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ORIGINAL ARTICLE

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Limited sampling models for simultaneous estimation of the pharmacokinetics of irinotecan and its active metabolite SN-38

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Abstract Irinotecan (CPT-11) is a novel topoisomerase I inhibitor with clinical activity in human malignancies. The objective of this study was to develop efficient limited sampling models (LSMs) to estimate simultaneously the area under the plasma concentration versus time curves (AUC) for both CPT-11 and its active metabolite SN-38. A total of 64 pharmacokinetic sets ($\geq$ 24-h sampling) were obtained in phase I studies at doses ranging from 50 to 750 mg/m^2 (0.5-h i.v. infusion). The patients were randomly assigned to a training data set ($n = 32$) and a test set ($n = 32$). Multiple linear regression analyses were used to determine the optimal LSMs based on the correlation coefficient (r), bias (MPE%, percentage of mean prediction error), and precision (RMSE%, percentage of root mean squared prediction error). Of these LSMs, the ones including maximal concentrations of CPT-11 (0.5 h, the end of the i.v. infusion) and metabolite SN-38 ($\approx$ 1 h) were favored along with predictive precision and clinical constraints. Several bivariate models including a 6-h time point as the last sampling time (or 7 h) were found to be highly predictive of either the CPT-11 AUC or the SN-38 AUC. The chosen sampling time points were the ones that allowed the best compromise between the accurate determination of either compound alone with the same sampling times. The simultaneously best prediction of both CPT-11 and SN-38 AUCs was obtained with sampling time points harvested at 0.5, 1, and 6 h (or 7 h). With these sampling time points a trivariate model was selected for the determination of CPT-11 AUC namely, CPT-11 AUC (ng h ml^{-1}) = $0.820 \times C_{0.5h} + 0.402 \times C_{1h} + 15.47 \times C_{6h} + 928$, and a corresponding model was selected for the determination of metabolite AUC, i.e., SN-38 AUC (ng h ml^{-1}) = $4.05 \times C_{0.5h} - 0.81 \times C_{1h} + 23.01 \times C_{6h} - 69.78$, where $C(t)$ is the concentration in nanograms per milliliter of either compound at a given time t. These models performed well with the test data sets for CPT-11 AUC ($r = 0.98$, MPE% = -1.4, RMSE% = 13.9) and for SN-38 AUC ($r = 0.95$, MPE% = -6.5, RMSE% = 37.7). In addition to the determination of AUCs (and hence clearance), these models also allow the determination of the maximal concentrations of both compounds, which might be needed for pharmacodynamics studies. Other bi- and trivariate models including other time points are also presented. These LSMs not only will facilitate ongoing and future clinical trials by significantly reducing the number of blood samples needed for pharmacokinetics studies but will hopefully contribute to a better knowledge of pharmacokinetic-pharmacodynamic relationships for both CPT-11 and its active metabolite SN-38.

Key words Pharmacokinetics · Limited sampling model Irinotecan · CPT-11 · Metabolite SN-38

Abbreviations *CPT-11* (7-Ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin · *SN-38* 7-ethyl-10-hydroxy-camptothecin · *AUC*, area under the plasma concentration versus time curve · *MPE%* percentage of mean prediction error (bias) · *RMSE%* percentage root mean squared prediction error (precision) · *MRT* mean residence time · *Vdss* volume of distribution at steady state · *CL* total body clearance

Introduction

The novel camptothecin analogue irinotecan (CPT-11) has demonstrated good antitumor activity against various experimental tumor models [3, 15, 22, 26], including multidrug-resistant lines [40]. CPT-11 has also shown antitumor activity in clinical studies [1, 4, 8, 9, 11, 25, 27–29, 32, 33, 35, 37].

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Like camptothecin, CPT-11 stabilizes the mammalian DNA-topoisomerase I cleavable complex, which can result in cell death during DNA replication [16–18, 21, 23, 42]. Although the precise mechanisms of resistance to camptothecins are presently not completely clear, decreased activity of the target enzyme DNA-topoisomerase I and/or mutation of the enzyme with decreased binding of the drug have been described in resistant cell lines [14, 19, 24, 36, 39]. Of particular interest is the lack of cross-resistance of camptothecin and the analogue CPT-11 with other drugs affected by the multidrug-resistance phenotype [7, 40].

The analogue CPT-11 is also converted in vivo to the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), whose in vitro cytotoxic activity is a $\geq$ 100-fold that of the parent compound [19, 20]. The contribution of this metabolite to the in vitro cytotoxicity and in vivo antitumoral activity is probably important [19, 20, 22, 38].

Prior clinical phase I–II studies have explored various schedules of CPT-11 administration [1, 4, 8, 9, 11, 25, 27–29, 32, 33, 35, 37], and the dose-limiting toxicities are mainly diarrhea and myelosuppression (reviewed in [4, 35]). CPT-11 has shown activity in several human malignancies, including refractory leukemia and lymphoma, lung, colon and gynecological cancers [1, 4, 8, 9, 11, 25, 27–29, 32, 33, 35, 37].

Early clinical studies have shown relationships between the pharmacokinetics and pharmacodynamics of this drug and those of its active metabolite SN-38 [1, 5, 6, 9, 12]. Since the determination of pharmacokinetic parameters usually requires more than ten venipunctures per patient, there is a need to develop strategies that would minimize the cost and burden to the patient, the clinical personnel, and the laboratory.

In this article we report the successful development of bi- and trivariate limited sampling models (LSM) for the simultaneous determination of the area under the plasma concentration versus time curve (AUC), total body clearance, and maximal concentration of CPT-11 and its active metabolite SN-38. These LSMs not only will facilitate ongoing and future clinical trials by significantly reducing the number of blood samples needed for pharmacokinetics studies but will also contribute to a more detailed study of pharmacokinetic-pharmacodynamic relationships in future trials. These LSMs have been implemented in phase II trials in Europe [12].

Patients and methods

Patient population

Complete pharmacokinetic data sets were obtained in 64 patients during their first receipt of CPT-11 during phase I studies [1, 9]. Only patients receiving the drug for the first time were entered in this study, because of the possible influence of prior CPT-11 administration on the pharmacokinetics of both CPT-11 and SN-38. All patients met standard phase I eligibility criteria [1, 9].

For the development of LSMs, this population was arbitrarily divided into two sets termed the *training set* ($n = 32$), to determine the model parameters, and the *test set* ($n = 32$), to validate the model. To ensure that the two sets of patients were well balanced in terms of the dose range given, the assignment to the training set or the test set was accomplished as follows: from a list of the 64 patients numbered according to the order of entry in the clinical study (and, thus, by increasing dosage for phase I trials), those with an odd number were assigned to the training group and those with an even number were assigned to the test group.

CPT-11 administration

CPT-11 was provided by Yakult Honsha Co. Ltd. (Tokyo, Japan) and by Laboratoire Bellon (Neuilly-sur-Seine, France) as a ready-for-use solution in 2-ml or 5-ml vials containing 40 or 100 mg of the drug, respectively. The required dose was diluted in 250 ml of 0.9% sodium chloride solution and given as a target 0.5-h i.v. infusion. In these phase I studies, CPT-11 was infused at doses ranging from 50 to 750 mg/m^2.

Pharmacokinetics

Chemicals

Pure standards of CPT-11, SN-38, and camptothecin were provided by Yakult Honsha Co. Ltd (Tokyo, Japan). Solvents and reagents were of the highest purity commercially available and were obtained from Farmitalia Carlo Erba (Milano, Italy).

Plasma collection for pharmacokinetics study

Heparinized blood samples (2 ml) were collected immediately predosing (time 0), at 10, 20 and 30 min during the 30-min i.v. infusion; and then at 5, 10, 15, 30, 45, and 60 min and 2, 4, 8, 12, 24, and 36 h postinfusion. To determine the terminal half-life more accurately, sampling was prolonged to 96 h in 20 cases. Blood was immediately centrifuged at 2,000 $\times$ *g* for 15 min, and the plasma was transferred to a 1.5-ml polypropylene tube and stored at -20°C until analysis. An error of 15% in the target sampling times was considered acceptable for the LSM development. Because of the 4-h lag time between the cumulative times of 4.5 and 8.5 h, additional model-independent time-point values were determined for the 6- and 7-h time points by linear interpolation on a semilogarithmic scale.

Determination of CPT-11 and SN-38 plasma levels

CPT-11 and SN-38 were assayed by reversed-phase high-performance liquid chromatography (HPLC) with fluorescence detection using a procedure that allowed the simultaneous determination of both compounds [2]. Briefly, after the addition of camptothecin as the internal standard, plasma samples (100 µl) were extracted using a solid-phase (C18) extraction step. The extracts were then chromatographed on a C18 reversed-phase analytical column using a mobile phase (1 ml/min) composed of 34% acetonitrile and 66% potassium dihydrogen phosphate (0.1 *M*) containing 3 m*M* sodium heptanesulfonic acid (pH 4). The fluorescence-detector wavelengths were set at 380 nm (excitation) and 500 nm (emission). Calibration curves were linear over a wide range of concentrations (from 1 ng/ml to 10 µg/ml), and the lower limit of quantification was 1 ng/ml for

both CPT-11 and SN-38. CPT-11 and SN-38 concentrations were determined from peak area ratios of either compound to the internal standard (camptothecin) in reference to a calibration curve performed daily. This assay measures total CPT-11 and SN-38, i.e., the lactone and the carboxylate forms.

Pharmacokinetic parameter determination

The following pharmacokinetic parameters were determined by model-independent procedures [13]: the terminal half-life, by linear regression analysis of the last phase aligned concentrations on a semilogarithmic scale; the total AUC, by the trapezoidal method to infinity; the mean residence time (MRT) and the volume of distribution at steady state (Vdss), according to the statistical moment theory; and the total body clearance (CL), as the dose divided by the total AUC.

Metabolite SN-38 plasma concentrations allowed the determination of the following parameters: the time of peak plasma level, the AUC, and the terminal apparent half-life as determined by linear regression analysis of the last three to four aligned concentration time points on a semilogarithmic scale.

Limited-sampling-strategy development

Stepwise regression method

Using the training data set, the concentrations of CPT-11 or SN-38 (independent variables) were first correlated to the AUC (dependent variable) using linear regression analysis. Using forward multiple regression analysis, the concentrations obtained at other time points were thereafter included as additional independent variables to try to improve the models. The F-test was used to select the optimal strategy. Backward elimination regression analysis was also performed on the training data set as a check for the forward regression analysis. Statistical calculations were performed using SigmaStat (Jandel Scientific GmbH, Germany).

LSM validation

The models obtained with the training data set were assessed for their ability to predict accurately the AUC of the test data set (or validation set) determined from the complete concentration-time curves.

Criteria used for the choice of the best models

The models considered as optimal for AUC prediction were the ones presenting the highest correlation coefficient r, the lowest predictive bias, and the highest precision [34]. Bias was estimated as the percentage of mean prediction error (MPE%), and the precision was evaluated as the percentage of root mean squared prediction error (RMSE%).

Clinical constraints were also considered in the choice of the optimal models; the models with the fewest sampling time points (≤ 3) that had been harvested as early as possible after the beginning of the i.v. infusion (i.e., ≤ 8 h) were considered optimal, provided they met the predictive performance criteria mentioned above. Also taken into account were the points showing the maximal concentration of CPT-11 (i.e., 0.5 h, or the end of the i.v. infusion) and its active metabolite SN-38 (≈ 1 h from the start of the 0.5-h i.v. infusion). Finally, the best models had to predict simultaneously for both CPT-11 and metabolite SN-38 AUCs, preferably with the same sampling time points, i.e., they needed to be the best compromise between the highest predictive models for either the CPT-11 AUC or the SN-38 AUC alone.

Results

Patient population

The characteristics of the population of patients from whom were derived the pharmacokinetic data sets used for the determination and the validation of the LSMs are presented in Table 1. The median age was 52 years; there were 41 men and 23 women; the performance status was 0 in 53% of patients (34/64), 1 in 42% (27/64), and 2 in 5% (3/64); and the tumor types were mostly head and neck, rectum, colon, lung, and breast malignancies. Most patients had received prior treatment, including surgery, radiotherapy, and chemotherapy.

This patient population was further divided into two subpopulations, hereafter termed the *training set* ($n = 32$), to determine the model parameters, and a *test set* ($n = 32$) independent of the training set, to validate the model. Because of the wide range of doses given in these pharmacokinetics studies (50–750 mg/m^2), special care was taken in the assignment of patients to the two groups so as to avoid bias in the predictive value of one set to another (see Patients and methods). The two patient subpopulations assigned to the training set or the test set were found to be very similar not only in terms of the dose range delivered (as expected) but also in terms of age, gender distribution, performance status, tumor types, and prior treatments.

CPT-11 pharmacokinetics

The mean plasma concentration profiles obtained for CPT-11 after the administration of a 350-mg/m^2 dose are depicted in Fig. 1. This dose level is the recommended dose for phase II studies using an every 3 week schedule. CPT-11 pharmacokinetic parameters obtained in this population of 64 patients presented a high interpatient variability as shown by the relatively high standard deviations observed (Table 2; Fig. 1). The mean terminal plasma half-life was 13.0 ± 8.2 h; the mean residence time (MRT), 11.2 ± 3.7 h; the total body clearance (CL), 13.3 ± 5.8 l h^{-1} m^{-2}; and the volume of distribution at steady state (Vdss), 149 ± 76 l/m^2. The pharmacokinetics was linear within the dose range given as indicated by a proportional increase in the CPT-11 total AUC as a function of increased CPT-11 dose and by the stable total body clearance. CPT-11 pharmacokinetic parameters were found similar in the two patient subpopulations, i.e., the training and the test sets (Table 2).

Table 1 Patients' characteristics

Characteristics	All patients ($n = 64$)	Training set ($n = 32$)	Test set ($n = 32$)
Dose range (mg/m^2)	50–750	66–750	50–750
Median age (years)	52	53.5	51.5
Gender (M/F)	41/23	21/11	20/12
Performance status:			
Grade 0	34	17	17
Grade 1	27	14	13
Grade 2	3	1	2
Tumor types:			
Head and neck	11	5	6
Rectum	11	3	8
Colon	10	5	5
Lung	8	6	2
Breast	5	3	2
Melanoma	2	1	1
Kidney	2	2	0
Liver	2	2	0
Cervix	2	1	1
Mesothelioma	2	0	2
Others	9	4	5
Prior treatment:			
None	3	1	2
Surgery	44	21	23
Radiotherapy	36	20	16
Chemotherapy	52	27	25

Table 2 Pharmacokinetic parameters of the patient population as well as the training and test sets.[a]

	Population ($n = 64$)	Training set ($n = 32$)	Test set ($n = 32$)
CPT-11:			
Dose range (mg/m^2)	50–750	66–750	50–750
Number of patients (50–260 mg/m^2)	33	16	17
Number of patients (300–500 mg/m^2)	22	11	11
Number of patients (600–750 mg/m^2)	9	5	4
Terminal half-life (h)[b]	13.0 ± 8.2	13.2 ± 8.6	12.7 ± 7.7
MRT (h)[b]	11.2 ± 3.7	11.5 ± 4.5	10.8 ± 2.6
CL (l h^{-1} m^{-2})[b]	13.3 ± 5.8	13.9 ± 4.4	12.8 ± 6.8
Vdss (l/m^2)[b]	149 ± 76	165 ± 88	133 ± 58
AUC range (ng h ml^{-1})	2,798–83,061	3,054–55,048	2,798–83,061
SN-38:			
Time of maximal levels (h)[b]	1.0 ± 0.7	0.8 ± 0.5	1.2 ± 0.9
Apparent half-life (h)[b]	12.0 ± 8.3	10.5 ± 6.1	13.4 ± 9.7
AUC range (ng h ml^{-1})	36–3,551	36–3,243	59–3,551

[a] CPT-11 was given as a 0.5-h i.v. infusion
[b] Mean values ± SD.

Pharmacokinetics of the active metabolite SN-38

The mean levels of metabolite SN-38 detected at a dose level of 350 mg/m^2 of CPT-11 are depicted in Fig. 1. Maximal levels were observed at $\approx$ 1 h from the start of the 0.5-h i.v. infusion (i.e., 0.5 h postinfusion). The SN-38 plasma decay closely followed that of CPT-11, with a mean apparent terminal half-life of 12.0 ± 8.3 h being noted. Interindividual levels of this metabolite were found to be highly variable at the same dose levels (Fig. 1). SN-38 pharmacokinetic parameters were found to be similar in the training and the test sets (Table 2).

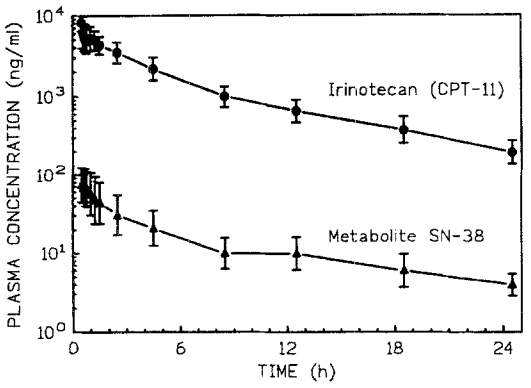

Fig. 1 Mean plasma concentrations of irinotecan (*CPT-11, circles*) and active metabolite SN-38 (*triangles*) measured following the administration of CPT-11 at the 350-mg/m^2 dose level as a 0.5-h i.v. infusion. Each point is the mean of 7 pharmacokinetic data sets; *error bars*, ± SD

LSM development for CPT-11

One-time-point models

Using the training data set, the CPT-11 concentrations at each time point correlated with the CPT-11 AUC. The linear regression analyses yielded correlation coefficients (r) that ranged from 0.821 at 0.5 h (i.e., the end of the i.v. infusion) to 0.962 at 6 h (Table 3). The two best sampling points were the 6- and 7-h time points, which had the highest correlation coefficient and the lowest RMSE% (highest precision). The predictive value of these single-time-point models was validated with the test set, and the points at 4.5, 6, and 7 h appeared to be the best, yielding the highest correlation coefficients, the lowest bias (MPE%), and the highest precision (lowest RMSE%).

Two-time-point models

Following the single-time-point analysis, the CPT-11 plasma concentrations of the training set were subjected to stepwise forward multiple linear regression analysis. As compared with the single-time-point models, precision was significantly improved with the two-time-point models, resulting in higher correlation coefficients and lower RMSE% (Table 3). Although the 4.5-h time point would have been more clinically convenient as the last time point, this point was found not to perform as well as models including the 6- or 7-h

Table 3 Limited sampling models for the estimation of CPT-11 plasma AUC[a]

	Time (h)			Coefficients				Training set			Test set		
Model	t_1	t_2	t_3	k_1	k_2	k_3	k_4	r	MPE%	RMSE%	r	MPE%	RMSE%
1-point models:													
A	4.5	–	–	13.56	3,877	–	–	0.931	0.025	23.6	0.974	− 3.2	17.8
B	6.0	–	–	19.89	2,607	–	–	0.962	− 0.009	17.6	0.973	− 2.4	18.2
C	7.0	–	–	23.48	3,163	–	–	0.958	0.102	18.6	0.967	− 0.4	19.6
2-point models:													
D	0.5	4.5	–	1.101	10.36	1,853	–	0.954	− 0.034	19.5	0.974	− 2.3	17.7
E	0.5	6.0	–	0.893	16.16	1,103	–	0.976	0.018	14.0	0.983	− 1.8	14.5
F	1.0	6.0	–	1.090	17.15	1,787	–	0.967	− 0.004	16.4	0.980	− 0.8	15.4
G	1.25	6.0	–	2.010	14.78	1,237	–	0.974	0.048	14.8	0.979	0.7	15.9
H	1.5	6.0	–	1.610	16.06	1,633	–	0.970	0.014	15.6	0.980	− 1.2	15.4
I	2.5	6.0	–	2.120	15.77	1,979	–	0.967	− 0.017	16.5	0.978	− 3.7	17.1
J	0.5	7.0	–	0.989	18.69	1,302	–	0.976	0.094	14.1	0.974	− 0.3	17.3
K	1.0	7.0	–	1.600	18.95	1,648	–	0.970	0.051	15.7	0.976	1.4	17.0
L	1.25	7.0	–	2.397	16.55	1,170	–	0.977	0.077	13.9	0.972	2.5	18.8
M	1.5	7.0	–	2.160	17.71	1,444	–	0.976	0.049	14.1	0.975	0.5	17.2
N	2.5	7.0	–	3.010	16.79	1,905	–	0.970	0.047	15.7	0.974	− 3.1	17.9
3-point models:													
O	0.5	1.0	6.0	0.820	0.402	15.47	928	0.977	− 0.070	13.8	0.984	− 1.4	13.9
P	0.5	1.25	6.0	0.675	1.264	13.87	611	0.980	− 0.019	12.9	0.986	− 0.1	12.9
Q	0.5	1.5	6.0	0.743	0.931	14.58	792	0.979	− 0.010	13.3	0.985	− 1.3	13.4
R	0.5	2.5	6.0	0.825	1.423	13.68	796	0.978	0.003	13.4	0.989	− 2.8	12.1
S	0.5	1.0	7.0	0.787	0.893	17.14	836	0.979	0.069	13.2	0.978	0.7	16.3
T	0.5	1.25	7.0	0.660	1.638	15.52	560	0.983	0.054	11.9	0.980	1.7	16.0
U	0.5	1.5	7.0	0.694	1.480	16.16	678	0.983	0.086	11.9	0.980	0.3	15.7
V	0.5	2.5	7.0	0.829	2.135	14.72	711	0.982	0.054	12.3	0.986	− 2.2	13.0

[a] All times are cumulative, i.e., they include the 0.5-h infusion time. The determination of the CPT-11 AUC (ng h ml^{-1}) in a given model is determined by entering the appropriate plasma concentration (C, ng/ml) at a given time t from the start of the 0.5-h i.v. infusion. The models are:
CPT-11 AUC = $k_1 \times C(t_1) + k_2$ (1 point model),
CPT-11 AUC = $k_1 \times C(t_1) + k_2 \times C(t_2) + k_3$ (2-point model), and
CPT-11 AUC = $k_1 \times C(t_1) + k_2 \times C(t_2) + k_3 \times C(t_3) + k_4$ (3-point model)

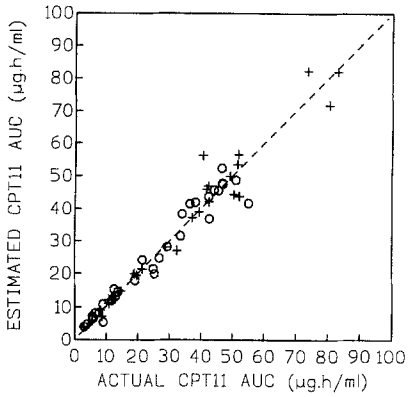

Fig. 2 Actual AUC generated for irinotecan (*CPT-11*), versus the estimated AUC for the trivariate model (model O) including sampling times at 0.5, 1, and 6 h (*Circles* Training data set, *crosses* test data set, *dashed line* identity line)

time point. The bivariate models including the 6-h time point with one time point at 0.5, 1, 1.25, 1.5, or 2.5 h yielded almost equivalent results in terms of precision for the determination of the CPT-11 AUC for the training set (Table 3, models E–I). Of these models, model E (0.5 and 6 h) performed the best with both the training and the test sets (highest r and lowest RMSE%). The bivariate models including the 7-h time point performed as well as those with the 6-h time point in terms of precision (r and RMSE%; Table 4, models J–N).

Three-time-point models

The addition of a third time point to the model with the 6-h time point as the last one (Table 3, models O–R) slightly improved the precision. The predictive value of model O is depicted in Fig. 2. A comparison of the bivariate and the trivariate models including the 7-h time point disclosed a maximal increase in r of less than 1% and a decrease in RMSE% of 2% (Table 3). The performance of these trivariate models with the test set appeared almost equivalent for all the intermediate time points (Table 3).

LSM development for metabolite SN-38

After the development of the above mentioned models for CPT-11, the same procedure was applied to develop models for the determination of the AUC for the active metabolite SN-38.

One-time-point models

With the training data set, SN-38 concentrations at each time point correlated with the SN-38 AUC. The linear regression analyses yielded the best correlation coefficients with the 6- and 7-h time points ($r = 0.957$ and 0.919, respectively; Table 4). The predictive value of these univariate models was validated with the test set, and the points at 4.5, 6, and 7 h were the most predictive, yielding the highest correlation coefficient ($r \geq 0.942$), the lowest bias (MPE% ≤ 16.2), and the highest precision (smallest RMSE%, ≤ 36.3; Table 4). Of these, the 6-h time point was the best, yielding the highest r (0.945) and the lowest MPE (4.5%) and RMSE (33.9%).

Two-time-point models

After the univariate analysis, the SN-38 plasma concentrations at each time point of the training set were subjected to stepwise forward multiple linear regression analysis. With a second time point, precision improved markedly as shown by a significant increase in r and a decrease in RMSE% (Table 4). Of the models with the 6-h time point as the last sample (Table 4, models *e–i*), model *e* (0.5 and 6 h) appeared the best, giving the highest r and the lowest RMSE%. Among the bivariate models including the 7-h time point (models *j–n*), model *j* performed the best with the training set (Table 4).

Three-time-point models

The introduction of a third time point to the model (—models *o–v*, Table 4) slightly improved the precision as compared with the bivariate models described above. The predictive value of model *o* (0.5, 1, and 6 h) for the determination of the AUC for the active metabolite SN-38 is depicted in Fig. 3.

Discussion

Our results demonstrate that several LSMs can be developed for the independent determination of the AUC of either CPT-11 or its active metabolite SN-38. Each of the models presented has its advantages or disavantages. The problem is to choose among the best models for either compound alone exactly, which one(s) will predict the best for the simultaneous determination of both the CPT-11 AUC and that of its active metabolite SN-38, keeping in mind that the metabolite is not only more cytotoxic than the parent compound in vitro [19, 20] but also appears to play a major role in the in vivo toxicity and/or anticancer effect [19, 20, 22, 38].

Since there was a 4-h lag time between the cumulative times of 4.5 and 8.5 h, additional model-independent

Table 4 Limited sampling models for the estimation of metabolite SN-38 plasma AUC[a]

	Time (h)			Coefficients				Training set			Test set		
Model	t_1	t_2	t_3	k_1	k_2	k_3	k_4	r	MPE%	RMSE%	r	MPE%	RMSE%
1-point models:													
a	4.5	–	–	20.73	17.18	–	–	0.850	− 0.017	65.9	0.944	6.1	35.2
b	6.0	–	–	25.79	42.65	–	–	0.957	− 0.002	36.2	0.945	4.5	33.9
c	7.0	–	–	22.00	161.04	–	–	0.919	− 0.009	49.3	0.942	16.2	36.3
2-point models:													
d	0.5	4.5	–	7.41	14.69	− 113.21	–	0.726	0.005	91.1	0.934	− 35.4	82.0
e	0.5	6.0	–	3.33	22.87	− 74.10	–	0.976	0.008	27.1	0.957	− 6.2	36.3
f	1.0	6.0	–	1.74	24.12	− 20.71	–	0.964	0.002	33.0	0.954	0.1	34.4
g	1.25	6.0	–	2.25	23.17	− 32.72	–	0.967	− 0.008	31.5	0.947	3.4	34.9
h	1.5	6.0	–	1.57	24.38	− 7.25	–	0.961	0.009	34.6	0.949	2.6	34.5
i	2.5	6.0	–	3.45	20.79	− 12.70	–	0.966	0.035	32.3	0.947	3.1	35.6
j	0.5	7.0	–	5.34	18.96	− 65.84	–	0.977	− 0.004	26.8	0.935	− 4.4	36.3
k	1.0	7.0	–	3.74	19.84	− 8.61	–	0.959	0.013	35.3	0.953	3.9	29.2
l	1.25	7.0	–	3.77	18.75	8.33	–	0.953	− 0.046	38.1	0.940	11.6	33.2
m	1.5	7.0	–	3.72	19.67	17.84	–	0.944	− 0.007	41.2	0.947	9.4	30.7
n	2.5	7.0	–	6.14	15.01	10.95	–	0.961	− 0.034	34.8	0.942	8.7	32.9
3-point models:													
o	0.5	1.0	6.0	4.05	− 0.81	23.01	− 69.78	0.977	0.002	26.7	0.951	− 6.5	37.7
p	0.5	1.25	6.0	3.31	0.03	22.85	− 74.25	0.976	− 0.016	27.0	0.957	− 6.2	36.3
q	0.5	1.5	6.0	3.58	− 0.49	23.09	− 67.27	0.977	− 0.007	26.9	0.955	− 6.4	36.7
r	0.5	2.5	6.0	3.01	0.87	21.89	− 76.68	0.977	− 0.022	26.8	0.959	− 5.5	35.5
s	0.5	1.0	7.0	4.82	0.57	18.93	− 69.26	0.977	− 0.055	26.6	0.942	− 4.3	34.5
t	0.5	1.25	7.0	5.52	− 0.22	19.04	− 64.68	0.977	0.059	26.7	0.933	− 4.8	37.1
u	0.5	1.5	7.0	5.08	0.46	18.82	− 72.46	0.977	0.006	26.6	0.939	− 4.2	35.3
v	0.5	2.5	7.0	4.20	2.35	16.93	− 74.81	0.980	0.004	24.8	0.953	− 2.8	31.3

[a] All times are cumulative, i.e., they include the 0.5-h infusion time. The determination of the SN-38 AUC (ng h ml^{-1}) in a given model is determined by entering the appropriate SN-38 plasma concentration (C, ng/ml) measured at a given time from the start of the 0.5-h i.v. infusion. The models are:

SN-38 AUC $= k_1 \times C(t_1) + k_2$ (1-point model),

SN-38 AUC $= k_1 \times C(t_1) + k_2 \times C(t_2) + k_3$ (2-point model),

SN-38 AUC $= k_1 \times C(t_1) + k_2 \times C(t_2) + k_3 \times C(t_3) + k_4$ (3-point model)

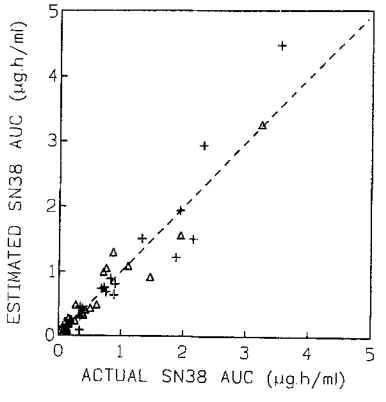

Fig. 3 Actual AUC generated for active metabolite SN-38 versus the estimated AUC for the trivariate model (model *o*) including sampling times at 0.5, 1, and 6 h (*Triangles*, Training data set, *crosses* test data set, *dashed line* identity line)

time-point values were determined for the 6-and 7-h time points by linear interpolation on a semilogarithmic scale. This is not uncommon in limited-sampling-strategy development, as it has been used by other investigators as well (e.g., [10, 31]). In the present case, the robustness of this method is guaranteed by the use of only interpolated points that were model-independent, because the determination of these time points by model analysis could have been biased by the frequent rebound concentrations observed with both CPT-11 and SN-38 at early time points [6].

In addition to meeting the predictive value criteria mentioned above (i.e., a high correlation coefficient, a low MPE%, and a low RMSE%), the best model in the present case would be the one that would predict accurately the AUC of both the parent compound CPT-11 and its active metabolite SN-38 with ideally the same, and the fewest, sampling time points. Clinical constraints also need to be taken into consideration in the choice of the best models, i.e., not only are the models with the fewest sampling time points considered optimal, but these samples also have to be harvested as early as possible after the beginning of the i.v. infusion (preferably at ≤ 8 h). Also, in the present case one should take into account the points showing the maximal concentrations of both CPT-11 (i.e., 0.5 h, or the

end of the i.v. infusion) and its active metabolite SN-38 ($\approx$ 1 h from the start of the 0.5-h infusion), because future pharmacokinetics-pharmacodynamics studies may need to include these maximal concentrations to evaluate possible relationships with the pharmacological effects of these compounds.

Considering the above-mentioned criteria, the trivariate models including sampling time points at 0.5, 1, and 6 h appear to be the best compromise for accurate simultaneous determination of CPT-11 and SN-38 AUCs with the same and the fewest sampling times. These models appear optimal for the simultaneous determination of both the CPT-11 and the SN-38 AUC for the following reasons: (a) they include the 0.5-h time point needed to determine the maximal CPT-11 concentration (i.e., the end of the 0.5-h i.v. infusion), (b) the 1-h time point is included (where near-maximal concentrations of SN-38 are observed), and (c) the last sampling time is clinically convenient.

Although the 0.5-h time point (end of the infusion) may be error-prone due to the difficulty of sampling exactly at the end of the i.v. infusion, this problem may be easily circumvented by monitoring closely the infusion status and taking the blood sample immediately prior to the end of the infusion.

However, several of the other models presented may be used, since many of them appear as predictive as the one selected above. For example, other sampling time points may be required due to clinical constraints, or they may be useful when actual sampling times do not exactly match the planned ones in the clinical situation. Also, if there is no need to determine the maximal (or near-maximal) SN-38 concentration, the sampling times may then be reduced to only 0.5 and 6 h, which would also predict accurately for both CPT-11 and SN-38 AUCs.

The determination of the CPT-11 AUC obviously may be used to determine the total body clearance of the drug (dose divided by AUC). These models can also be used to determine the metabolic ratio in a given patient (i.e., the SN-38 AUC divided by the CPT-11 AUC) so as to study pharmacodynamics as a function of the extent of metabolism in a patient population.

The LSMs presented for CPT-11 AUC estimation compare favorably in terms of precision and bias with models developed for other anticancer drugs [10, 30, 31, 41]. However, the performance of the best models for estimation of the AUC of metabolite SN-38 was not as precise as that of the best models for the parent compound. This is probably due to the influence both of enterohepatic recycling of the metabolite and of the numerous enzymatic steps that SN-38 may undergo (e.g., carboxyesterase, glucuronidation, and hydrolysis of glucuronide, among others). The model precision for the metabolite of another camptothecin analogue (topotecan) has also been reported to be less precise than that of models for the parent compound [41]. Although they are less precise, we consider the best models for SN-38 AUC determination to be clinically acceptable until more knowledge is gained on the pharmacokinetics of this compound that could allow the development of better models.

Although only patients receiving the drug for the first time were entered in this study, it is now known that prior exposure to CPT-11 does not influence the pharmacokinetics of either CPT-11 or SN-38 [6] and that, consequently, the models presented in this article are also valid for repeated administration of the drug.

In conclusion, the development and validation of LSMs for the simultaneous determination of both the CPT-11 AUC and that of its active metabolite SN-38 has led to the choice of a sampling strategy including three time points, i.e., 0.5, 1, and 6 h. The trivariate model for the determination of CPT-11 AUC is CPT-11 AUC (ng h ml^{-1}) $= 0.820 \times C_{0.5h} + 0.402 \times C_{1h} + 15.47 \times C_{6h} + 928$; and the trivariate model for the determination of metabolite SN-38 AUC is SN-38 AUC (ng h ml^{-1}) $= 4.05 \times C_{0.5h} - 0.81 \times C_{1h} + 23.01 \times C_{6h} - 69.78$, where $C(t)$ is the concentration in nanograms per milliliter of either compound at a given time t. In addition to the determination of AUCs (and hence clearance), these models also allow the determination of the maximal concentrations of these two compounds at 0.5 h for CPT-11 and at 1 h for SN-38. Other models (bivariate or trivariate) were also found suitable for the precise determination of CPT-11 and SN-38 AUCs. These models have been implemented as a part of ongoing clinical studies with CPT-11 [12] and will probably prove valuable in the study of population pharmacokinetics due to the minimal burden they impose on the patient, the clinical staff, and the laboratory. More importantly, these models may lead to a better knowledge of the relationships between the pharmacokinetics and pharmacodynamics of both CPT-11 and its active metabolite SN-38 [5, 6, 12], and they may ultimately lead to a better use of this clinically active drug by allowing treatment optimization for a given patient on the basis of individual pharmacokinetic characteristics.

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