (a); non-Hodgkin-lymphoma, $n = 20$ (b); small cell lung cancer, $n = 5$ (c); and malignant teratoma, $n = 4$ (d). The following chemotherapy regimes were used: (a) daunorubicin, cytarabine, etoposide; (b) cyclophosphamide, doxorubicin, vincristine, etoposide, prednisolone; (c) vincristine, etoposide; and (d) etoposide, bleomycin, cisplatin.

On three consecutive days etoposide was given daily as the first drug of each chemotherapy cycle as a 45 min intravenous (i.v.) infusion (dose range 80–150 mg/m^2). From each patient, only one 24 h kinetic was analysed. When a patient received cisplatin, this and the following day's data were excluded from the study. Plasma samples were obtained at nine time points following drug administration (end of infusion = 0, plus 15, 30, 60 min, and 2, 4, 8, 16, 24 h) [7]. After 24 h, the measured plasma concentration was so low and uncertain, that its value was omitted in further analyses. From each set of eight plasma samples, pharmacokinetic parameters were determined according to the method described in detail in previous publications [7, 8].

Etoposide assay

The etoposide analysis was performed in four stages: sample extraction, sample purification by thin-layer chromatography (TLC), target preparation and quantitative sample detection by ^{252}Cf-plasma desorption mass spectrometry (PDMS). The quantitative result was obtained by comparing the two mass lines corresponding to the two molecular ions of etoposide at *m/z* 588 and of the internal standard, teniposide, at *m/z* 656 [9]. The feasibility of the method has been tested by varying the concentration ratio of etoposide/teniposide in blank plasma samples over four orders of magnitude. The calibration curves for the TLC/PDMS assay are linear and reproducible [9].

Pharmacokinetics

In order to determine the AUC, all plasma etoposide concentrations were normalised to the standard dose 100 mg/m^2. The pharmacokinetic parameters, describing etoposide distribution, were calculated by standard compartmental methods from serial plasma concentrations versus time data fitted by a bi-exponential curve [8, 9].

Statistical analysis

Data from the first 15 patients formed the training set, and data from the remaining 15 patients formed the test set. A separate univariate analysis was performed for each time point versus the AUC in the training data set. Stepwise forward multiple regression was used to develop five different limited sampling models (LSMs) with two time pionts, and another model with three time points for the training data set. The *F*-test was used to select the best models. These models were validated using the test data set, correlating the estimated and actual AUC. Mean predictive error (MPE) and root mean square predictive error (RMSE) were also calculated as a measure of bias and precision [10–17].

RESULTS

Interpatient variability

The analysis of the complete data set (30 patients) resulted in the following mean values: AUC 5013 ± 688 min μg/ml (range 3413–7500), mean residence time (MRT) 383 ± 34 min (range 317–452), long-term half life ($t_{\frac{1}{2}}\beta$ 295 ± 35 min (range 228–363), $t_{\frac{1}{2}}\alpha$ 23 ± 7 min (range 11–40), total body clearance (CL) 22 ± 3 ml/min/m^2 (range 13–29), steady state distribution volume (Vd_{ss}) 7.8 ± 1.3 l/m^2 (range 4.3–9.8). Table 1 shows a comparison with mean values of the two groups (training and test data sets) and data obtained with the same analytical method in two previous studies [7, 8], where the individual variability in etoposide pharmacokinetics has been described.

In summary, there was a significant interpatient variability in the etoposide plasma concentration at all time points (Table 2). The lowest variability was observed within the first hour after the end of infusion, the highest beyond 2 h.

Model development

Using the training data, etoposide plasma concentrations at each time point were correlated with the total AUC (Table 3). The two single time points with the best univariate correlation were 4 and 8 h with a correlation coefficient of 0.86 and 0.89, respectively ($P < 0.0001$). For the multiple regression analysis, the four time points with highest univariate correlation (end of infusion, 15 min, 4 h and 8 h) were used. Five different models were generated by varying the choice of the variables, i.e. time points entered in the multiple regression programme [12] (Table 4). The following model (model E) included the two best time points from univariate analysis:

$$\text{AUC} = 343\ (\text{min})\ C_{4h}\ (\mu\text{g/ml}) + 650\ (\text{min})\ C_{8h}\ (\mu\text{g/ml}) + 1252\ (\text{min}\ \mu\text{g/ml}),$$

where C_{4h} is the plasma concentration of etoposide at 4 h after end of infusion and C_{8h} at 8 h, both concentrations normalised to standard dose 100 mg/m^2. The *F*-value of this model was significantly higher ($F = 75.9$) than the *F*-value for the best univariate model using only C_{8h} ($F = 55.6$). Model E was tested on the training data set, and Figure 1 illustrates the high correlation ($r = 0.96$) and the excellent fit of the estimated AUC and the actual measured AUC.

Model validation

For the development of a mathematical model, the regression parameters of the training data set have to be validated on a distinct test data set. As shown in Table 1, both groups, training and test data, were distinct and comparable to the whole group and also comparable to previous publications [7, 8]. The five LSMs were each validated on the 15 patients of the test data set. As a sign of the interpatient pharmacokinetic variability, the measured AUC ranged from 4033 to 5689 min μg/ml, MRT from 326 to 452 min and CL from 18.0 to 27.0 ml/min/m^2. The correlation coefficient *r* between the estimated AUC values of the LSM and the actual measured AUC values ranged from 0.86 to 0.93. All five models had a minor bias (MPE range −2.4–+2.0%) and a very high precision (RMSE 4.7–6.5%; Table 4). Model E was selected as the "most accurate" model, because of its very low bias (MPE = −0.2%) and its excellent precision (RMSE = 4.7%). Figure 2 shows that model E estimates the total AUC very precisely for the test data, although the correlation coefficient ($r = 0.93$) is slightly less than for the training data ($r = 0.96$). This comparison is independent of the normalisation procedure as both values, i.e. actual and estimated AUC, are corrected in the same sense. The data points in Figure 2 clustered into two groups, but this was not related to previous exposure to cisplatin or renal function impairment, because such patients were excluded.

Additional model

An additional model, which included a third plasma etoposide concentration at 15 min (C_{15min}, also normalised to standard dose), was developed to estimate AUC values for patients with different malignancies, concomitant diseases, various ages and different polychemotherapy protocols:

$$\text{AUC} = 63.7\ (\text{min})\ C_{15min}\ (\mu\text{g/ml}) + 301\ (\text{min})\ C_{4h}\ (\mu\text{g/ml}) + 522\ (\text{min})\ C_{8h}\ (\mu\text{g/ml}) + 751\ (\text{min}\ \mu\text{g/ml}).$$

Table 1. Comparison of all data from this study and previous publications for the pharmacokinetic parameters: AUC (min μg/ml), AUC normalised to standard dose, MRT (min), $t_{\frac{1}{2}}\beta$ (min), $t_{\frac{1}{2}}\alpha$ (min), CL (ml/min/m²) and Vd_{ss} (l/m²)

	Training data set (n = 15)	Test data set (n = 15)	Complete data (n = 30)	Köhl *et al.* ($n \leq 30$) [8]	Pflüger *et al.* (n = 62) [7]
AUC	5054 ± 742	4973 ± 652	5013 ± 688	—	—
AUC (100 mg/m²)	—	—	4715 ± 782	4494 ± 1060 (n = 22)	5430 ± 1740
MRT	382 ± 35	385 ± 33	383 ± 34	372 ± 104 (n = 30)	402 ± 84
$t_{\frac{1}{2}}\beta$	291 ± 31	298 ± 40	295 ± 35	281 ± 82 (n = 30)	294 ± 72
$t_{\frac{1}{2}}\alpha$	22 ± 5	24 ± 8	23 ± 7	—	—
CL	22 ± 4	22 ± 3	22 ± 3	23 ± 5 (n = 22)	19 ± 5
Vd_{ss}	7.6 ± 1.4	7.9 ± 1.1	7.8 ± 1.3	7.0 ± 1.3 (n = 10)	6.8 ± 2.7

n, number of kinetics; mean values of listed pharmacokinetic parameters in training and test data set deviate less than 1% from mean values of the complete data. AUC, area under concentration–time curve; MRT, mean residence time; $t_{\frac{1}{2}}\beta$, long-term half life; CL, total body clearance; Vd_{ss}, steady state distribution volume.

Table 2. Interpatient variability shown by mean values and standard deviations (S.D.) of plasma etoposide concentrations C (μg/ml) plus coefficients of variation (CV) at the end of infusion and at seven further time points

	C_{0min}	C_{15min}	C_{30min}	C_{60min}	C_{2h}	C_{4h}	C_{8h}	C_{16h}
Mean	24.1	16.9	13.8	11.8	8.7	5.7	2.8	0.96
S.D.	2.3	2.6	2.0	1.7	1.4	1.0	0.6	0.2
CV (%)	18.8	33.7	37.7	26.8	35.1	34.2	46.5	41.7

Table 3. Correlation of the plasma etoposide concentration with the total AUC for the training data set (n = 15) at the end of infusion and at seven further time points

	Time after end of infusion							
	0 min	15 min	30 min	60 min	2 h	4 h	8 h	16 h
Correlation coefficient r	0.76	0.76	0.72	0.66	0.64	0.86	0.89	0.61

Table 4. Limited sampling models for estimation of the etoposide AUC based on two concentration measurements at the time points T_1 and T_2

	Time (h)		Coefficients			Training data set			Test data set		
Model	T_1	T_2	K_1	K_2	K_3	r	MPE	RMSE	r	MPE	RMSE
A	0	4	120	461	−533	0.92	−0.3	5.8	0.86	0.5	6.5
B	0	8	73.1	818	1014	0.91	−0.4	6.7	0.90	−2.4	6.0
C	0.25	4	107	460	529	0.93	−0.3	5.3	0.88	2.0	6.5
D	0.25	8	86.7	779	1392	0.93	−0.3	5.6	0.93	−1.1	4.9
E	4	8	343	650	1252	0.96	−0.2	4.4	0.93	−0.2	4.7
M	2	4	49.2	578	1272	0.86	−0.5	7.7	0.80	1.0	8.4

AUC (min μg/ml) = K_1(min) C (T_1) (μg/ml) + K_2 (min) C (T_2) (μg/ml) + K_3 (min μg/ml). Model E is "most accurate", while model M, based on the two time points given in [18], is listed for comparison.

For the training data set, this model was significantly better than the bivariate models ($P < 0.0001$): the correlation coefficient r increased from 0.96 to 0.98; validation on the test data resulted in a correlation coefficient of 0.96 (in the bivariate model r = 0.93). However, the most important statistical parameters MPE and RMSE exhibited only a marginally decrease (e.g. RMSE in bivariate model E: 4.7%, in additional trivariate model: 3.8%).

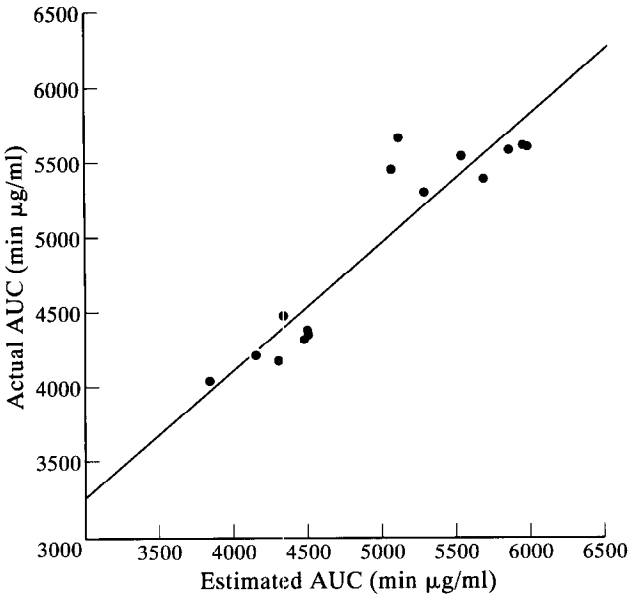

Figure 1. Development of the limited sampling model E ("most accurate" bivariate model) with the training data set (15 patients, correlation coefficient $r = 0.96$).

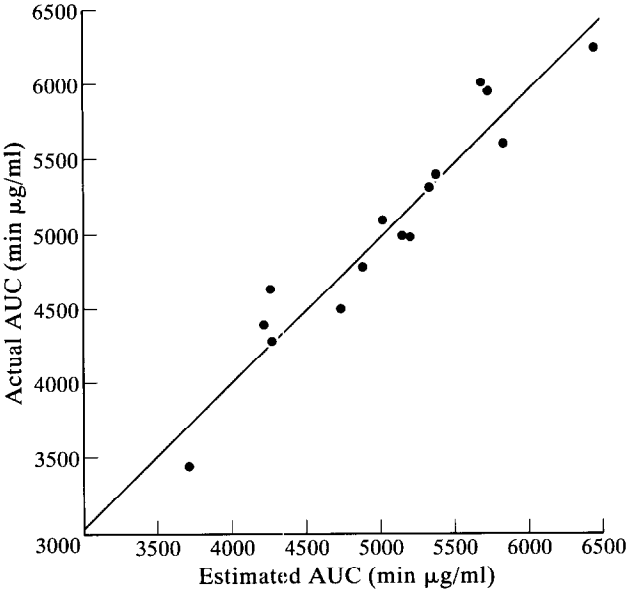

Figure 2. Validation of the limited sampling model E with the test data set (15 patients, correlation coefficient $r = 0.93$).

Analysis of the model

The bivariate model E with the time points at 4 and 8 h after the end of infusion estimated the total AUC in a very accurate way, although there was a minimal trend to underestimate the actual AUC. The mean predictive error for the test data set was −0.2% (range −5.7% to 10.5%), the root mean square predictive error was 4.7%. We found a correlation between MRT, $t_{\frac{1}{2}}\beta$ and the predictive error ($r = 0.79$, $P = 0.0003$ versus $r = 0.81$, $P = 0.0001$). This suggests that this model might be less useful for patients with a higher MRT (delayed clearance). There was also a minor correlation between the volume of distribution and the predictive error ($r = 0.53$, $P = 0.04$), which is another indication that one has to pay attention to patients with renal impairment. The coefficient of variation (CV) of the fraction of the AUC in the terminal phase (β-AUC) was higher than the CV of the total AUC (CV of the β-AUC averaged 34%, CV of the total AUC averaged 29%), but we did not find a significant correlation between the β-fraction of the total AUC and the MPE. Therefore, we conclude that this model tolerates a higher variability in the fraction of the total AUC in the terminal phase without loss of precision.

DISCUSSION

This study illustrates that a limited sampling model can be developed for etoposide, which estimates the total AUC from plasma concentrations measured at only two time points. A univariate analysis of all time points with the actual AUC in the training data set was initially started. Using forward stepwise multiple regression, five bivariate models based on two time points were developed, and tested by F-test and error estimation for the statistical best fit [12]. All five models were validated on the test data and model E with lowest bias (MPE) and highest precision (RMSE) was chosen as the "most accurate model". One model based on three time points was also developed, which was not substantially better, with bias and precision not increasing significantly. Thus, for practical reasons, the bivariate model E based on two plasma concentrations measured 4 and 8 h after the end of infusion is preferable.

A comparison with other LSMs for etoposide [18, 19] reveals some interesting differences. Miller and colleagues [18] also developed a model estimating the etoposide AUC with two time points for patients with extensive stage, small cell lung cancer. Their model was developed for a fixed dose of etoposide (150 mg/m^2), but we were not able to verify the two time points, 2 and 4 h, as optimal (model M in Table 4). It seems that these two time points used by Miller and coworkers estimate the AUC accurately for a well defined population of patients only—in this case patients with lung cancer—and a specific chemotherapy protocol with a fixed dose of etoposide. Our best bivariate model (model E) estimates the AUC very accurately for a heterogeneous population, different protocols and for various dose levels of etoposide. The best time point obtained from the univariate analysis, i.e. 4 h after the end of infusion, was found first by Strömgren and associates [19], and confirmed independently by this work. However, our second time point at 8 h rather than 21 h, and also our third time point at 15 min rather than 5 min, resulted in better precision (smaller MPE).

Comparing the bivariate model E to the recent LSM for etoposide [19] and several LSMs with other cytotoxic drugs [10, 14, 16, 20] in terms of accuracy, the mean predictive error (MPE) −0.2% and root mean square predictive error (RMSE) 4.7% of the AUC estimated here are very small and range within the interpatient variability (Table 1). The quality of our LSM study can be related to three criteria which reduced systematic and experimental errors: fixed duration of infusion; precisely timed blood sampling and precisely determined plasma concentrations by quantitative mass spectrometry. These conditions for the development of the LSM allow a wide range of dosages and altered tumour types without loss of quality.

As reported in a previous publication, etoposide pharmacokinetics are unchanged in high-dose chemotherapy cycles [8], and are rarely susceptible to various modalities of drug infusion [4, 5]. Thus, the model E could probably be employed in different studies with a wide range of dose and application schemes, however, the infusion time cannot be altered due to its influence on the pharmacokinetics. Applying this model for

drug monitoring in clinical routine, the aim of an individual etoposide dosage scheme for each patient is achievable, but, in addition, questions of etoposide pharmacodynamics, in particular efficacy and toxicity, could be answered in prospective studies. Future investigations should look at further chemotherapy regimens, including different cytotoxic drugs, to evaluate if possible changes in etoposide pharmacokinetics are limiting the performance of LSMs.

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