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A limited-sampling strategy for estimation of etoposide pharmacokinetics in cancer patients

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Abstract Etoposide (VP16), a widely used anticancer drug, is a topoisomerase II inhibitor. A number of studies have highlighted a correlation between hematologic toxicity and pharmacokinetic or physiological parameters. Other studies have also suggested that the anti-tumor response could be related to the plasma etoposide concentration. Therefore, it would seem of interest to individualize VP16 dose regimens on the basis of pharmacokinetic parameters. The aim of this study was to develop and validate a limited-sampling strategy allowing VP16 pharmacokinetic evaluation with minimal disturbance to the patient. A total of 34 patients (54 kinetics) received VP16 at various dose regimens, with doses ranging between 30 and 200 mg and infusion times varying between 0.5 and 2 h. The statistical characteristics of the pharmacokinetic parameters were assessed from the first courses of treatment performed in 23/34 patients; then the following three-sample protocol was designed: the end of the infusion and 5 and 24 h after the start of the infusion. For validation of the model the main pharmacokinetic parameters (clearance, half-lives, volume of distribution) were estimated in the 11 remaining patients by maximum-likelihood estimation (ML) and by Bayesian estimation (BE) using the three sampling times designed. Statistical comparison showed a good concordance between ML and BE estimates (the bias for clearance was –1.72%). The limited-sampling strategy presented herein can thus be used for accurate estimation of VP16 pharmacokinetic parameters.

Key words Bayesian estimation · Pharmacokinetics · Etoposide

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Introduction

The podophyllotoxin derivative etoposide (VP16) has been extensively evaluated in malignant disease [35]. This drug has clinical value in the treatment of a variety of neoplasms, such as small-cell lung cancer, Kaposi's sarcoma, testicular cancer, leukemias, and lymphomas (Hodgkin's and non-Hodgkin's) [1]. VP16 is given either as a single agent or in combination with other anticancer drugs, particularly cisplatin [25, 35]. VP16 is also used in high-dose treatments, especially transplantation regimens [8, 34]. After i.v. dosing, the VP16 plasma concentration-time curve is biexponential, with the distribution half-life being 30–60 min and the terminal half-life ranging from 5 to 12 h in adults and from 2 to 6 h in children [12, 26, 32].

A major side effect of etoposide is myelosuppression, including both neutropenia (generally the dose-limiting effect) and thrombocytopenia [1, 3]. Considerable inter-individual differences are observed in the myelotoxic response to similar doses of VP16. Studies have been carried out to examine the pharmacokinetics of etoposide and to correlate these parameters with myelotoxic effects. The results of these studies established a significant correlation between the VP16 area under the concentration-time curve (AUC) and the hematologic toxicity [20, 36]. A population model quantifying the leukopenic effect of etoposide, taking pharmacokinetic and physiological parameters into account, has been proposed [21]. Moreover, some studies have shown that the percentage of response would increase with the etoposide AUC [11, 20]. From these results it appears that further studies are

required to specify the relationship between the pharmacokinetics of VP16 and its pharmacodynamics. When VP16 is given as a long-term infusion the AUC can be estimated from the steady-state concentration, which exhibits a good correlation with the relative leukocyte decrease [23]. After a short-term infusion there is obviously no such steady state, and one has to take between seven and ten blood samples to calculate the individual pharmacokinetic parameters in a given patient.

To minimize the number of samples required for determination of the individual pharmacokinetic parameters, limited-sampling methods were developed. Such methods allowed the estimation of some VP16 pharmacokinetic parameters with minimal cost and minimal disturbance to the patient. These methods have been developed for VP16 using multiple linear regression [14, 19, 37], but they cannot safely be applied to all kinds of administration schedules. Each one is applicable for a specific duration of infusion. Moreover, they require an accurate sampling schedule.

More reliable, the Bayesian approach proposed by Sheiner et al. [28, 30] authorizes the estimation of individual pharmacokinetic parameters by combining population characteristics of the pharmacokinetic parameters with a limited number of individual drug concentrations.

Studies comparing the classic maximum-likelihood estimation (ML) method with the Bayesian estimation (BE) procedure have been realized for estimation of the pharmacokinetic parameters of several drugs, such as amikacin [6], methotrexate [5, 16], ceftazidime [38], and others [7, 15, 31], and have demonstrated the efficiency of BE. We applied a BE procedure associated with an optimal sampling time (OST) protocol so as to operate with the more informative samples.

Patients and methods

Patient populations

A total of 34 patients (8 women, 26 men) were included in the study. Their main characteristics and chemotherapy regimens are summarized in Table 1. All patients gave their informed consent to the studies. The patients were treated according to 3 different administration schedules as follows: (1) 15 patients received 1 course of VP16 given as a 2-h i.v. infusion at a dose ranging from 30 to 200 mg (group I); (2) 11 patients received 1 course of VP16 given as a 1-h infusion, 10 received 200 mg, and 1 received 100 mg (group II); and (3) 8 patients received 120 mg/m^2 given as a 30-min i.v. infusion once daily for 2 days and as a 2-h i.v. infusion at the same dose on day 3, successive courses being repeated every 2 or 3 weeks (group III). In groups I and II, patients received VP16 as a single agent or in combination with cisplatin (CDDP); in group III, patients received polychemotherapy including doxorubicin, etoposide, and ifosfamide.

Population characteristics of VP16 were assessed on the first courses of treatment performed in 23 randomly selected patients (T1–T23). The OST protocol was determined from these characteristics. Validation was then undertaken with the 11 remaining patients (V1–V11) proceeding from the 3 different trials. For this purpose the values obtained for the pharmacokinetic parameters using either ML or BE associated with an OST protocol were compared.

Blood sampling

In group I, blood samples were collected at 1, 2, 3, 6, 9, and 12 h after the start of the 2-h infusion, and three subjects were sampled for up to 24 h. In group II, samples were taken at 1, 1.16, 1.5, 2, 3, 5, 9, and 25 h after the start of the 1-h VP16 infusion. In group III the sampling schedule was adapted as follows, taking the treatment constraints into account: at 0.5, 1, 1.5, 2.5, 6, 18, 21, and 24 h after the start of the 30-min infusion on day 1; at 0.5, 1.5, 6, 18, and 20 h on day 2; and only at the end of infusion on day 3. In all cases, blood samples were collected in heparinized tubes. One test sample was taken from each patient prior to the injection. Blood samples were rapidly centrifuged and plasma was frozen at −20 °C until analysis.

Analytical method

Etoposide was assayed by reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection using a previously described method [9] with modifications as follows. In brief, etoposide and the internal standard (teniposide) were extracted from plasma with dichloromethane. The organic phase was evaporated to dryness and the residue was dissolved in 200 µl of the chromatographic eluent acetonitrile / bidistilled water (35/65). Then, 20 µl was injected onto a 10-cm reversed-phase column (Lichrospher 100 5 µm C18) with an eluent flow rate of 0.8 ml/min. The compounds were detected by a UV detector applying an absorbance wavelength of 230 nm. Retention times were 3.6 and 11 min for etoposide and the internal standard, respectively. The limit of quantification for 0.5-ml samples was 20 ng/ml [coefficient of variation (CV) $< 10\%$]. In the working range (0.1–20 µg/ml) the average CV was 9% (range 8–10%).

Pharmacokinetic analysis

Estimation of individual characteristics

Kinetic data analysis was performed by mathematical modeling; individual concentration-time profiles of VP16 were fitted by a two-compartment model. Consequently, observed data will be characterized by the model parameters. The VP16 concentration predicted by the model, $y_M(t,\underline{x})$, is given by

$$y_M(t,\underline{x}) = \frac{D}{\delta}\cdot\sum_{j=1}^{2}\frac{A_j}{a_j}\cdot\left[e^{-a_j(t-\delta)\cdot u(t-\delta)} - e^{-a_j\cdot t}\right]$$

at time t and involves four parameters, A_1, A_2, a_1, and a_2. The model parameters are compiled in the vector form $\underline{x}^T = [A_1\ A_2\ a_1\ a_2]$, where the superscript T denotes vector transpose and $u(t-\delta)$ is the step function valued at 0 for $t \le \delta$ and at 1 elsewhere. In the previous expression, D and δ are the total infused amount of VP16 and the administration duration, respectively.

For the 23 courses in the training group, numerical values were assigned to $\underline{x}$ by fitting of the model to the m observed data y_i $i = 1,\ldots,m$ according to the ML principle. To apply this we assumed that residual error $y_i - y_M(t_i,\underline{x})$ follows a zero mean Gaussian distribution with heteroscedastic variance model $K^2\cdot y_M^2(t,\underline{x})$; K is the coefficient of variation of residual error (see Appendix 1).

From the estimated model parameters we computed usual pharmacokinetic parameters such as clearance (CL), half-lives for the two decreasing phases in the semilogarithmic plot ($t_{1/2a}$, $t_{1/2b}$), and the volume of distribution of the central compartment (V_1). The area under the curve was calculated as $AUC = D/CL$.

Estimation of population characteristics

Population characteristics were evaluated using a two-stage method [28]. In the first step we estimated individual parameters as indicated above. In the second step, from these estimates we computed

Table 1 Patient's characteristics (*T1–T23* Training group, *V1–V11* test group, *CUP* carcinoma unknown primary, *Endomet. adeno.* endometrial adenocarcinoma, *SCLC* small-cell lung carcinoma, *AVI* doxorubicin/ etoposide/ ifosfamide, *CDDP* cisplatin)

Patient / sex	Age (years)	Diagnosis	Treatment	Number of kinetics	VP16 (mg)	Infusion time (h)	Group
T1 / M	67	Lung adenocarcinoma	VP16	1	36	2	I
T2 / M	71	CUP	VP16	1	34	2	I
T3 / F	72	CUP	VP16	1	30	2	I
T4 / F	73	Endomet. adeno.	VP16	1	34.4	2	I
T5 / M	41	Lung adenocarcinoma	VP16	1	30	2	I
T6 / M	70	Lung adenocarcinoma	VP16	1	35	2	I
T7 / M	62	Lung adenocarcinoma	VP16 + CDDP	1	200	2	I
T8 / F	60	Breast carcinoma	VP16	1	34	2	I
T9 / M	80	CUP	VP16	1	37	2	I
T10 / M	69	Lung adenocarcinoma	VP16 + CDDP	1	200	1	II
T11 / M	69	Sarcoma	VP16	1	200	1	II
T12 / M	58	Kaposi's sarcoma	VP16	1	200	1	II
T13 / F	27	Sarcoma	VP16	1	200	1	II
T14 / M	68	Lung adenocarcinoma	VP16 + CDDP	1	200	1	II
T15 / F	54	CUP	VP16 + CDDP	1	200	1	II
T16 / M	67	SCLC	AVI	4	220	0.5[a]	III
T17 / M	51	SCLC	AVI	4	200	0.5	III
T18 / F	56	SCLC	AVI	2	232	0.5	III
T19 / M	55	SCLC	AVI	2	184	0.5[a]	III
T20 / M	61	SCLC	AVI	4	240	0.5	III
T21 / M	49	Liposarcoma	VP16	1	36.8	2	I
T22 / M	63	Lung adenocarcinoma	VP16 + CDDP	1	200	1	II
T23 / M	23	Embryonic carcinoma	VP16 + CDDP	1	164	1.83	I
V1 / M	41	Urothelial carcinoma	VP16 + CDDP	1	200	2	I
V2 / M	48	Lung adenocarcinoma	VP16	1	36	2	I
V3 / M	70	Lymphoma	VP16	1	34.5	2	I
V4 / M	58	Leiomyosarcoma	VP16	1	40	2	I
V5 / M	85	Lymphoma	VP16	1	100	1	II
V6 / M	25	Dysgerminoma	VP16 + CDDP	1	200	1	II
V7 / M	65	Lung adenocarcinoma	VP16 + CDDP	1	200	1	II
V8 / F	67	Lung adenocarcinoma	VP16	1	200	1	II
V9 / F	70	SCLC	AVI	4	200	0.5[a]	III
V10 / M	54	SCLC	AVI	4	180	0.5[a]	III
V11 / M	55	SCLC	AVI	4	230	0.5[a]	III

[a] The planned infusion time was 0.5 h, but in some cases the effective infusion time increased significantly as follows:

Patient	Course	Infusion time (h)
T16	Day 2 of course 2	1.85
T19	Day 1 of course 2	1
V9	Day 2 of course 1	1.57
V9	Day 1 of course 2	2.17
V10	Day 2 of course 1	1.5
V11	Day 1 of course 1	1

the parameters of a probability density model expressing dispersion among individuals. We assumed that the multivariate normal distribution model would adequately describe the underlying dispersion, i.e., we had to compute the mean population vector $\underline{x}_0$ and the dispersion matrix D_0. For population the CV of residual error was obtained by averaging of squared individual Ks (see Appendix 2).

Optimal sampling times

After specifying a small number of samples m' ($m' < m$), we seek the most informative sampling times $\tau_i \; i = 1, \ldots, m'$ that allow precise estimation of $\underline{x}$ parameters. This is done by using the D-optimality criterion minimizing the volume of the confidence ellipsoid in the parameters space [2, 10] (see Appendix 3). This criterion involves predictions $y_M(t, \underline{x})$ and, therefore, unknown parameter values $\underline{x}$ that are to be estimated from the optimized samples. To overcome this difficulty we set these values equal to the mean population values $\underline{x}_0$.

Bayesian estimation

As for the training group, we obtain individual parameters for the 11 first courses of the test group (V1–V11); these ML estimates, $\underline{x}_{ML}$, will be used as reference values for assessment of the Bayesian procedure. From the same courses of the test group we selected m' data, those for which the sampling times t_i were the closest to the optimal sampling times $\tau_i \; i = 1, \ldots, m'$. These partial individual data were combined with the population information into a Bayesian estimation criterion to supply the Bayesian estimates of the model parameters, $\underline{x}_{BE}$ (see Appendix 4). Then, two sets of

parameters, $\underline{x}_{ML}$ and $\underline{x}_{BE}$, were available for the test set, obtained by the ML and the BE procedure, respectively.

Validation of the BE procedure

For assessment of the performance of the Bayesian procedure, paired statistical comparisons (Wilcoxon nonparametric test) were done between $\underline{x}_{ML}$ and $\underline{x}_{BE}$. For a parameter, absolute errors over the individuals in the test set, i.e., for $CL(CL_{ML} - CL_{BE})_j$ on the j-th individual, the bias (mean, median), and the precision (standard deviation, interquartile range) were also evaluated [29].

All pharmacokinetic calculations (ML and BE, population-characteristic computation, and OST determination) were done by means of the pharmacokinetics software package APIS [17]. Statistical calculations were done by the statistical package BMDP [13].

Results

In all cases the lowest VP16 plasma concentration was found to be greater than the lowest concentration of the working range (100 ng/ml). The two-compartment model adequately described the plasma concentration versus time data.

Estimation of population characteristics

The population characteristics computed from 23 courses are summarized in Table 2. The four pharmacokinetic parameters exhibited a CV ranging from 33% (a_2) to 76% (A_1). Mean values and standard deviations recorded for the half-lives of the quick ($t_{1/2a}$) and slow ($t_{1/2b}$) phases were found to be 0.80 ± 0.51 and 6.15 ± 2.40 h, respectively, the mean clearance was 2.43 ± 0.88 l/h, and the mean volume of distribution (V_1) of the central compartment was 8.37 ± 3.551.

On the basis of these results and taking into account practical constraints (cost, duration of analysis, and nursing organization), we designed a three-sample protocol with optimal sampling times as follows: t_1 – the end of the infusion, t_2 – 5 h after the beginning of the infusion, and t_3 – 24 h after the beginning of the infusion.

Table 2 Population characteristics computed from 23 concentration-time curves generated for the training group

	Mean parameter	Variance-covariance matrix			
A_1 (l^{-1})	1.04 E-1	6.22 E-3			
A_2 (l^{-1})	4.63 E-2	9.51 E-4	4.50 E–4		
a_1 (h^{-1})	1.30	5.14 E-2	1.26 E-2	8.66 E-1	
a_2 (h^{-1})	1.28 E-1	6.95 E-4	4.63 E-4	2.09 E-2	1.75 E-3
Residual error	7.70 E-2				

Validation

Validation was achieved by application of the ML and BE procedures to the 11 remaining patients. The sampling times retained for BE calculations were those closest to the OST. As an example, Fig. 1 shows for

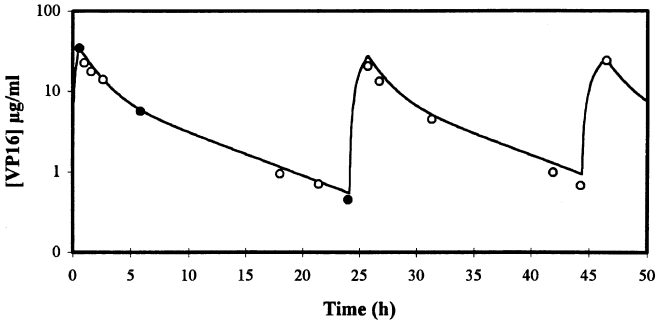

Fig. 1 Concentration-time curve (patient V9, course 1) determined by the BE procedure combined with plasma concentrations corresponding to the OST (•) overlayed with all available data (•,○)

Table 3 ML and BE values recorded for the pharmacokinetic parameters of the test group

	CL		$t_{1/2a}$		$t_{1/2b}$		V_1	
	ML	BE	ML	BE	ML	BE	ML	BE
1	2.02	1.90	0.46	1.76	6.21	9.62	5.56	6.59
2	3.85	3.55	0.29	1.42	4.44	10.68	2.86	6.62
3	4.28	4.10	0.48	1.43	9.85	13.01	3.47	6.51
4	3.55	3.29	0.04	1.77	1.49	10.44	2.73	7.55
5	3.10	3.10	0.86	0.81	14.33	12.01	9.00	9.46
6	2.23	2.19	0.96	0.66	8.12	7.96	6.89	6.25
7	2.41	2.19	0.47	0.95	9.17	10.38	6.49	6.51
8	2.31	2.51	1.12	1.52	12.93	13.12	5.38	6.11
9	1.79	1.90	0.91	0.40	5.68	4.30	5.14	4.98
10	2.45	2.32	0.61	0.57	6.08	5.53	5.91	6.34
11	2.15	2.24	0.63	1.93	7.48	10.74	4.82	5.57
Min	1.79	1.90	0.04	0.40	1.49	4.30	2.73	4.98
Max	4.28	4.10	1.12	1.93	14.33	13.12	9.00	9.46
Mean	2.74	2.66	0.62	1.20	7.80	9.80	5.29	6.59
SD	0.82	0.73	0.32	0.54	3.69	2.84	1.85	1.15
Median	2.41	2.32	0.61	1.42	7.48	10.44	5.38	6.51
IQR	1.13	1.01	0.42	0.91	3.63	2.59	2.06	0.43

Table 4 Bias (mean and median) and precision (SD and IQR) of absolute errors between ML and BE values recorded for the pharmacokinetic parameters of the test group

	CL	$t_{1/2a}$	$t_{1/2b}$	V_1
Wilcoxon *P* value	0.1141	0.0618	0.1095	0.0208
Mean	0.0786	−0.5807	−2.0014	−1.2955
SD	0.1636	0.7470	3.4108	1.7651
Median	0.125	−0.48	−1.21	−0.73
IQR	0.2475	1.26	3.6875	1.81

patient V9 the concordance between the observed data and the values predicted by the model using $\underline{x}_{BE}$. ML and BE estimates of the pharmacokinetic parameters are presented in Table 3. The variability observed in the test group was of the same magnitude as that seen in the training group; the most dispersed parameter was $t_{1/2a}$, and the least dispersed was *CL*. The bias and precision of absolute errors between ML and BE are presented in Table 4. The statistical test revealed no bias for *CL*, $t_{1/2a}$, or $t_{1/2b}$ ($P = 0.1141$, 0.0618, and 0.1095, respectively). A significant difference between the two sets of estimates was found only for V_1 ($P = 0.0208$).

The precision of the absolute error as evaluated either by the standard deviation (SD) or the interquartile range (IQR) was quite satisfactory for CL, because it was 5-fold lower than the dispersion of parameters (0.16 versus 0.82 for SD and 0.25 versus 1.13 for IQR). Thus, BE allows discrimination of CL values even for two resembling individuals. In contrast, neither for $t_{1/2a}$ nor for $t_{1/2b}$ was the precision of the absolute error satisfactory because it was of the same magnitude as, or even larger than, the dispersion of parameters.

Figure 2 presents regression analysis of the AUC values obtained after BE versus ML (correlation coefficient $r = 0.992$). The estimated values of the slope ($a = 0.981 \pm 0.041$) and the intercept ($b = 1.755 \pm 3.121$) are not significantly different from 1 and 0, respectively, showing the statistical equivalence between ML and BE for the AUC values. Moreover, for the further VP16 administrations in patients T16–T20 and V9–V11, Fig. 3 shows the concordance between ML and BE AUC estimates.

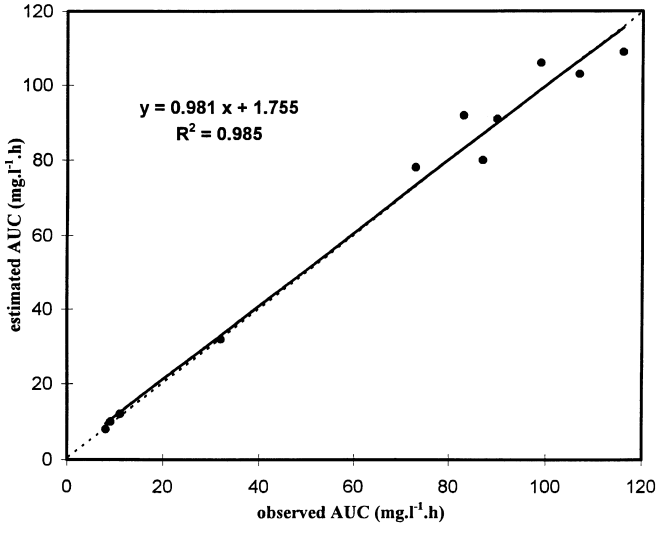

Fig. 2 Estimated etoposide AUCs versus observed AUCs for the 11 courses used to validate the BE methodology. Dashes represent the line of identity

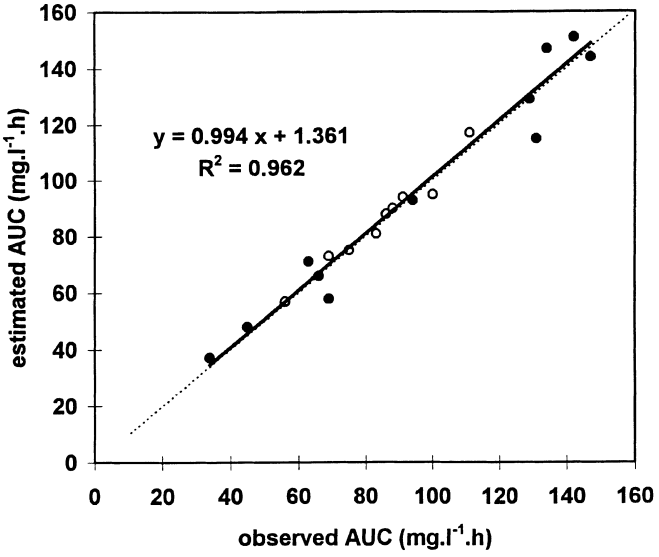

Fig. 3 Estimated etoposide AUCs versus-observed AUCs for the 21 additional administrations (• Patients T16–T20 in the training group, ○ patients V9–V11 in the test group). Dashes represent the line of identity

Discussion

The existence of relationships between VP16 pharmacodynamics (PD) and pharmacokinetics (PK) have been shown in a number of studies [18, 24, 36]. On the clinical side, Slevin et al. [33] stressed the importance of the dose schedule of etoposide, as was further reported by Berry and Erlichman [4]. Ratain et al. [27] showed the lack of correlation between VP16 clearance and body surface area and concluded that pharmacologically based dosing could increase the dose intensity without affecting life-threatening toxicities. Mick and Ratain [22] reported a nonlinear pharmacodynamic model to be capable of predicting hematological toxicity.

Hence, it would seem of interest to individualize VP16 dose regimens on the basis of pharmacokinetic parameters. The BE procedure is of great interest because it minimizes the number of blood samples required and enables quick and efficient PK assessment for further PK/PD studies. This report describes a limited-sampling protocol allowing efficient estimation of individual PK parameters of VP16 for each patient using the BE procedure following three different administration schedules. VP16 population characteristics were estimated according to an open two-compartment model.

The large interindividual variability exhibited by the PK parameters emphasized the interest of individual VP16 PK follow-up. The PK parameters we obtained by ML estimation are in the range of the values generally reported in the literature [12, 26, 32] i.e., $t_{1/2a}$ – 0.5–1 h, $t_{1/2b}$ – 5–12 h, V_1 – 7–15 1/m^2, and CL – 20–30 ml min^{-1} m^{-2}.

As can be seen from the example illustrated in Fig. 1, a PK model using parameters estimated by the BE procedure provides accurate predictions of the plasma concentration of VP16 at any time and could be used to achieve the desired plasma concentration in a given patient. This methodology is also a good predictor of the PK parameters, mainly CL and AUC, regardless of the dose injected, the type of administration, and the type of treatment applied in our study (VP16 given as a single agent or in combination). The CL values found using both methodologies (i.e., BE and ML procedures) appeared to be similar (2.66 ± 0.73 versus 2.74 ± 0.82 1 h^{-1}, respectively). The analysis of individual results revealed that in all cases a difference between AUC BE and ML estimates follows the dispersion of CL values and never exceeds 12%. These results are of clinical importance for further PK/PD studies and for patients' dose individualization.

High deviations in the BE of $t_{1/2b}$ and V_1 were observed in 2 of the 11 cases (V2 and V4). This could be explained by the differences existing between the OST and the closest available observations. Indeed, in these cases the closest available observation to t_3 (24 h after the beginning of the infusion) was always about 12 h earlier. This could explain the unexpected increase in BE estimates obtained with these data sets. The poor performance for $t_{1/2a}$ determination could have been attributable to the large variability associated with the first decline phase (A_1 and a_1 CV 76% and 72%, respectively). Nonetheless, our model allows an accurate estimation of parameters of importance for the determination of drug exposure, mainly CL and AUC.

In conclusion, the BE procedure combined with an OST protocol as presented in this report supplies efficient estimations of the PK parameters of etoposide at minimal cost and a level of disturbance acceptable to the patient. Using the population characteristics presented herein, we think that this methodology should lead to the development of other PK/PD studies using different drug administration schedules and appropriate OST protocols.

Appendix 1

From m observed data (t_i, y_i) $i = 1, \ldots, m$, ML estimates $\underline{x}_{ML}$ and K_{ML} of the model parameters $\underline{x}$ and of the coefficient of variation K of the heteroscedastic variance model of the residual error, respectively, are:

$$[\underline{x}_{ML}, K_{ML}] = \arg\min\left\{\sum_{i=1}^{m}\log\left[K^2\cdot y_M^2(t_i,\underline{x})\right] + \frac{1}{K^2}\cdot\sum_{i=1}^{m}\left[\frac{y_i - y_M(t_i,\underline{x})}{y_M(t_i,\underline{x})}\right]^2\right\} .$$

Appendix 2

For a specific individual j these estimates may be noted $\underline{x}_{MLj}$ and K_{MLj}. From individuals belonging in the training group, population characteristics for a multivariate normal distribution (mean vector $\underline{x}_0$ and dispersion matrix D_0) may be computed as:

$$\underline{x}_0 = \frac{1}{n}\cdot\sum_{j=1}^{n}\underline{x}_{MLj} \quad D_0 = \frac{1}{n}\cdot\sum_{j=1}^{n}\left(\underline{x}_{MLj} - \underline{x}_0\right)\cdot\left(\underline{x}_{MLj} - \underline{x}_0\right)^T .$$

For population the CV of the residual error is given by:

$$K_0^2 = \frac{1}{n}\cdot\sum_{j=1}^{n}K_{MLj}^2 .$$

Appendix 3

The weighted sensitivity of the model prediction $y_M(t,\underline{x})$ with respect to the k-th model parameter $(k = 1, \ldots, p)$ is defined as:

$$S_k(t,\underline{x}_0) = \frac{1}{y_M(t,\underline{x}_0)}\cdot\left.\frac{\partial y_M(t,\underline{x})}{\partial x_k}\right|_{\underline{x}=\underline{x}_0} .$$

For the p parameters the sensitivity evaluated at m' sampling times t_i $i = 1, \ldots, m'$ leads to the $(m' \times p)$ sensitivity matrix having as elements $S_k(t_i,\underline{x}_0)$. The sensitivity matrix is noted $S(\underline{t},\underline{x}_0)$, where $\underline{t}$ is the vector containing the m' sampling times. The D-optimal m' sampling times $\underline{\tau}$ allowing precise $\underline{x}$ estimation are:

$$\underline{\tau} = \arg\max\left|\frac{1}{K_0^2}\cdot S^T(\underline{t},\underline{x}_0)\cdot S(\underline{t},\underline{x}_0) + D_0^{-1}\right| .$$

Absolute value $|.|$denotes the determinant of a square matrix and superscript T, the matrix transpose.

Appendix 4

Let $\underline{t}$ be the m' sampling times out of the m available on a given individual that are the closest to the m' optimal $\underline{\tau}$ ones. Bayesian estimates $\underline{x}_{BE}$ of the model parameters $\underline{x}$ are:

$$\underline{x}_{BE} = \arg\min\left\{(\underline{x}-\underline{x}_0)^T\cdot D_0^{-1}\cdot(\underline{x}-\underline{x}_0) + \sum_{i=1}^{m'}\log[K_0^2\cdot y_M^2(t_i,\underline{x})] + \frac{1}{K_0^2}\cdot\sum_{i=1}^{m'}\left[\frac{y_i - y_M(t_i,\underline{x})}{y_M(t_i,\underline{x})}\right]^2\right\} .$$

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