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PREDICTION OF THE SYSTEMIC EXPOSURE TO ORAL 9-AMINO-20(*S*)-CAMPTOTHECIN USING SINGLE-SAMPLE ANALYSIS

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ABSTRACT:

The purpose of this study was to develop and validate limited-sampling strategies for prediction of the area under the plasma-concentration time curves (AUCs) of the lactone and total (i.e., lactone plus carboxylate) forms of the novel topoisomerase-I inhibitor 9-amino-20(*S*)-camptothecin (9-AC). Complete pharmacokinetic curves for both drug species were obtained from 32 patients who received the drug orally in a clinical phase I setting at dose levels ranging from 0.25 to 1.10 mg/m^2. The concentrations of the lactone and carboxylate forms of 9-AC in plasma were measured by HPLC. Using data from 20 randomly selected patients, forward-stepwise multivariate regression analysis was used to generate modeling strategies incorporating data from one, two, or three plasma samples. The simultaneous optimal prediction of both 9-AC lactone and 9-AC total AUCs was obtained with sample time points at 0.33, 3.0, and 11.0 h after drug dosing. Validation of the models on an independent data set comprising data of the remaining 12 patients demonstrated that 9-AC lactone and 9-AC total AUCs could be predicted sufficiently unbiased and precise using one and two time points: [AUC (ng · h/ml) = 7.103*C_3 + 4.333] for 9-AC lactone and [AUC (ng · h/ml) = 9.612*C_3 + 13.77*C_{11} − 44.11] for 9-AC total, where C_3 and C_{11} represent the 9-AC plasma concentrations in ng/ml at 3 and 11 h after drug dosing. Application of the proposed models will be valuable in the determination of 9-AC population pharmacokinetics and permits treatment optimization for patients on the basis of individual pharmacokinetic characteristics through restricted drug monitoring in clinical routines.

9-Amino-20(*S*)-camptothecin (9-AC; NSC 603071)[1] is a synthetic derivative of the cytotoxic plant alkaloid camptothecin that does not produce hemorrhagic cystitis associated with the parent compound (Potmesil, 1994; Takimoto and Arbuck, 1996; Gerrits et al., 1997). The mechanism of action of 9-AC involves stabilization of a cleavable complex between the intranuclear enzyme topoisomerase-I and DNA, thereby inhibiting resealing of enzyme-mediated single-strand breaks required for DNA replication and RNA transcription (Hsiang et al., 1985, 1989; Hertzberg et al., 1989a). In preclinical studies, complete remissions have been obtained with 9-AC in nude mice bearing human tumor xenografts resistant to common antineoplastic agents (Giovanella et al., 1989, 1991; Pantazis et al., 1992, 1993). These animal studies further demonstrated that a prolonged duration of exposure and a higher frequency of administration were necessary to maximize drug efficacy.

Although many schedules of drug administration for camptothecin analogs have been evaluated (reviewed in Creemers et al., 1994; Gerrits et al., 1997), the optimal schedule and route of administration of 9-AC have not yet been defined. Based on favorable results of preclinical studies of 9-AC in mice given on an intermittent protracted intragastric or oral schedule (Potmesil et al., 1995; Pastori et al., 1997; De Souza et al., 1997) and our observation of significant intestinal absorption of the drug in patients (Sparreboom et al., 1998), we recently performed a clinical phase I study of oral 9-AC in a daily-times-fourteen schedule (De Jonge et al., 1999a). The dose-limiting myelotoxicity in that study was demonstrated to be significantly correlated with the area under the plasma concentration-time curve (AUC) of the closed lactone form of 9-AC, which suggests that kinetic-dynamic relationships of the drug may be important for future dosing strategies (De Jonge et al., 1999b). In addition, we have shown that 9-AC delineates dose-independent pharmacokinetics with substantial interindividual differences in the maximum plasma concentration (C_{max}) as well as in the AUC with both i.v. and oral drug administration (De Jonge et al., 1999b), further indicating that tailoring 9-AC dosage to a patient's individual needs could be of crucial importance. Accurate estimation of the AUC of 9-AC, however, requires analysis of 9 to 12 samples after drug administration, which is in general considered inconvenient and expensive. In view of these problems inherent to the drug, it was the aim of the present report to investigate the utility of limited-sampling strategies for prediction of the systemic exposure to oral 9-AC. These strategies would eventually enable estimation of the risk of hematological toxicity and/or convenient use of adaptive controlled dosing, using a limited number of samples drawn on the first day of oral 9-AC chemotherapy.

[1] Abbreviations used are: 9-AC, 9-amino-20(*S*)-camptothecin; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; %MPE, percent mean predictive error; %RMSE, percent root mean-squared predictive error.

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Materials and Methods

Patients and Treatment. The pharmacokinetic models were developed and validated in 32 patients with a histologically or cytologically proven malignant solid tumor that participated in a phase I and pharmacokinetic evaluation of 9-AC given in a repeated oral schedule (De Jonge et al., 1999a). Eligibility criteria included the following: 1) age between 18 and 75 years; 2) an Eastern Cooperative Oncology Group (ECOG) performance status <2; 3) life expectancy of at least 12 weeks; 4) adequate hematopoietic (absolute peripheral granulocyte count >2000 μl^{-1} and platelets >100,000 μl^{-1}), hepatic (total bilirubin within normal limits and aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase <2 times upper normal limits), and renal functions (creatinine: <133 μM); and 5) provision of informed written consent according to guidelines of the institutional review board before treatment.

9-AC was provided by Pharmacia & Upjohn (Milan, Italy) as hard gelatin capsules in a matrix containing PEG1000 as excipient (see Sparreboom et al., 1998 for descriptive characteristics of the dosage form). The drug was given with 150 to 200 ml of water by single-daily oral administration for 14 days at 0.25 mg/m^2 (n = 8), 0.40 (n = 3), 0.60 (n = 3), 0.84 (n = 7), 1.0 (n = 7), or 1.1 mg/m^2 (n = 3). Treatment cycles were repeated every 21 days, and all patients had fasted at least 8 h before and 0.5 h after 9-AC administration.

Pharmacokinetic Analysis. Blood sampling for 9-AC pharmacokinetic analysis was performed on day 1 of the first chemotherapy course, making 32 pharmacologic data sets evaluable for analysis. Heparinized blood samples were drawn from an indwelling cannula at 0 (predose), 0.33, 0.66, 1, 2, 3, 5, 7.5, 11, and 24 h (Fig. 1) after dosing. Determination of 9-AC lactone and 9-AC total (i.e., lactone plus carboxylate) plasma concentrations was performed by reversed-phase HPLC with fluorescence detection as described in detail previously (Loos et al., 1997). The lower limits of quantitation using 1-ml samples were 50 and 100 pg/ml for the lactone and total forms, respectively, with the percent deviation (accuracy) and precision of the assay always being less than 10%.

Individual 9-AC lactone concentration-time data were fitted to a triexponential equation after extravascular bolus with lag time using the Powell-minimization algorithm and weighted (1/y) least-squares regression analysis, using Siphar v4.0 (Simed, Creteil, France) as described (Sparreboom et al., 1998). The AUC for 9-AC total from time zero to the last measurable level (C_{last}) was calculated by the trapezoidal rule. Extrapolation to infinity was obtained by dividing C_{last} by the elimination rate constant (k_{el}), estimated by a log-linear fit of the terminal phase. The terminal elimination half-life [$T_{1/2}(\gamma)$] was estimated by ln2/k_{el}. C_{max} was estimated by visual inspection of the semilogarithmic plot of the concentration-time curve. Interpatient and intrapatient variability in pharmacokinetic parameters was assessed by the coefficient of variation, expressed as the ratio of the standard deviation and the observed mean. Pharmacokinetic parameters were Gaussian distributed as judged by normality plots and the Kolmogorov-Smirnov test.

Model Development and Validation. Limited-sampling models were constructed on a training data set that comprised 20 complete pharmacokinetic curves from randomly assigned patients. Using this data set, the 9-AC concentrations at each time point (independent variable) were correlated with the corresponding AUC (dependent variable) by univariate linear regression analysis, as assessed by Pearson's correlation coefficient (r), to find the interval with the optimal single-sample time point. Forward-stepwise multivariate regression analyses were performed to include one or two additional sample time points, if necessary. Backward-elimination regression analysis, the F-test statistic, and the coefficient of determination were used to select the optimal modeling strategy. The models obtained with the training data set were validated on an independent data set composed of 12 pharmacokinetic curves from the remaining patients. The predictive performance of the developed models was evaluated on the basis of bias (percent mean predictive error, %MPE) and precision (percent root mean-squared predictive error, %RMSE) as described (Sheiner and Beal, 1981). Pearson's correlation coefficient was used to rank the concordance between measured and predicted pharmacokinetic parameters. Differences in patient demographics and pharmacokinetics between the training and validation data sets were evaluated with the two-tailed Student's t test or the Fisher's exact probability test, if required. All statistical computations were performed with the software package Number Cruncher Statistical System (NCSS v5.×; J.L. Hintze, Kaysville, UT, 1992) running on an IBM-compatible computer.

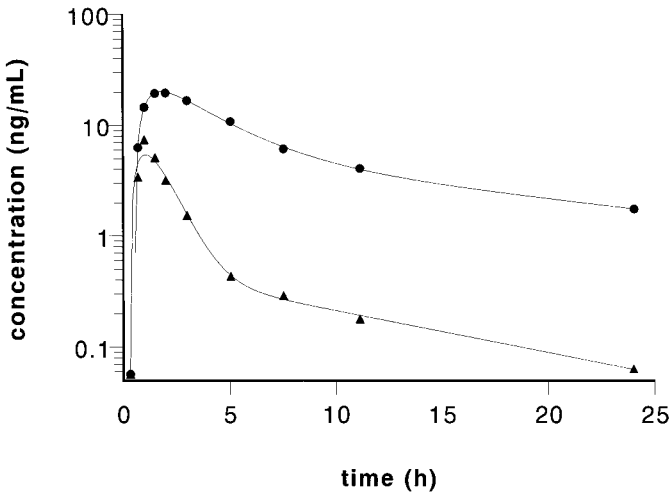

FIG. 1. *Representative plasma concentration-time profiles of 9-AC lactone (▲) and 9-AC total (●) measured on day 1 of the first treatment course in a single patient after oral administration of 9-AC at a dose level of 0.84 mg/m^2 in a daily-times fourteen schedule.*

Pharmacokinetic curves were fitted to a triexponential equation using the Siphar v4.0 computer program, assuming a three-compartment model for the distribution and elimination of the drug.

Results

Thirty-two patients with various types of solid tumors were entered in a phase I and pharmacokinetic study with 9-AC given orally. Patients were randomly divided in a training-data set (20 patients) and a validation-data set (12 patients) (Table 1). There were no significant differences in baseline patient characteristics or pharmacokinetic parameters between the two cohorts (not shown). There was large interpatient pharmacokinetic variability in the concentrations of 9-AC at each of the sample time points, as well as with the AUC, with values for the coefficient of variation up to 99% (see also De Jonge et al., 1999b). The 9-AC lactone and total concentrations at each of the sample time points were correlated with the AUC using the training-data set by univariate regression analysis (Table 2). Overall, Pearson's correlation coefficients ranged from 0.241 to 0.989, with the best correlations observed at the 3-h sample time point for both the lactone and total forms of the drug, which was considered the most informative variable. The measured drug concentrations were subsequently subjected to multivariate regression modeling, with a restriction to

TABLE 1

Patient demographics

Characteristic	Training Set	Validation Set
No. of patients	20	12
Age (years)	63 (54–74)[a]	55 (39–74)[a]
Sex (female/male)	8:12	7:5
Eastern Cooperative Oncology Group performance status	1 (0–1)[b]	1 (0–2)[b]
Primary tumor		
Ovarian	2	3
Colorectal	8	3
Pancreas	1	1
Lung (non-small cell)	1	1
Breast	1	1
Miscellaneous	7	3

[a] Mean value with range in parentheses.
[b] Median value with range in parentheses.

TABLE 2

Univariate correlation of 9-AC lactone and 9-AC total concentrations at each sample time point with the corresponding AUC in the training-data set

Time Point	9-AC Lactone		9-AC Total	
h	*n*	*r*	*n*	*r*
0.33	14	0.389	17	0.241
0.67	19	0.733	20	0.571
1.0	20	0.854	20	0.628
1.5	20	0.849	20	0.754
2.0	20	0.841	20	0.862
3.0	20	0.959	20	0.989
5.0	20	0.921	20	0.943
7.5	19	0.717	20	0.943
11	19	0.699	20	0.931
24	12	0.514	19	0.899

Abbreviation: *n*, number of data sets with complete pharmacokinetic curves.

models with one or two additional time points. In the bivariate models, sample-time couples with the highest correlation and lowest %RMSE were composed of drug concentrations at 3 h and 0.33 h, and 3 and 11 h for AUCs of the lactone and total respectively (Table 3). Strategies for estimation of the 9-AC total AUC using more conveniently timed samples were all associated with significantly worse predictive ability as compared with the modeling that included the 3- and 11-h samples. For example, models based on inclusion of the 5- and 7.5-h samples demonstrated correlation coefficients of only 0.969 and 0.971 with corresponding %RMSE values as high as 63.4 and 62.2%, respectively. The best models with three time points included strategies with addition of the 11-h and 0.33-h concentration. In the training-data set, all models demonstrated little bias with the absolute value of the %MPE ranging from 0.10 to 0.64% of the measured AUC (Table 3).

Concentrations of 9-AC for use in univariate and bivariate models to predict the terminal elimination half-life showed poor correlation coefficients ($r \leq 0.01$) combined with low accuracy (%RMSE $\geq$ 46.1%) and were not used in the limited-sampling model validation. We also considered the use of 9-AC lactone concentrations for prediction of 9-AC total AUC or 9-AC carboxylate AUC, as we had previously demonstrated a significant linear relationship ($r = 0.87$) between 9-AC lactone and 9-AC total AUCs, with the drug administered orally at a dose level of 1.50 mg/m^2 (Sparreboom et al., 1998). In both cases, the best models included three sample time points with acceptable correlation coefficients (up to $r = 0.91$), but in the end were rendered highly inaccurate, with values for the %RMSE ranging from 106 to 288%.

Prospective evaluation of the proposed models was performed in the validation-data set composed of the remaining 12 patients. In the case of 9-AC lactone, all three models had minor bias (%MPE range: -0.71 to -0.23%) and excellent precision (%RMSE range: 6.05–8.24%), indicating that the addition of a second and third variable did not substantially improve the model (Table 3). Furthermore, a strong linear correlation was observed between the measured and predicted 9-AC lactone AUCs in all models (Fig. 2A). There was some bias noticed in the single-point model for prediction of 9-AC total AUC with an absolute value of the %MPE of 7.8%, accompanied by a %RMSE of $\geq$30%. Validation of the bivariate and trivariate models, however, resulted in predictions of 9-AC total AUC that were sufficiently unbiased and precise to warrant clinical application (Table 3 and Fig. 2B).

As in all validated models, computations were made without dose-normalization. We also performed an additional analysis with the univariate and bivariate models by including dose in milligrams per square meter of body-surface area (mg/m^2) in the AUC prediction. For both 9-AC lactone and 9-AC total models, similar results were obtained with and without dose as an additional variable, as indicated by equivalent correlation coefficients and values for the %RMSE of 8.24 versus 8.12 and 14.5 versus 16.2, respectively, for lactone and total forms in the two-sample models.

Discussion

In the present study we have shown that several limited-sampling strategies can be developed for reliable and accurate prediction of the systemic exposure to 9-AC after oral drug administration. Using stepwise forward regression analysis, univariate and bivariate models for independent estimation of 9-AC lactone AUC values based on one and two sample time points, respectively, were developed and tested for the statistical best fit. Results of models using three time points did not show improved results in terms of bias and precision. In view of logistical and economical reasons, the single-sample strategy is clearly preferred to those using two samples, particularly with respect to application in large-scale studies of population pharmacokinetics, which require methods that are both accurate and practical in a daily routine.

Pharmacokinetic studies with camptothecin analogs, including 9-AC, were previously shown to be complicated by a chemical, pH-dependent instability of the terminal E-lactone ring of the compounds, generating a ring-opened carboxylate, which is over 1000-fold less active as an inhibitor of topoisomerase-I (Hertzberg et al., 1989b). The importance of this nonenzymatic hydrolysis reaction of the lactone moiety in the pharmacology and toxicology of 9-AC is not yet fully understood. The clinical pharmacokinetics of the lactone and total (i.e., lactone plus carboxylate) forms of 9-AC has been extensively studied in patients receiving the drug by i.v. infusion over 72 h (Rubin et al., 1995; Dahut et al., 1996; Takimoto et al., 1997; Eder et al., 1998). From these studies, pharmacodynamic correlations have been suggested between the 9-AC lactone steady-state concentration in plasma and the degree of leukocytopenia. We have recently observed similar relationships for 9-AC lactone AUC and myelotoxicity with the drug administered orally (De Jonge et al., 1999b), which is in line with the lactone being the pharmacological active species of the drug. Applying a limited-sampling strategy, questions of 9-AC pharmacodynamic outcome relating to lactone-carboxylate interconversion could be answered conveniently in prospective studies. It is noteworthy, however, that model prediction for 9-AC total AUC was less precise than that of models for the lactone, including slight bias in the validation-data set toward underestimation of the AUC with all three strategies. Nevertheless, the best model for prediction of the 9-AC total AUC (including two sample time points) still can be considered acceptable and clinically useful.

In recent years, limited-sampling strategies have also been developed for several other antineoplastic agents (reviewed in Van Warmerdam et al., 1994a), including the camptothecin analogs irinotecan (CPT-11; Campto; Yamamoto et al., 1994, 1997; Sasaki et al., 1995; Nakashima et al., 1995; Chabot, 1995; Mick et al., 1996; Mathijssen et al., 1999) and topotecan (Hycamtin; Van Warmerdam et al., 1994b; Minami et al., 1996). In some of these models, drug-dose levels as measured in milligrams per square meter of body-surface area (mg/m^2) are included in the AUC estimate, by dose normalization of each patient's pharmacokinetic data to a constant dose. The rationale for this procedure is to be able to discriminate between variability in dose and interindividual variation in pharmacokinetics as the primary cause for variability in measured concentrations. To test whether the administered dose would improve the validity of the presented models, this parameter was also included in the single and two-sample strategies by multivariate regression analysis. For both approaches, correlation

TABLE 3

Limited-sampling models for prediction of 9-AC lactone AUC (ng · h/ml) and 9-AC total AUC (ng · h/ml) in patient plasma

Modeling Strategy		Training Set			Validation Set		
		r	%MPE	%RMSE	*r*	%MPE	%RMSE
9-AC lactone							
A	$AUC = 7.103*C_3 + 4.333$	0.959	−0.10	8.75	0.943	−0.71	8.18
B	$AUC = 1.031*C_{0.3} + 6.212*C_3 + 4.858$	0.982	+0.21	5.11	0.972	−0.15	8.24
C	$AUC = 1.381*C_{0.3} + 6.711*C_3 - 1.731*C_{11} + 4.135$	0.989	+0.21	4.55	0.977	−0.23	6.05
9-AC total							
D	$AUC = 16.12*C_3 - 55.54$	0.989	−0.30	35.6	0.854	−7.8	30.1
E	$AUC = 9.612*C_3 + 13.77*C_{11} - 44.11$	0.991	−0.38	13.4	0.941	−4.8	14.5
F	$AUC = 1.561*C_{0.3} + 9.776*C_3 + 1.571*C_{11} - 45.79$	0.993	+0.64	11.1	0.942	−4.9	15.1

Abbreviations: $C_{0.3}$, C_3, and C_{11}, plasma concentrations of 9-AC in ng/ml at 0.33, 3.0, and 11 h, respectively, after oral drug administration.

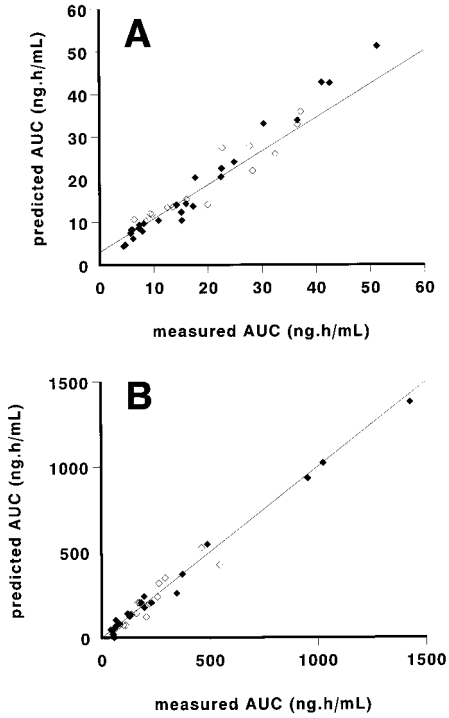

FIG. 2. *Correlation between the measured 9-AC lactone AUC (A) or 9-AC total AUC (B) and the AUC predicted from modeling strategies incorporating one or two plasma samples for lactone and total forms, respectively, in the training-data set (♦) and the validation-data set (◇).*

Pearson's correlation coefficients in the training and validation-data set were, respectively, 0.959 and 0.943 (A) and 0.991 and 0.941 (B). The solid line represents the line of identity.

coefficients remained unchanged, whereas the %MPE and the %RMSE in the training as well as the validation-data set of the two-sample approach slightly decreased by an absolute maximum of 0.4%. Thus, the use of the dose in mg/m^2 as an additional independent variable did not contribute significantly to prediction of the AUC for oral 9-AC. This conclusion is consistent with our recent observation that oral 9-AC delineates linear and dose-independent pharmacokinetics within the examined dose interval, i.e., 0.25 to 1.5 mg/m^2 (De Jonge et al., 1999b).

The presented models have proven both valid and acceptable in terms of bias and precision in a heterogeneous group of cancer patients given 9-AC over a wide range of dose levels. Furthermore, our current finding of extremely low intrapatient variability in oral 9-AC pharmacokinetics indicates that the models are valid also for prediction of the AUC with repeated administration of the drug. The clinical significance and the ultimate utility of the models, however, remain to be explored in future studies. In addition, use of the models in chemotherapy regimens other than the one investigated in the current study should be done with caution, as the potential for pharmacokinetic interactions between 9-AC and coadministered drugs, e.g., phenytoin, phenobarbital, and/or valproic acid (Grossman et al., 1998), cannot be ruled out.

In conclusion, the feasibility and validity of prediction of the systemic exposure to oral 9-AC using limited-sampling strategies were demonstrated. The optimal strategies included an univariate model with one sample time point at 3 h for 9-AC lactone AUC and a bivariate model with two sample time points at 3 and 11 h for 9-AC total AUC. Application of the proposed models will be valuable in the determination of 9-AC population pharmacokinetics and investigations of the clinical implications of the 9-AC lactone-carboxylate interconversion with regard to pharmacodynamics. In addition, with the strategies, routine drug monitoring is feasible, thereby allowing treatment optimization for a given patient on the basis of individual pharmacokinetic characteristics. This could be achieved after oral drug administration of an appropriate starting dose of 9-AC (e.g., the maximum tolerated dose in a 14-day schedule of 1.0 mg/m^2) by measuring the 9-AC lactone plasma concentration at 3 h after drug dosing. Using the limited-sampling model and the linear regression relationship between drug dose and AUC (De Jonge et al., 1999b), the optimal dose leading to the target AUC determined according the toxicity considered acceptable, can then be calculated.

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