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Original Paper

Phase I Pharmacokinetics and Limited Sampling Strategies for the Bioreductive Alkylating Drug EO9

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EO9 is a synthetic indoloquinone which was designed to undergo redox cycling and formation of alkylating intermediates under bioreductive conditions. As part of a phase I clinical trial, EO9 plasma disposition was evaluated in 20 patients receiving 2.7–15 mg/m² i.v. weekly for 3 weeks. Pharmacokinetic studies were performed with the first and third dose of therapy and nine blood samples were obtained over 30 min postinfusion. Plasma EO9 was detected using HPLC UV and the disposition described by a two-compartment model. Wide variability in EO9 pharmacokinetics was observed. EO9 was rapidly eliminated from plasma with a median systemic clearance of 3.5 l/min/m² (range 1.2–9.8), apparent volume of distribution of 6.2 l/m² (1.0–34.9) and $t_{1/2}\beta$ of 10.1 min (2.2–63.0). Substantial intrapatient variability was observed for all pharmacokinetic parameters. Linear regression and Bayesian methods were developed and validated for estimation of EO9 plasma AUC using up to three samples postinfusion. The use of two or three plasma samples provided precise estimation with acceptable prediction bias. In addition, a Bayesian algorithm offered more robust estimation of AUC and is preferable to linear regression models for future EO9 population pharmacokinetic analysis. Copyright © 1996 Elsevier Science Ltd

Key words: EO9, phase I, pharmacokinetics, limited sampling methods, bioreductive agents, chemotherapy

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INTRODUCTION

EO9 is a synthetic indoloquinone that is structurally related to mitomycin C [1]. EO9 appears to exert activity through redox cycling and formation of alkylating intermediates under bioreductive conditions. In preclinical studies, EO9 has demonstrated activity against a number of solid tumour cell lines, including colon, brain, melanoma, renal cell and non-small cell lung cancers [2]. EO9 has been shown to be rapidly eliminated in rodents with a plasma half-life of 1.9–3 min [3]. A large apparent volume of distribution has also been observed [3]. A primary metabolite, the aziridine ring opened analogue EO5A, has been identified in rodent plasma along with several additional unidentified species.

Clinical evaluation of EO9 has been initiated using a 5 min infusion in cycles of either one dose every 3 weeks or weekly doses for 3 weeks [1,4,5]. Antitumour activity has also been observed in phase I analysis, with minor or partial responses in a small number of patients [4]. Dose-limiting proteinuria was observed in patients receiving EO9 as a 5 min infusion every 3 weeks [4].

The contribution of pharmacokinetic variability to toxic and therapeutic drug effects has been recognised for a number of anticancer agents [6]. This principle is evident with EO9, as the occurrence of proteinuria has been associated with an elevated area under the plasma concentration–time curve (AUC) (>9000 ng/ml min) [4]. In addition, pharmacological evaluation of EO9 in EORTC ECTG phase II trials of breast,

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colorectal, gastric, pancreatic and non-small cell lung cancer are currently ongoing [7]. Therefore, the present study characterised the inter- and intrapatient variability of EO9 pharmacokinetics and developed limited sampling strategies for future therapeutic studies.

PATIENTS AND METHODS

The pharmacokinetics of EO9 were evaluated in 20 patients (11 male: 9 female) with a median age of 49 years (range 19–64 years). The patients had a variety of solid tumours including colorectal carcinoma (n = 9), melanoma (n = 5), non-small cell lung cancer (n = 2), sarcoma (n = 2), liver carcinoma (n = 1) and adenocarcinoma of unknown primary origin (n = 1). EO9 was administered intravenously over 5 min, weekly for 3 consecutive weeks at a starting dose of 2.7 mg/m^2 (1/10 mouse LD_{10}). The actual infusion length varied from 4 to 10 min. The dose was escalated in cohorts of at least 3 patients to the maximum tolerated dose of 15 mg/m^2 [15]. At least 5 patients were entered at a given dose level if toxicity greater than grade 2 occurred.

Plasma pharmacokinetic studies were performed on the first and third doses of the first cycle. Thirty-four data sets were available for analysis. Blood samples were obtained prior to, at the end of infusion, and 1, 2, 3, 5, 7, 10, 15, 20 and 30 min postinfusion. Blood samples were immediately centrifuged, taking great care to avoid erythrocyte contamination or lysis. Plasma was removed and stored at −70°C until analysis.

EO9 was quantified in human plasma using the method of Schellens and colleagues [8]. EO9 was extracted from 1 ml plasma using a C18 Octadecyl solid phase extraction column (Applied Separations, Lehigh Valley, Pennsylvania, U.S.A.). The eluate was evaporated and resuspended in mobile phase. Following injection (100 µl), separation was achieved using a Lichrospher 60RP precolumn (4×4 mm, 5 µM) and Lichrospher 60RP analytical column (125×4 mm, 5 µM; Hewlett-Packard, Germany). Mobile phase was 89% 0.02 M phosphate buffer, pH 7:10% acetonitrile: 1% tetrahydrofuran (v/v) at 1 ml/min. EO9 was detected by ultraviolet detection at λmax = 270 nM. A linear standard curve in human plasma from 10 ng/ml to 1000 ng/ml was used with a limit of quantitation of 5 ng/ml. In a parallel study as part of the assay method validation, the stability of EO9 was investigated in whole blood. Human whole blood and plasma was spiked with EO9 100 ng/ml and incubated at 37°C for 0, 0.5, 1, 2 and 3 h. Plasma was removed and stored at −70°C until analysis. EO9 concentrations from whole blood rapidly declined with a degradation $t_{1/2}$ = 2.5 h. Plasma EO5A concentrations increased proportionately to the decline in EO9. Several unidentified peaks were also formed. EO9 was stable (<10% degradation) at 37°C in human plasma over 3 h.

Pharmacokinetic analysis

An iterative two-stage analysis was used to estimate EO9 plasma pharmacokinetic parameters in a two-compartment open model, using ADAPT II software [9]. An initial analysis with maximum likelihood estimation generated a covariance matrix for volume of distribution in the central compartment (Vd), the elimination constant (k_e), and intercompartmental constants (k_{cp} and k_{pc}). Repeated Bayesian analysis, using an updated covariance matrix and parameter values from the previous iteration, was then conducted until less than a 1% change in mean values for all pharmacokinetic parameter estimates occurred. This method is sensitive to data best fit by a one-compartment model, as estimates of k_{cp} in individual data sets will become extremely small (i.e. 1×10^{-6} min^{-1}). Five iterative cycles were required to obtain the final Bayesian parameters for analysis of EO9 in this study. EO9 AUC was calculated as dose/systemic clearance (CLs).

Limited sampling approaches for estimating EO9 AUC were developed using Best Subsets Regression and Bayesian estimation algorithms. The 34 data sets were randomly separated into two groups: 17 for model development and 17 for validation. The optimal time points for one, two and three sample AUC prediction models were determned by best subsets regression, using all time points, dose, infusion length and rate of infusion. A covariance matrix was also developed from the test data set only, using the iterative two-stage method. Model precision and bias for predication of AUC were determined using root mean squared error (RMSE) and mean prediction error (MPE), respectively.

Statistics

Differences in pharmacokinetic parameters from individual patients between the first and third dose of EO9 were evaluated using the Wilcoxon test. The relationship between CLs or Vd determined with the first and third dose was assessed by the Spearman rank test. Gender differences were calculated using the Mann–Whitney test. The influence of dose (mg, mg/m^2) or age on EO9 pharmacokinetic parameters was evaluated by linear regression analysis.

RESULTS

EO9 pharmacokinetics were best described by a two-compartment model for 31 of 34 patients (Figure 1). The curve fit for the remaining three data sets was not improved beyond a one-compartment model. The final covariate matrix used for Bayesian analysis was [mean (%CV)]:Vd 9.3 l/m^2 (50), k_e 0.55 min^{-1} (50), k_{cp} 0.12 (30) and k_{pc} 0.087 (30). Pharmacokinetic parameters are shown in Table 1. EO9 was rapidly eliminated from plasma with a median CLs of 3.5 l/min/m^2 (range 1.2–9.8) and a median elimination half-life of 10.1 min (range 2.2–63.0). The apparent volume of distribution in the central compartment (Vd) was 6.2 l/m^2 (range 1.0–34.9). The EO9 plasma area under the concentration–time curve (AUC) extended from 286.7 to 8326.4 ng/ml min over the dose range 2.7–15 mg/m^2, increasing in a dose-dependent manner (r^2 = 0.29; Figure 2). However, a wide variability in EO9 disposition was observed. For example an 8-fold range in

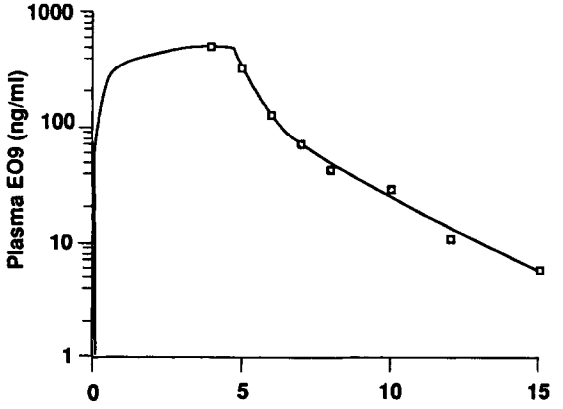

Figure 1. EO9 plasma disposition in a representative patient receiving 7 mg/m^2 over 5 min. Plasma EO9 was not detectable beyond 15 min after the start of infusion in this patient.

Table 1. EO9 pharmacokinetic parameters after the first and third dose

Parameter	Dose 1 (n = 20) Median	Dose 1 (n = 20) Range	Dose 3 (n = 14) Median	Dose 3 (n = 14) Range
Systemic clearance (l/min/m^2)	3.5	(1.2–9.8)	3.9	(2.3–7.4)
Systemic clearance (l/min)	7.0	(2.2–16.0)	6.8	(3.7–15.6)
Vd*c* (l/m^2)	6.2	(1.0–34.9)	6.5	(2.1–23.1)
Vd*c* (l)	11.1	(1.7–57.3)	10.7	(3.5–43.9)
Vd*ss* (l/m^2)	17.5	(3.8–67.7)	12.9	(3.1–53.1)
Vd*ss* (l)	35.9	(6.1–115.2)	22.6	(4.9–111.5)
$t_{1/2}\alpha$ (min)	1.1	(0.4–2.6)	0.9	(0.5–3.2)
$t_{1/2}\beta$ (min)	10.1	(2.2–63.0)	7.9	(2.8–22.3)

Vd*c*, volume of distribution in central compartment; Vd*ss*, volume of distribution at steady state.

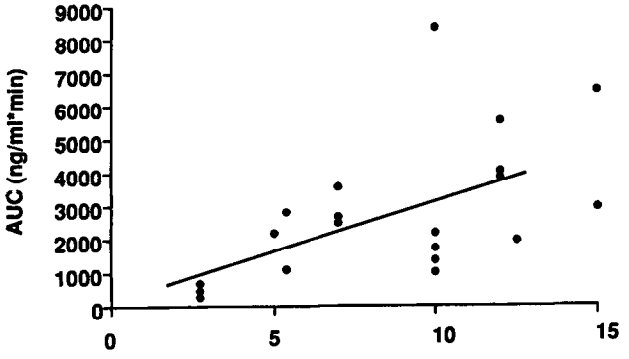

Figure 2. The relationship between EO9 dose (mg/m^2) and plasma AUC (ng/ml min). The linear regression line fit is shown (r^2 = 0.29).

AUC was found among the 5 patients receiving 10 mg/m^2. Wide interpatient variability was observed for CLs (CV = 57.4%).

EO9 pharmacokinetic parameters were not influenced by dose, patient age, hepatic function (bilirubin, alkaline phosphatase, SGOT, SGPT, SGGT, lactate dehydrogenase) or renal function (serum creatinine, blood urea nitrogen). Median CLs and Vd were slightly higher in female than male patients, but this difference was not statistically significant (5.1 versus 3.0 l/min/m^2; P = 0.1 and 9.2 versus 5.0 l/m^2; P = 0.06, respectively).

Substantial intrapatient variability in all pharmacokinetic parameters was observed between the first and third administered dose. A median 11.4% increase in CLs (range −88 to +25.9%; P = 0.76) and 32.0% increase in Vd (−186.3 to +66%; P = 0.35) was observed between the first and third dose. The absence of statistical significance for intrasubject variability in EO9 disposition reflects the extreme range of the alterations. A correlation was observed between dose one and dose three CLs (r_s = 0.58) and Vd (r_s = 0.19).

Best subsets regression analysis found the infusion length, concentration at the end of infusion, the concentration 2 min postinfusion, and the concentration 3 min postinfusion provided the greatest impact for predicting EO9 AUC. The linear regression equations for the best one, two and three sample predictive models, as well as a previously reported equation, are listed in Table 2. The inclusion of EO9 dose in the linear regression equations did not improve the predictive ability of the models. The performance (bias and precision) for the model development and validation data sets is detailed in Table 3, with two and three sample models providing a similar degree of bias. The models were favourable to naive predictors (e.g. three sample test data set RMSE = 480.1 versus 1207.3). There was no major difference in model bias or precision with increasing sample size (Table 3). The performance of a Bayesian model using the above time points was also evaluated (Table 3). The Bayesian model provided more precision and less bias than the regression model for both the model generation and validation data sets. The Bayesian model was favourable to naive predictors (e.g. three sample test data set RMSE = 466.7 versus 1207.3). Model precision or bias improved with increasing number of samples (Table 3). In all cases, the Bayesian model was more precise with similar bias to the linear regression models (e.g. three sample validation data set %RMSE 16 versus 47%).

DISCUSSION

EO9 is rapidly eliminated from plasma in humans with a median systemic clearance of 7.0 litre per minute (range 22.6–16 l/min). A large Vd was also observed (median 35.9 l, range 6.1–115.2 l). Both parameters were similar to that described in the previous phase I study (CLs 3.2–24 l/min, Vd 22–273 l) [4]. The CLs was greater than liver blood flow (1.5 l/min) in all subjects, implying extrahepatic metabolism or degradation was occurring. Indeed, *in vitro* evaluation of EO9 stability in human blood found degradation in whole blood ($t_{1/2}$ = 2.5 h), but not plasma. This reduction is considerably slower than that observed *in vivo* (elimination $t_{1/2}$ = 2–63 min) suggesting that degradation mechanisms exist in erythrocytes but are not necessarily a primary site of metabolism. Wide interpatient variability was observed for CLs in the current study (coefficient of variation = 57.4%). The 8-fold range in CLs is similar to that previously reported (6.9-fold) [4]. A similar level of intersubject variation was found for Vd in both studies (19-fold versus 10.6-fold) [4].

A large degree of intrapatient variability in CLs and Vd was observed in the current study. There was no apparent trend for the changes in pharmacokinetics from the first to third dose and no influence of biochemical parameters was found. This is in contrast to the small range in intrapatient variability for CLs found by Schellens and colleagues (range 1–29% change) [4]. One possible explanation for the discordant results is the schedule differences between the two trials. In the current study, EO9 was administered weekly for 3 weeks, with pharmacokinetic studies during the first and third doses of the first cycle. This is in contrast to the study by Schellens and colleagues where EO9 was administered every 3 weeks

Table 2. Best regression models generated from the test data set for prediction of EO9 AUC from one, two and three plasma samples

Infusion length + one sample	
AUC = 7.36(EOI) + 418(Infus) −2488	R^2 = 93.3
Infusion length + two samples	
AUC = 6.56(EOI) + 6.85(3 min) + 475(Infus) −3002	R^2 = 94.7
Infusion length + three samples	
AUC = 7.02(EOI) −0.0419(2 min) + 7.3(3 min) + 464(Infus) −3084	R^2 = 95.7
Infusion length + two samples, Schellens and colleagues [12]	
AUC = 6.93(EOI) + 10.2(5 min) + 465(Infus) −2931	R^2 = 94.6

EOI, concentration at the end of infusion; Infus, infusion length.

Table 3. Comparison of model performance for the regression and Bayesian limited sampling methods

A. Test data set precision (RMSE)

	Regression	Bayesian
Infusion length+EOI	529.9	490.1
Infusion length+EOI, 3 min	455.1	474.8
Infusion length+EOI, 5 min	458.2	424.8
Infusion length+EOI, 2 min, 3 min	480.1	466.7

B. Validation data set

	Regression			Bayesian		
	MPE	RMSE	R^2	MPE	RMSE	R^2
Infusion length+EOI	−265.8	496.1	89.4	−224.5	16.9	94.9
Infusion length+EOI, 3 min	−241.2	535.0	87.8	−177.6	335.7	94.6
Infusion length+EOI, 5 min	−212.4	509.9	88.4	−157.4	308.5	95.1
Infusion length+EOI, 2 min, 3 min	−263.2	567.4	89.2	−147.7	308.8	94.7

EOI, end of infusion.

and intrasubject changes evaluated with the first and second cycle (i.e. at least 21 days from the previous dose). It is possible that the frequent drug administration influenced the pharmacokinetics of EO9, either through altered enzyme activity, organ blood flow or such parameters. The differences in methodology for pharmacokinetic modelling between the two trials (non-compartmental versus two-compartment approach) should not be responsible for the disparate intrapatient variation results. Further *in vivo* analysis, either in humans or animal models, is required to gain insight into the mechanisms responsible for the observed alterations.

Although EO9 AUC increased with dose, individualised drug dosage based on body surface area did not substantially reduce interpatient differences in drug exposure, due to the variability in drug clearance. This may have important implications for both patient toxicity and response. Schellens and colleagues observed a correlation between the degree of proteinuria and EO9 systemic exposure, suggesting that patients with inherently low drug clearance will be at risk from toxicity at proposed phase II doses [4]. The plasma AUCs obtained in the present study were not in the range previously associated with proteinuria, as a result of the lower maximum tolerated dose in the weekly for 3 weeks schedule. The relationship between EO9 systemic exposure and drug activity is currently under evaluation in the EORTC Early Clinical Trials Group phase II trials [7].

A relationship between the anticancer drug systemic exposure (e.g. AUC, steady-state concentration) and toxicity has been observed in most phase I clinical trials performed to date. However, similar correlations have not been found for antitumour response, as the patient population which participates in phase I trials is generally heavily pretreated and relatively resistant to therapy. A more appropriate place for pharmacokinetic–pharmacodynamic studies of anticancer drugs is in phase II–IV trials. However, pharmacological evaluation is hindered in this setting by the need for large numbers of blood samples in large numbers of patients, often in the context of a multicentre study. This has led to strategies for assessing patient systemic exposure with only a limited number of samples [10]. To date, limited sampling strategies have focused on step-wise linear regression to determine 'optimal' time points for calculation of systemic clearance, AUC, or such parameters. This approach has limitations, in that it assumes the sampling time point of most influence in a one-sample model will continue to be important in models with a greater number of sample points. This is not always the case, as a one-sample model may select a mid-elimination time point while a two-sample model selects time points which reflect multiple elimination phases.

Regression models are developed for specific infusion length, sample times, and often, dosages. This limits the practical application of such models, especially where drugs are rapidly eliminated. Flexibility in sample times of up to 10% are acceptable for drugs with long $t_{1/2}$ (i.e. hours). The same degree of imprecision for blood sampling of rapidly eliminated drugs, such as EO9, will negatively alter precision and bias and render the model of little value. In clinical practice, the 4 h timepoint, for example, may actually be obtained 2–6 h after the infusion. Linear mathematical equations do not have the flexibility to accommodate such deviations, restricting its application. Such difficulties have led to Bayesian algorithms which can better manage data with imprecise sampling and are more amenable to adaptive control trials [11].

There are no published comparisons of regression and Bayesian methods for limited sampling studies. In the current study, Bayesian models had greater precision with a lower degree of bias compared with the regression equations. Prediction bias improved with increased sample number in the Bayesian model, but was not influenced by sampling in the regression approach (Table 3). Retrospective optimal sample time analysis, using the mean population pharmacokinetic parameters in the SAMPLE [9] program, suggested the end of infusion, 13 and 45 min postinfusion would yield the greatest information on EO9 disposition from three samples. However, the application of this analysis was restricted as EO9 was undetectable or near the limit of quantitation by 20–30 min postinfusion.

This study demonstrates the significant inter- and intrasubject variability in EO9 pharmacokinetics in humans. By defining limited sampling models for estimation of EO9 systemic exposure with ≤ 3 plasma samples, prospective assessment of the clinical significance of variable EO9 disposition can be performed.

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