Assessment of Toxicokinetics and Toxicodynamics Following Intravenous Administration of Etoposide Phosphate in Beagle Dogs

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The toxicokinetics and toxicodynamics of etoposide phosphate (BMY-40481), a water soluble phosphate ester derivative of etoposide, were investigated in beagle dogs (N = 4) following 5 min i.v. infusion doses equivalent to 57, 114 and 461 mg/m^2 of etoposide. The doses were administered in sequence starting with the low dose. There was a 28 day wash-out period between the doses. Serial blood samples were collected over 32 hr and the levels of intact BMY-40481 and etoposide in plasma were measured using validated HPLC assays. Hematology profiles were obtained at pre-dose, and twice a week post-dose for 28 days to correlate systemic exposure to etoposide and hematologic toxicity. Following i.v. administration, plasma concentrations of BMY-40481 declined rapidly. For the 3 doses, mean $t_{1/2}$ of BMY-40481 ranged from 0.11 - 0.17 hr (6.6-11 min). The mean C_{max} and AUC values of BMY-40481 ranged from 1.72 - 40.5 μg/ml and 0.16 - 4.14 hr.μg/ml, respectively. Both systemic clearance and steady state volume of distribution of BMY-40481 decreased significantly at the high dose. In contrast, the mean C_{max} and AUC values of etoposide ranged from 5.46 - 39.4 μg/ml and 2.28 - 22.6 hr.μg/ml, respectively. C_{max} occurred at the end of infusion (5 min) at all dose levels, indicating that etoposide was rapidly formed from BMY-40481. The apparent systemic clearance (range: 342 - 435 ml/min/m^2) and apparent steady state volume of distribution (range: 21.5 - 26.6 l/m^2) of etoposide were dose-independent. The AUC of etoposide was significantly correlated with hematologic toxicity, i.e., percent decreases in white blood count (WBC), absolute neutrophil count (ANC) and platelets. The relationship was best described by the sigmoid E_{max} model for WBC and ANC, and by a simple linear model for platelets. Hemoglobin showed slight decreases which did not correlate with etoposide AUC. In summary, BMY-40481 is rapidly and extensively converted to etoposide; etoposide exhibits linear kinetics; and except for hemoglobin, hematologic toxicity is significantly correlated with etoposide exposure.

KEY WORDS: BMY-40481; etoposide phosphate; etoposide; pharmacokinetics; pharmacodynamics; dogs.

INTRODUCTION

Etoposide phosphate (BMY-40481, Figure 1) is a new water soluble phosphate ester derivative of the widely used anti-tumor agent, etoposide (VP-16). Unlike etoposide, it does not need to be dissolved in a large volume of fluid for administration, and it is formulated without Tween 80, polyethylene glycol or ethanol. These improved characteristics make BMY-40481 easier to use, and allow more flexible doses and schedules. Currently, the drug is undergoing clinical development as a pro-drug of etoposide.

In animal (1) and human (2,3) studies, BMY-40481 was rapidly and extensively converted to etoposide, *in vivo*. The half-life of disappearance of the intact drug from the systemic circulation was less than 10 min. The rapid conversion of BMY-40481 to etoposide, *in vivo*, is known to be mediated by phosphatases present in serum and tissues, and possibly by hepatic metabolism (4). The drug has shown a similar activity to etoposide against a variety of *in vivo* murine tumor models (5); however, the *in vitro* cytotoxic potency was significantly less than that of etoposide (5), indicating that dephosphorylation to etoposide may be necessary for activity.

The purpose of this study was to assess the toxicokinetics and toxicodynamics of intravenous BMY-40481.

MATERIALS AND METHODS

Study Design and Animals. Four pure bred male beagle dogs (White Eagle Farms, DoylesTown, PA) received three escalating doses of BMY-40481 following an 18 hr fast, with a 28 day washout period between the doses. The doses were 57, 114 and 461 mg/m^2 of etoposide equivalents, and were within the dose range used in the toxicological evaluation of etoposide phosphate (4). Doses were not randomized since there was a potential for severe toxicity at the high dose. The dogs had a mean age of 28 months (range: 26 - 32 months), a mean weight of 12.3 kg (range: 11.1 - 14.6 kg), and a mean body surface area of 0.54 m^2 (range: 0.51 - 0.60 m^2). Prior to initiation of the study, the animals were judged to be healthy by physical examination and laboratory tests.

Drug Preparation and Administration. The i.v. formulation of the drug was prepared for each dog on the day of study by dissolving an appropriate amount of BMY-40481 (Lot #C92D809; 99.4% pure) in 20 ml of normal saline. The nominal concentrations of the resulting solutions ranged from 2.1 - 22.1 mg/ml. The dosing solutions were sterilized by filtration through a 0.22 μm sterile filter prior to use. Each dose of BMY-40481 was administered into the saphenous vein through an indwelling catheter, by constant rate infusion (3 ml/min) over 5 min, using a calibrated pump.

Biological Sample Collection and Handling. Blood samples (5 ml) for the determination of pharmacokinetic parameters were collected through an indwelling catheter placed in the jugular vein. The samples were collected into Vacutainer® tubes containing K_3EDTA as an anti-coagulant at 0, 5 (end of infusion), 10, 15, 20, 25, 30 and 45 min, and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24 and 32 hr. K_3EDTA prevents the hydrolysis of BMY-40481 to etoposide by serum phosphatases. Following blood collection, the tubes were immediately placed on chipped ice (4°C). The samples were centrifuged within 30 min of collection at 1000 × g for 10 min using a refrigerated centrifuge (4°C). Plasma was separated and transferred into labeled screw-cap tubes and stored frozen at −20°C until analyzed for BMY-40481 and etoposide.

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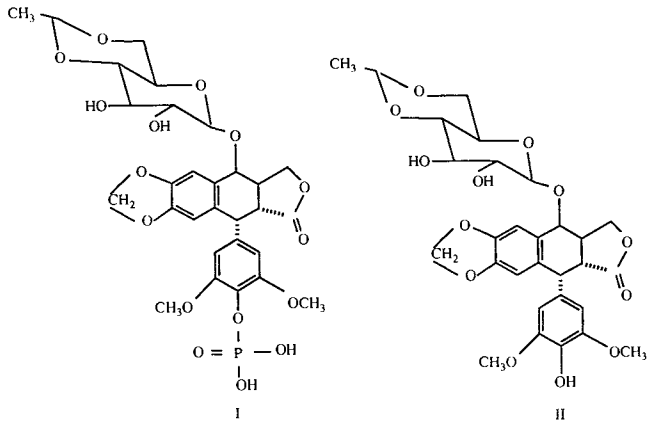

Fig. 1 Chemical structures of etoposide phosphate (BMY-40481, I) and etoposide (II).

Blood samples (1 ml) for the determination of hematology profile were obtained via jugular venipuncture into labeled Vacutainer® tubs containing K_3EDTA. Samples were collected twice (3-4 days apart) during the week preceding dosing to establish baseline values. Sample collection was continued twice a week post-dose for 28 days.

Assay of Study Samples. The assay of BMY-40481 in plasma samples was performed using a validated HPLC method. The assay involved solid phase extraction of 0.5 ml of plasma using 1 ml C-18 Bond Elut® column, followed by elution of BMY-40481 with 1% triethylamine in methanol. The eluate was evaporated to dryness under a gentle stream of nitrogen and the residue was reconstituted in 200 μl of CH_3CN:H_2O (10:90 v/v). A 50 μl aliquot of this solution was injected onto the HPLC Deltabond Phenyl (5 μm) column. The mobile phase was CH_3CN:H_2O (12:88, v/v), containing 0.01 M tetramethylammonium hydroxide and 0.02 M ammonium phosphate (pH = 3.0), and was pumped at a flow rate of 1.0 ml/min. Detection was accomplished by fluorescence (excitation at 200 nm and emission at 325 nm). Fresh plasma standards were prepared and analyzed during each analytical run. The linear range of the standard curve was 0.01-1 μg/ml, and quantitation was done using peak height response. The standard curves were linear with a coefficient of determination (r^2) ≥ 0.995. The predicted concentrations of the quality control (QC) samples, prepared prior to the initiation of the study, were within 15% of their nominal values. The between day and within day assay variabilities were less than 10%.

The concentrations of etoposide in the plasma samples were determined using a validated HPLC assay. The assay involved the addition of a 50 μl aliquot of 17β-estradiol solution (internal standard; 1 μg/ml) and 0.2 ml of 0.2 M Na_2HPO4 (pH = 8.0) to 0.5 ml of plasma, followed by extraction into ethylene dichloride. The sample was centrifuged for 5 min and the organic phase transferred into a clean polypropylene tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 200 μl of CH_3CN:CH_3OH:H_2O (30:15:55) and 50 μl were injected onto the HPLC column. The stationary phase was a Zorbax Phenyl column (5 μm). The mobile phase was CH_3CN:CH_3OH:H_2O:CH_3COOH (30:15:54.5:0.5, v/v) containing 10 mM tetramethylammonium hydroxide, and was delivered at a flow rate of 1 ml/min. Detection of the analyte was by electrochemical oxidation at +0.5 volts. Fresh plasma standards were prepared and analyzed during each analytical run. All study samples were initially assayed using a standard curve with a linear range of 0.1-10 μg/ml. Study samples with concentrations less than the lower limit of the curve (0.1 μg/ml) were reassayed using a standard curve range of 0.01-1 μg/ml. Quantitation was done with peak height ratios. The standard curves were linear with $r^2 \geq 0.995$. The predicted concentrations of the QC samples were within 11% of their nominal values. The between day and within day assay variabilities were less than 11%.

Stability of BMY-40481 in Dog EDTA Whole Blood, EDTA Plasma and Serum. The stability of BMY-40481 in dog whole blood and plasma containing K_3EDTA, and in serum, was investigated. Briefly, fresh dog K_3EDTA whole blood, K_3EDTA plasma or serum, in 10 ml volumetric flasks, was spiked with aliquots of stock solutions of BMY-40481 to give a final concentration of 4 μg/ml. The samples were mixed and immediately placed on chipped ice (4°C). An additional serum sample of BMY-40481 (4 μg/ml) was prepared and placed in a water bath at 37°C. Aliquots (0.1 ml) of the samples were collected at 0, 15, 30, 45, 60, 90 and 120 min. The plasma and serum samples were diluted 10-fold with control K_3EDTA plasma. For EDTA whole blood, 1 ml aliquots were collected and centrifuged at 1000 × g for 5 min at 4°C and plasma separated. Aliquots (0.1 ml) of the plasma samples were diluted 10-fold with control K_3EDTA plasma. The samples were immediately vortexed for 5 seconds and stored at −20°C until analyzed. The concentrations of BMY-40481 in the samples were determined using the HPLC assay described above. The stability of BMY-40481 was evaluated by determining the percentage of BMY-40481 remaining at each sample collection time, relative to the measured concentration of the sample taken at time 0.

Hematology Profiles. Hematology profiles were determined using standard methods. Hemoglobin, white blood count and platelet count were determined using automated cell counters (Baker Instruments, Corp). For the differential WBC count, slides were prepared with Hematek Slide Stainer® and Stain Pak® (Ames Company), followed by microscopic evaluation.

Pharmacokinetic Analyses. Plasma concentration versus time data of BMY-40481 and etoposide were evaluated by a noncompartmental method (6,7). The terminal log-linear phase of the plasma concentration-time curve was identified by least squares linear regression analysis of at least three data points which yielded a minimum mean square error. The slope of this log-linear phase was the terminal elimination rate constant, K. The following pharmacokinetic parameters were determined using previously reported equations (6,7): area under the plasma concentration versus time curve from 0 to infinity (AUC), area under the first moment curve (AUMC), terminal elimination half-life ($t_{1/2}$), mean residence time (MRT_{iv}), total systemic clearance (CL), and steady state volume of distribution (V_{ss}). The maximum plasma concentration, C_{max}, and time to achieve C_{max}, T_{max}, were the observed values from the tabulated data. The 5-min infusion time was accounted for in the determination of MRT_{iv} of BMY-40481. The MRT_{iv} of etoposide was determined as suggested by Weiss (8), where the mean transit time of etoposide (MTT) was ≈ 0 as the T_{max} of

etoposide and BMY-40481 were identical. Since the fraction of BMY-40481 converted to etoposide (F) is not known, CL and V_{ss} for etoposide were designated as apparent clearance (CL/F) and apparent steady state volume of distribution (V_{ss}/F), respectively. In the calculation of clearance and volume parameters of BMY-40481, the dose used was adjusted to reflect the molecular weight difference between BMY-40481 and etoposide.

Pharmacokinetic-Pharmacodynamic Relationships. The pharmacodynamic response variables, percent decreases in white blood count (WBC), absolute neutrophil count (ANC), hemoglobin and platelet count, were determined using baseline and nadir values (9). The relationships between the AUC of etoposide and percent decrease in hematologic parameters were evaluated with the following pharmacodynamic models published in the literature (9,10): a) linear model: % Decrease = a*AUC + b, where a and b are the slope and intercept, respectively; b) sigmoid E_{max} model: % Decrease = $[E_{max}*(AUC)^h]/[(AUC_{50})^h + (AUC)^h]$, where E_{max} (maximal effect) is fixed at 100%, AUC_{50} is the AUC producing half of the maximal effect and h is a parameter affecting the sigmoidal shape of the curve. Data analysis was performed using PCNONLIN (11). Initial estimates were obtained by visual examination of the plots of the data. Inspection of the fittings, Akaike Information Criterion (AIC), correlation coefficient (r), residual sum of squares, and plots of residuals were used to determine the model that best described the pharmacodynamic data.

Statistical Data analysis. Weighted (1/dose) linear regression was used to determine whether C_{max} and AUC were dose linear and/or dose proportional for each compound. In these analyses for repeated measures, dog was used as a blocking factor. For $t_{1/2}$, CL and V_{ss}, an analysis of variance (ANOVA) appropriate for a randomized block design (12) was used to investigate differences among doses. If statistically significant differences were observed among dose groups, then Tukey's procedure (13) was used to make pairwise comparisons among means. Statistical analyses were performed with the SAS software package (14). Levene's test (15) evaluated homogeneity of variance at the P = 0.001 level. All other tests of significance were performed at the P = 0.05 level.

RESULTS

Safety Assessment. Except for vomiting, which occurred in all dogs 3-4 hr after receiving the 461 mg/m^2 dose, no overt signs of adverse drug effects were observed. Hematology profiles showed significant decreases in WBC, ANC and platelets over the dose range studied. Hemoglobin, however, exhibited only minimal changes following drug administration. In all dogs, nadir blood counts occurred between days 1-5 post-dose, with counts recovering to predose values by day 19.

Stability of BMY-40481 in Dog EDTA Whole Blood, EDTA Plasma and Serum. Figure 2 is a plot of percent of BMY-40481 remaining over 2 hr following incubation in dog whole blood or plasma containing K_3EDTA at 4°C, or serum at 4°C and 37°C. The concentration of BMY-40481 did not decrease from the zero time concentration in whole blood or plasma at 4°C for 2 hr; however, in serum, the concentration

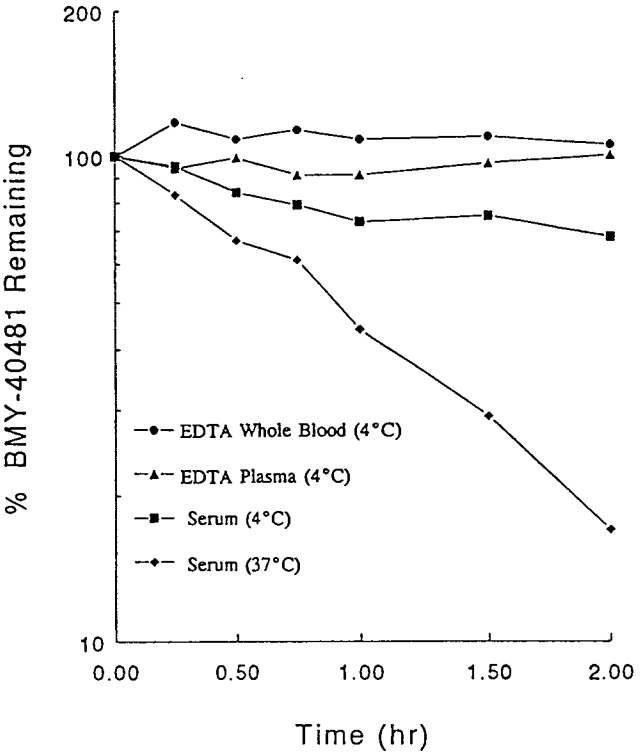

Fig. 2 Mean percent of BMY-40481 remaining in dog EDTA whole blood and EDTA plasma at 4°C, and in serum at 4°C and 37°C, over 2 hr. Each data point is the mean of 3 experiments.

of BMY-40481 decreased by approximately 32% and 87% at 4°C and 37°C, respectively.

Pharmacokinetics of BMY-40481. Figure 3A shows the mean plasma concentration-time profiles of BMY-40481 after i.v. administration. The mean pharmacokinetic parameters are summarized in Table I. Following i.v. administration, plasma BMY-40481 concentrations declined very rapidly. At all dose levels, C_{max} occurred at the end of infusion (5 min), and decreased by greater than 100-fold within 30 min of administration. The mean C_{max} and AUC values ranged from 1.72-40.51 μg/ml and 0.16-4.14 hr.μg/ml, respectively, for the three doses. Weighted linear regression analysis showed that both C_{max} and AUC of BMY-40481 increased in a dose linear, but not in a dose proportional manner.

Mean CL (Table I) at the high dose (2292 ml/min/m^2), was significantly smaller than the values at the two lower dose levels (7514 and 5724 ml/min/m^2 at 57 and 114 mg/m^2 doses, respectively). Mean V_{ss} at the 461 mg/m^2 dose, 12.2 l/m^2, was significantly smaller than mean V_{ss} at the 57 and 114 mg/m^2 doses (31.0 and 29.6 l/m^2, respectively). The mean $t_{1/2}$ values of BMY-40481 ranged from 0.11 - 0.17 hr (6.6 - 10.2 min). Although $t_{1/2}$ increased at the high dose relative to the two lower doses, the differences were not statistically significant. The C_{max}, AUC, CL and V_{ss} parameters all indicated that the pharmacokinetics of BMY-40481 was dose-dependent.

Pharmacokinetics of Etoposide. The mean plasma concentration-time profiles of etoposide after i.v. infusion of BMY-40481 are shown in Figure 3B. The mean pharmacokinetic parameters are summarized in Table II. Etoposide C_{max} occurred at the end of infusion (5 min) at all doses,

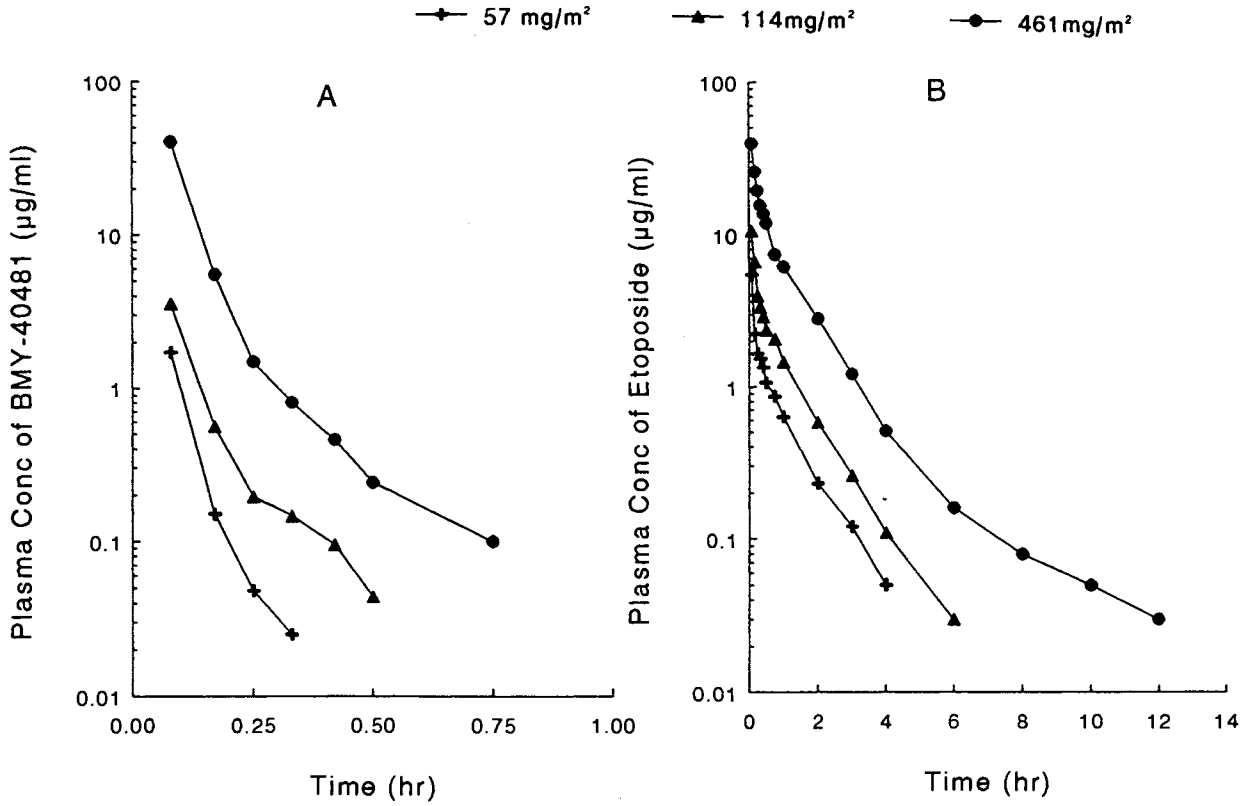

Fig. 3 Mean plasma concentration versus time profiles of BMY-40481 (A) and etoposide (B) after i.v. administration of 57, 114 and 461 mg/m^2 doses of BMY-40481. Doses are expressed as etoposide equivalents. (N = 4 dogs at each dose level).

indicating a rapid formation of etoposide. Mean C_{max} values at the different dose levels ranged from 5.46 - 39.44 µg/ml. C_{max} was proportionately related to dose. Mean etoposide AUC values ranged from 2.28 - 22.60 hr.µg/ml, and also showed a proportionate relationship with dose. Both C_{max} and AUC indicated that the predominant species in the systemic circulation following i.v. infusion of BMY-40481 is etoposide.

Etoposide exhibited a relatively longer $t_{1/2}$ than BMY-40481. Mean $t_{1/2}$ values ranged from 1.33 - 2.64 hr. The $t_{1/2}$ values were comparable at the two lower doses (1.43 and 1.33 hr for the 57 and 114 mg/m^2 doses, respectively), but increased significantly ($P < 0.004$) to 2.64 hr at the high dose. Mean values for apparent systemic clearance (CL/F) ranged from 342 - 435 ml/min/m^2, while mean apparent steady state volume of distribution (V_{ss}/F) ranged from 21.5 - 26.6 l/m^2. Both CL/F and V_{ss}/F were independent of dose.

Pharmacokinetic-Pharmacodynamic Relationships. Plots of percent decrease in hematologic parameters against etoposide AUC are depicted in Figure 4. Except for hemoglobin, significant relationships were observed between percent decreases in hematologic parameters and etoposide AUC values. Based on examination of the model fits, AIC, correlation coefficient, residual sum of squares, and the dispersion of residuals, the relationship between percent decreases in WBC or ANC and the AUC of etoposide

Table I. Mean (±S.D.) Pharmacokinetic Parameters of BMY-40481 Following i.v. Administration of 51–461 mg/m^2 Doses

Dose (mg/m^2)	C_{max} (µg/ml)	T_{max} (hr)	AUC (hr · µg/ml)	$t_{1/2}$ (hr)	CL[b] (ml/min/m^2)	CL[b] (ml/min/kg)	V_{ss} (l/m^2)	V_{ss} (l/kg)
57 (N = 4)	1.72 ± 0.51	0.08 ± 0.00	0.16 ± 0.05	0.11 ± 0.11	7515 ± 2721	332 ± 130	31.0 ± 10.8	1.36 ± 0.46
114 (N = 4)	3.55 ± 0.82	0.08 ± 0.00	0.39 ± 0.06	0.11 ± 0.02	5724 ± 1039	251 ± 51	29.6 ± 10.8	1.28 ± 0.43
461 (N = 4)	40.5 ± 16.3	0.08 ± 0.00	4.14 ± 1.27	0.17 ± 0.04	2295 ± 854	99 ± 33	12.2 ± 9.6	0.52 ± 0.39
Analysis of variance	—[a]	NS	—[a]	NS	57 114 461[c]	57 114 461[c]	57 114 461[c]	57 114 461[c]

[a] The relationship between C_{max} or AUC and dose was analysed by weighted linear regression. The regression equations were: C_{max} = 0.095 * Dose-4.826 (R^2 = 0.85) and AUC = 9.783 * Dose-0.503 (R^2 = 0.90). C_{max} and AUC were dose linear but not dose proportional.
[b] CL expressed in ml/min were 3986 ± 1235, 3080 ± 511 and 1280 ± 572 ml/min for the 57, 114 and 461 mg/m^2 doses, respectively.
[c] Tukey lines: means not connected by a common line are statistically significantly different ($p < 0.05$).
NS = Not significant.

Table II. Mean (±S.D.) Pharmacokinetic Parameters of Etoposide Following 57–461 mg/m² i.v. Doses of BMY-40481

Dose (mg/m²)	C_{max} (μg/ml)	T_{max} (hr)	AUC (hr · μg/ml)	$t_{1/2}$ (hr)	CL^c/F^d (ml/min/m²)	CL^c/F^d (ml/min/kg)	V_{ss}/F^d (l/m²)	V_{ss}/F^d (l/kg)
57 (N = 4)	5.46 ± 1.97	0.08 ± 0.00	2.28 ± 0.54	1.43 ± 0.50	435 ± 92	19.1 ± 4.51	24.7 ± 4.4	1.09 ± 0.23
114 (N = 4)	10.55 ± 2.10	0.08 ± 0.00	5.15 ± 0.45	1.33 ± 0.80	373 ± 38	16.3 ± 1.33	19.6 ± 3.5	0.86 ± 0.16
461 (N = 4)	39.44 ± 5.16	0.08 ± 0.00	22.60 ± 2.26	2.64 ± 0.82	342 ± 23	14.9 ± 1.48	21.8 ± 2.8	0.95 ± 0.15
Analysis of variance	—[a]	NS	—[a]	57 114 461[b]	NS	NS	NS	NS

[a] The relationship between C_{max} or AUC and dose was analysed by weighted linear regression. The regression equations were: C_{max} = 0.084 * Dose + 0.706 (R^2 = 0.96) and AUC = 0.050 * Dose-0.605 (R^2 = 0.99). C_{max} and AUC were dose proportional.

[b] Tukey lines: means not connected by a common line are statistically significantly different ($p < 0.05$).

[c] CL expressed in ml/min were 232 ± 41, 202 ± 33 and 187 ± 9 ml/min for the 57, 114 and 461 mg/m² doses, respectively.

[d] F = Fraction of BMY-40481 converted to etoposide.

NS = not significant.

was best described by the sigmoid E_{max} model (Figures 4A, 4B). The model estimates (95% confidence intervals) of AUC_{50} and the Hill coefficient (h) for WBC were 8.29 (3.77-12.8) hr.μg/ml and 1.15 (0.43-1.88) respectively. The corresponding estimates for ANC were 7.71 (2.44-13.0) hr.μg/ml and 1.8 (0.03-3.57), respectively. For platelets (Figure 4C), the best fit was obtained with a simple linear model (r = 0.97). The model estimates (95% confidence intervals) of slope and intercept were 3.12 (2.14-4.10) and 0 (−13.2-13.2), respectively. Hemoglobin showed only slight decreases over the dose range studied, and this did not appear to be related to etoposide exposure (Figure 4D).

DISCUSSION

Etoposide is an important chemotherapeutic agent for the treatment of various malignancies (16). Because of its poor aqueous solubility, the intravenous formulation contains organic solvents, such as Tween 80, polyethylene glycol and alcohol. These solvents have been implicated in some of the adverse effects experienced by patients during therapy with etoposide (17,18). BMY-40481 was developed in an attempt to improve the aqueous solubility of etoposide. Since BMY-40481 is a prodrug of etoposide, it is desirable that it be readily converted to etoposide *in vivo*. However, the conduct of meaningful pharmacokinetic/biopharmaceutic studies depends on the ability to prevent further hydrolysis of BMY-40481 once blood samples are collected. Previous *in vitro* studies have shown that the rapid hydrolysis of BMY-40481 in serum is catalyzed by the enzyme, alkaline phosphatase (4). Phosphatases are ubiquitous in nature and found in many tissues (19). They are zinc metalloproteins requiring Zn^{++} for their activity (20,21). For this reason, the chelating agent and anticoagulant EDTA was evaluated for its effectiveness in preventing the hydrolysis of BMY-40481 *ex vivo*. The present results showed that in the presence of EDTA, BMY-40481 was stable in dog whole blood or plasma at 4°C for up to 2 hr. In serum, where there was no EDTA, temperature-dependent hydrolysis of BMY-40481 was observed over the same time. These results indicate that EDTA prevents the hydrolysis of BMY-40481 to etoposide by deactivating the enzyme, alkaline phosphatase. Therefore, the sample collection procedure employed in the present study maintained the integrity of the samples.

The results of this study demonstrate that following i.v. administration, the phosphate ester of etoposide (BMY-40481) is cleaved *in vivo*, generating plasma levels of etoposide. The very short $t_{1/2}$ of BMY-40481 (<11 min), the fact that the C_{max} of etoposide occurred at the end of infusion (5 min), and C_{max} of BMY-40481 decreased by greater than 100-fold within 30 min of administration, and the several fold greater AUC of etoposide in comparison to BMY-40481, all indicate that BMY-40481 is rapidly and extensively converted to etoposide *in vivo* due to first pass metabolism.

At the 461 mg/m² dose, the increases observed for C_{max} and AUC of BMY-40481 were approximately 3-fold greater than those predicted from a proportional increase in dose. These results suggested that there was possible saturation of the enzymes (phosphatases) responsible for the conversion of BMY-40481 to etoposide. The V_{ss} of BMY-40481 also decreased significantly at the high dose, probably due to saturation of extravascular binding sites (22). The $t_{1/2}$ of BMY-40481 did not increase significantly at the high dose since both CL and V_{ss} decreased at this dose.

The C_{max} and AUC values for etoposide were proportionately related to dose. The apparent clearance (CL/F) and apparent steady state volume of distribution (V_{ss}/F) were dose-independent, while $t_{1/2}$ at the 461 mg/m² dose was higher than those at the two lower doses probably because of the longer duration of quantitation. The incongruity between the dose proportionality in the C_{max} and AUC of etoposide and BMY-40481 suggests that, unlike the kinetics of BMY-40481, the kinetics of etoposide is not sensitive to the changes in the extent of first pass metabolism of the prodrug (23).

The $t_{1/2}$ values of etoposide obtained in this study are in general agreement with the value of 1.7 hr reported in the literature following i.v. administration of 2 mg/kg dose of etoposide to beagle dogs (24). However, the $t_{1/2}$ of etoposide in the dog is shorter than that reported in humans, which ranges from 4-8 hr (25).

In toxicologic studies of BMY-40481 in beagle dogs, myelosuppression was one of the major toxic effects observed (4). Myelosuppression was also the dose-limiting toxicity observed following treatment of cancer patients with etoposide phosphate (2) and etoposide (26). The results from the current study demonstrated highly significant correlations be-

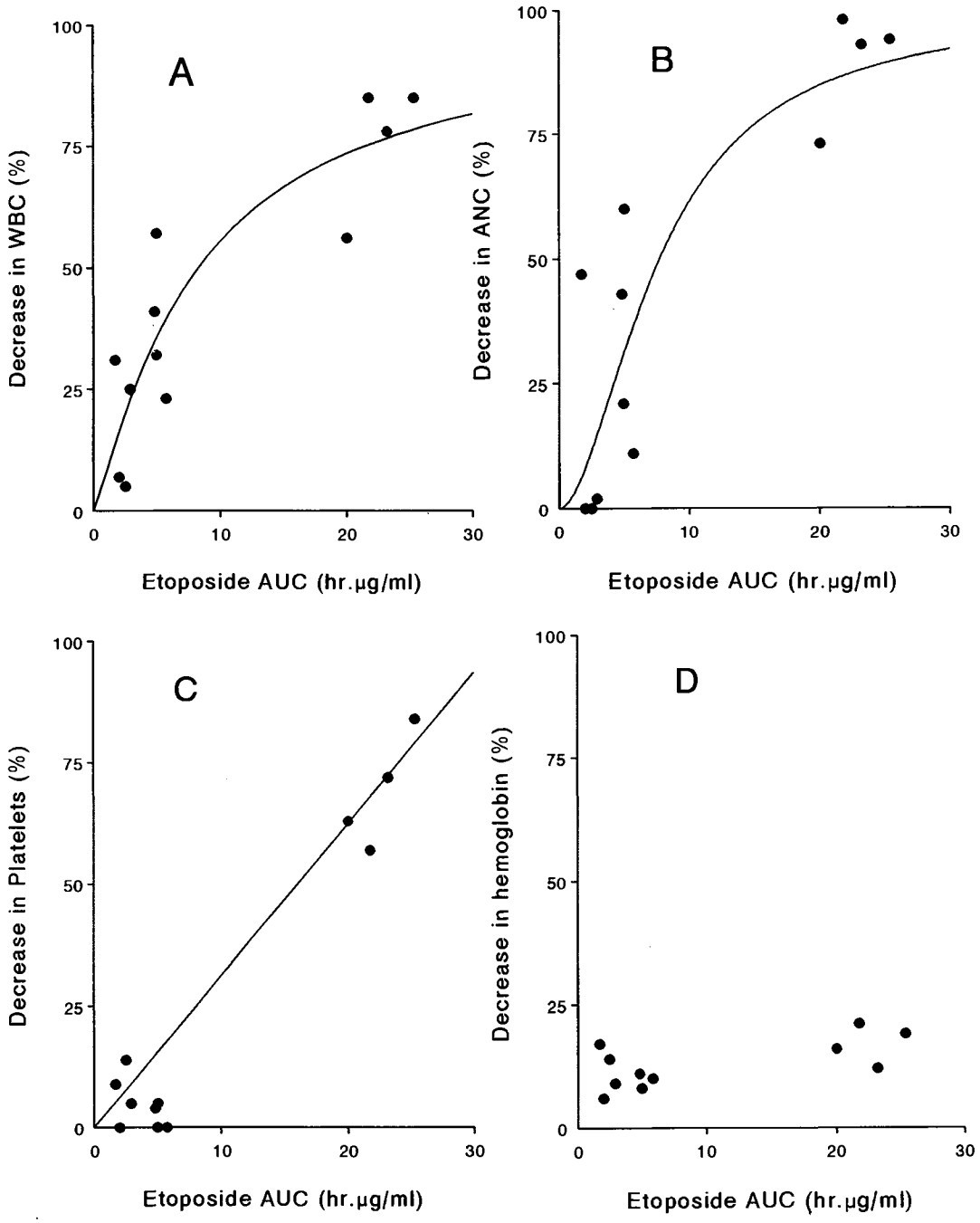

Fig. 4 The relationships between AUC of etoposide and percent decrease in WBC (A), ANC (B), platelets (C) and hemoglobin (D). The symbols represent experimental data. For WBC and ANC, the curves are the fits to the sigmoid E_{max} model; for platelets, the straight line is the fit to the linear model.

tween etoposide exposure and percent decreases in WBC, ANC and platelets following drug treatment. The relationship between etoposide systemic exposure and percent decrease in WBC has been adequately described by the sigmoid E_{max} model in cancer patients; the values for AUC_{50} and h were 104 hr.μg/ml and 0.53, respectively (10). The AUC_{50} value in humans is approximately ten fold great than that observed in dogs. Although the reasons for this difference is not clear, it may be due to the interspecies differences in the disposition of etoposide (24,25,27), extent of protein binding (28) and/or species differences in the sensitivity of hematologic toxicity (29,30).

In conclusion, BMY-40481 is rapidly and extensively converted to etoposide following i.v. administration. Etoposide generated from i.v. BMY-40481 exhibits linear kinetics over the dose range of 57 - 461 mg/m^2 of etoposide equivalents, and except for hemoglobin, myelosuppression is significantly correlated with exposure of dogs to etoposide.

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REFERENCES

1. L. N. Igwemezie, S. Kaul, A. Dean and R. H. Barbhaiya. Pharmacokinetics and pharmacodynamics of etoposide phosphate in dogs (Abstr). *Pharmaceutical Research*. 10:S316, 1993.
2. M. J. Milward, D. R. Newell, K. Balmanno, C. J. Charlton, L. Gumbrell, M. J. Lind, F. Chapman, M. Proctor, D. Simmonds, B. Cantwell, G. A. Taylor, C. McDaniel, B. Winograd and A. H. Calvert. Clinical and pharmacokinetic study of BMY-40481 (Abstr). *Proceedings of the American Association for Cancer Research*. 33:529, 1992.
3. K. D. Hande, L. N. Igwemezie, G. Garrow, F. A. Greco, L. Schacter and S. Kaul. Kinetics of etoposide phosphate (Abstr). *Clinical Pharmacology and Therapeutics*. 53:233, 1993.
4. Bristol-Myers Squibb Company: Data on file.
5. W. C. Rose, G. A. Basler, P. A. Trail, M. Saulnier, A. R. Crosswell and A. M. Casazza. Preclinical antitumor activity of a water soluble etoposide analog, BMY-40481-30. *Investigational New Drug*. 8:S25-S32, 1990.
6. M. Gibaldi and D. Perrier. *Pharmacokinetics*, 2nd Edition, Marcel Dekker, New York, 1982.
7. S. Riegelman and P. Collier. An application of statistical moments to the evaluation of in vivo dissolution time and absorption time. *Journal of Pharmacokinetics and Biopharmaceutics*. 8:509-534, 1980.
8. M. Weiss. A general model of metabolite kinetics following intravenous and oral administration of the parent drug. *Biopharmaceutics and Drug Disposition*. 9:159-176, 1988.
9. M. J. Egorin, D. A. Van Echo, M. Y. Whitacre, A. Forrest, L. M. Sigman, K. L. Engisch, and J. Aisner. Human pharmacokinetics, excretion and metabolism of the anthracycline antibiotic menogaril (7-OMEN, NSC 269148) and their correlation with clinical toxicities. *Cancer Research*. 46:1513-1520, 1986.
10. C. F. Stewart, S. G. Arbuck, R. A. Fleming and W. E. Evans. Relation of systemic exposure to unbound etoposide and hematologic toxicity. *Clinical Pharmacology and Therapeutics*. 50: 385-393, 1991.
11. Statistical Consultants, Inc.: PCNONLIN and NONLIN84. Software for statistical analysis of nonlinear models. *American Statistician*. 40:1, 1986.
12. B. J. Winer. *Statistical principles in experimental design*, 2nd edition, McGraw-Hill Book Company, New York, 1971.
13. J. L. Gill. *Design and analysis of experiments in the animal and medical sciences*, Iowa State University Press, Ames, 1978.
14. SAS Institute: *SAS Users Guide*: Statistical Version, 5th ed., SAS Institute Incorporation, Cary, North Carolina, 1985.
15. G. W. Snedecor and W. G. Cochran. *Statistical Methods*, 7th Edition, Iowa State University Press, Ames, 1980.
16. P. J. O'Dwyer, B. Leyland-Jones, M. T. Alonzo, S. Marsoni and R. E. Wittes. Etoposide (VP-16): Current status of an active anticancer drug. *New England Journal of Medicine*. 312:692-700, 1985.
17. P. J. O'Dwyer and R. B. Weiss. Hypersensitivity reactions induced by etoposide. *Cancer Treatment Report*. 68:959-961, 1984.
18. S. N. Wolff, M. F. Fer, C. M. McKay, K. R. Hande, Hainsworth, J. D. and F. A. Greco. High Dose VP-16-213 and autologous bone marrow transplantation for refractory malignancies: A phase I study. *Journal of Clinical Oncology*. 1:701-705, 1983.
19. M. M. Kaplan: Progress in hepatology: Alkaline phosphatase. *Gastroenterology*. 62:452-468, 1972.
20. C. N. Corder and O. H. Lowry: The Zinc requirement of dinucleotide pyrophosphatase in mammalian organs. *Biochimica et Biophysica Acta*. 191:579-587, 1969.
21. D. W. Moss: The influence of metal ions on the orthorphosphatase and inorganic pyrophosphatase activities of human alkaline phosphatase. *Biochemical Journal*. 112:699-701, 1969.
22. M. Rowland and T. N. Tozer: *Clinical Pharmacokinetics: Concepts and Applications*, Lea and Febiger, Philadelphia, 1989.
23. A. T. Chow and W. J. Jusko. Michaelis-Menten metabolite kinetics: equations relating area under the curve and metabolite recovery to administered dose. *Journal of Pharmaceutical Sciences*. 79:902-906, 1990.
24. N. Savaraj, K. Lu, L. G. Feun, M. A. Burgess and T. L. Loo. Comparison of CNS penetration, tissue distribution and pharmacology of VP16-213 by intracarotid and intravenous administration in dogs. *Cancer Investigation*. 5:11-16, 1987.
25. R. A. Fleming, A. A. Miller and C. F. Stewart. Etoposide: An update. *Clinical Pharmacy*. 8:274, 1989.
26. A. A. Miller, E. A. Tolley, H. B. Niell, C. F. Stewart, and J. P. Griffin. Pharmacodynamics of three daily infusions of etoposide in patients with extensive small cell lung cancer. *Cancer Chemotherapy*. 31:161-166, 1992.
27. R. M. J. Ings. Interspecies scaling and comparisons in drug development and toxicokinetics. *Xenobiotica*. 20:1201-1231, 1990.
28. R. A. Fleming, S. G. Arbuck and C. F. Stewart. Interspecies differences in *in vitro* etoposide plasma protein binding. *Biochemical Pharmacology*. 42:2246-2249, 1991.
29. J. C. Marsh. Correlation of hematologic toxicity of antineoplastic agents with their effects on bone marrow stem cells: interspecies studies using an in vivo assay. *Experimental Hematology*. (suppl. 16) 13:16-22, 1985.
30. P. S. Schein, R. D. Davis, S. Carter, J. Newman, D. R. Schein and D. P. Rall. The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. *Clinical Pharmacology and Therapeutics*. 11:3-40, 1970.