Cancer Chemother Pharmacol (2005) 56: 145–153
DOI 10.1007/s00280-004-0928-5

ORIGINAL ARTICLE

Amrita V. Kamath · Ming Chang · Francis Y. Lee
Yueping Zhang · Punit H. Marathe

Preclinical pharmacokinetics and oral bioavailability of BMS-310705, a novel epothilone B analog

Received: 21 July 2004 / Accepted: 21 September 2004 / Published online: 14 April 2005

Abstract *Purpose*: BMS-310705, a novel semisynthetic derivative of epothilone B, is a tubulin-polymerization agent currently in phase I clinical trials for anticancer therapy. The in vitro and in vivo pharmacokinetics and oral bioavailability of BMS-310705 were investigated in mice, rats, and dogs. In addition, comparison of the pharmacokinetics of BMS-310705 using various formulations was conducted in rats. *Methods*: The permeability of BMS-310705 was evaluated in Caco-2 cells, an in vitro model of the human intestinal epithelium. Human liver microsomes were used to determine the cytochrome P450 enzymes involved in the metabolism of BMS-310705. Plasma protein binding of BMS-310705 was determined in mouse, rat, dog, and humans. BMS-310705 was administered to female nude mice as single doses of 5 mg/kg intravenously or 15 mg/kg orally. Male Sprague-Dawley rats were treated with single doses of BMS-310705 either intraarterially (2 mg/kg) or orally (8 mg/kg). The effect of Cremophor on the pharmacokinetics of BMS-310705 was evaluated in rats using various formulations with and without Cremophor. Male dogs were treated with 0.5 mg/kg intravenously or 1 mg/kg orally in a crossover study design. *Results*: Systemic clearance of BMS-310705 was high in mice (152 ml/min/kg), rats (39 ml/min/kg), and dogs (25.7 ml/min/kg). The volume of distribution (Vss) in mice, rats, and dogs was 38, 54, and 4.7 l/kg, respectively, and greater than total body water. BMS-310705 showed moderate binding to plasma proteins in all four species tested. The clearance in humans may be intermediate to high based on both allometric scaling using parameters obtained from three species, and in vitro human liver microsomal stability data. In rats, the presence of Cremophor in the formulation resulted in a significant increase in exposure compared to buffered vehicles not containing Cremophor. Inhibition of p-glycoprotein and/or CYP3A4 by Cremophor may be responsible for this phenomenon, and studies in Caco-2 cells and human liver microsomes suggested that BMS-310705 may be a substrate for both p-glycoprotein and CYP3A4. The oral bioavailability of BMS-310705 in pH buffered formulations was 21% in mice, 34% in rats and 40% in dogs. *Conclusion*: In summary, BMS-310705 is cleared rapidly and distributes extensively in mice, rats, and dogs. The presence of Cremophor in the formulation could significantly increase exposure in rats, possibly due to interactions with p-glycoprotein and/or CYP3A4. Oral bioavailability using formulations not containing Cremophor were found to be adequate, suggesting potential for development of BMS-310705 as an oral anticancer drug.

Keywords Pharmacokinetics · Bioavailability · Formulation · Metabolism epothilone · Allometric scaling

A. V. Kamath (✉) · M. Chang · F. Y. Lee · Y. Zhang
P. H. Marathe
Department of Metabolism and Pharmacokinetics,
Bristol-Myers Squibb Pharmaceutical Research Institute,
P.O. Box 4000, Princeton, NJ 08543, USA
E-mail: amrita.kamath@bms.com
Tel.: +1-609-2525303
Fax: +1-609-2526802

Introduction

The epothilones are a novel class of microtubule-stabilizing compounds which have potential for anticancer therapy [2, 7, 10]. The epothilones were isolated from the fermentation of the myxobacterium *Sorangium cellulosum* [7]. The cytotoxic activities of epothilones have been linked to binding with tubulin and stabilization of microtubules resulting in mitotic arrest at the G_2/M transition and consequent cell death [2, 10]. The epothilone chemotype offers an alternative to the taxanes, as activity is seen against paclitaxel-resistant cancer cells, including both the MDR and tubulin mutation modes of resistance [2, 10].

The natural products epothilone A and B show only modest in vivo activity despite their potent in vitro

activities [11]. Analogs to improve the in vivo activity of this chemical class have been pursued and over 300 semisynthetic analogs have been made and tested [3, 9, 11, 17]. BMS-310705 is an aqueous soluble, chemically stable C-21 substituted derivative of epothilone B (Fig. 1) [12]. In studies in mice implanted with human tumor xenografts, BMS-310705 has shown antitumor activities that were superior to paclitaxel, epothilone B, or epothilone D [12]. BMS-310705 is currently in phase I clinical trials [13, 18].

In this report we describe the in vitro and in vivo pharmacokinetic characteristics of BMS-310705 in mice, rats, and dogs. Since Cremophor-containing formulations were utilized in early studies, the effect of Cremophor on the pharmacokinetics of BMS-310705 was evaluated in rats.

Materials and methods

Chemicals

BMS-310705 was synthesized in the Oncology Chemistry Department at Bristol-Myers Squibb Pharmaceutical Research Institute. Hank's balanced salt solution (HBSS) and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals used were reagent grade or better.

p-Glycoprotein substrate assay using Caco-2 cells

Caco-2 cells (American Type Culture Collection, Rockville, Md.) were seeded onto 24-well polycarbonate filter membranes at a density of 100,000 cells/cm^2. The permeability studies were conducted with the monolayers cultured for approximately 21 days in culture. Prior to all experiments, the transepithelial electrical resistance (TEER) was measured to ensure the integrity of the Caco-2 monolayers. The transport medium buffer was modified Hank's balanced salt solution containing 10 m*M* HEPES. The pH of both the apical and basolateral compartments was 7.4. The concentration of BMS-310705 used in the p-glycoprotein substrate assay was 50 μ*M* (20 m*M* stock solution made in DMSO). The bidirectional permeability studies were initiated by adding an appropriate volume of buffer containing BMS-310705 to either the apical (apical to basolateral transport) or basolateral (basolateral to apical transport) side of the monolayer ($n = 3$). Samples were taken from both the apical and basolateral compartments at the end of a 2-h incubation period and the concentrations of test compound were analyzed for BMS-310705 using a HPLC method with UV detection. Permeability coefficient (Pc) was calculated according to the following equation: Pc = dA/(dt×S×Co), where dA/dt is the flux of BMS-310705 across the monolayer (nmol/s), S is the surface area of the cell monolayer, and Co is the initial concentration of BMS-310705 in the donor compartment. The Pc values were expressed as nanometers per second.

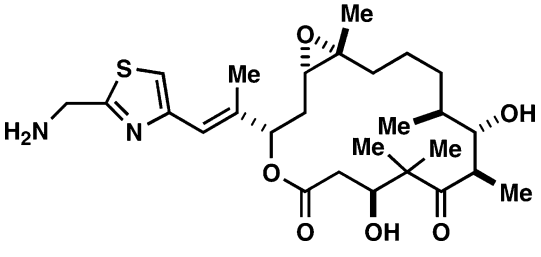

Fig. 1 Chemical structure of BMS-310705

Incubations with human liver microsomes and Supersomes

BMS-310705 was incubated with human liver microsomes along with compounds specific for the inhibition of individual cytochrome P450s (CYPs) commonly involved in drug metabolism. Human liver microsomes were purchased from In Vitro Technologies (Baltimore, Md.) and were pooled from seven individual donors. The rates of oxidative metabolism were measured under the following conditions: 20 μ*M* BMS-310705 (20 m*M* stock solution made in acetonitrile), 1 mg/ml microsomal protein, 1 m*M* NADPH, and 56 m*M* phosphate buffer (pH 7.4) ($n = 2$). Reaction phenotyping studies to determine the CYPs involved in the metabolism of BMS-310705, were carried under similar conditions using a 10 μ*M* starting concentration of BMS-310705 ($n = 1$). The inhibitors used in the reaction phenotyping assays were: furafylline (CYP1A2), sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6), troleandomycin (CYP3A4), and ketoconazole (CYP3A4). The inhibitors were dissolved in either acetonitrile (furafylline, sulfaphenazole, tranylcypromine) or methanol (quinidine, troleandomycin, ketoconazole). Inhibitor concentrations were selected to give maximum specific inhibition [15]. Organic solvent content from the addition of inhibitors was 0.5% and control incubations containing the appropriate solvent compositions were run. BMS-310705 was also incubated with human CYPs obtained from insect cells that expressed a single CYP enzyme (Supersomes obtained from Gentest Co., Woburn, Mass.). The conditions for these incubations were as follows: 50 pmol/ml CYP enzyme, 10 μ*M* BMS-310705 (20 m*M* stock solution made in acetonitrile), 1 m*M* NADPH, and 56 m*M* potassium phosphate (pH 7.4) ($n = 1$). The extent of metabolism was calculated based on disappearance of the parent compound. Samples were analyzed by LC/MS/MS.

Plasma protein binding

The extent of protein binding of BMS-310705 was determined in fresh mouse, rat, dog, and human plasma

by ultrafiltration using the Amicon Centrifree micropartition device (Amicon, Beverly, Mass.), type 50A (molecular weight cutoff 50,000). For determination of plasma protein binding, plasma samples (approximately 1 ml) containing BMS-310705 at a concentration of 20 μ*M* ($n=2$) were prepared, transferred to the micropartition device and centrifuged at 1000 *g* for 20 min at room temperature to obtain ultrafiltrate. The free fraction was determined from the ratio of BMS-310705 concentration in the ultrafiltrate to that in the plasma. The nonspecific binding to the ultrafiltration unit was determined by performing the same procedure in buffer.

In vivo animal studies

All procedures used in animal studies were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee.

Pharmacokinetics and oral bioavailability in mice

Female nude mice were obtained from Harlan Sprague Dawley Co. (Indianapolis, Ind.). A total of 30 mice were dosed, 15 by the intravenous (IV) route and 15 by the oral route. BMS-310705 was dissolved in a mixture of Cremophor/ethanol/water (1:1:8, v/v) for the IV dose (5 mg/kg) and Cremophor/ethanol/phosphate buffer, 1 *M*, pH 8 (1:1:8, v/v) for the oral dose (15 mg/kg). Plasma concentrations in nude mice ($n=3$ at each time point) after IV bolus and oral administration of BMS-310705 were measured at 10 min, 45 min, and 2, 4 and 6 h after dosing.

Pharmacokinetics and oral bioavailability in rats

Adult male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, Mass.). Rats were surgically prepared with an indwelling jugular vein cannula. Rats that were given an intraarterial (IA) dose had an additional indwelling carotid artery cannula. Rats were fasted overnight prior to dosing and fed approximately 4 h after dosing. Water was provided ad libitum throughout the study. The pharmacokinetics of BMS-310705 were investigated in rats following a single dose either IA (2 mg/kg) as a 10-min infusion or orally by gavage (8 mg/kg). A total of nine rats were divided into three groups, two IA groups and one oral group, with three rats in each group. The vehicles used for the IA dosing were Cremophor/ethanol/water (1:1:8, v/v) and ethanol/phosphate buffer, 0.05 *M*, pH 7.4 (6:94, v/v). The vehicle for the oral dose was ethanol/phosphate buffer, 0.3 *M*, pH 8 (18:82, v/v). Plasma levels of BMS-310705 were monitored for 48 h after dosing. Samples were collected at 10, 15, 30, and 45 min, and 1, 2, 4, 6, 8, 10, 24, and 48 h. Approximately 250 μl of blood was collected from the jugular vein catheter into heparinized tubes and plasma was obtained by centrifugation at 4°C.

Pharmacokinetics and oral bioavailability in dogs

The pharmacokinetics of BMS-310705 were investigated in fasted male beagle dogs following a single IV dose ($n=3$, 0.5 mg/kg) as a 10-min infusion and as an oral dose by gavage ($n=3$, 1 mg/kg) in a cross-over study design. The vehicle used for the IV dose was propylene glycol/ethanol/phosphate buffer, 0.05 *M*, pH 7.4 (40:5:55, v/v) and the vehicle for the oral dose was propylene glycol/ethanol/phosphate buffer, 0.3 *M*, pH 8 (40:5:55, v/v). The dogs were fed 6 h after dosing. Plasma levels of BMS-310705 were monitored for 24 h after dosing. Samples were collected at 0.167, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, and 24 h after dosing. Approximately 1 ml of blood was collected into heparinized tubes and plasma was obtained by centrifugation at 4°C.

Sample analysis

BMS-310705 was unstable in mouse, rat, and dog plasma at 37°C, but was found to be stable when placed on ice for up to 2 h. Addition of acetonitrile to plasma followed by freezing also stabilized the compound, and this procedure was adopted for handling of plasma samples from all studies. Plasma concentrations of BMS-310705 were analyzed by a specific LC/MS/MS method. Plasma samples were deproteinized with three volumes of acetonitrile. Standard and quality control (QC) samples were prepared in plasma and deproteinized in the same manner as the study samples. All samples were centrifuged and 200 μl of each resulting supernatant was mixed with 50 μl internal standard (500 ng/ml of BMS-247550 in acetonitrile). The resulting solution was evaporated to dryness under a stream of nitrogen at 40°C. The dried extracts were reconstituted in 50 μl of the mobile phase A (described below), a 10-μl portion of which was analyzed by LC/MS/MS.

The HPLC system consisted of two Shimadzu LC10AD pumps, a Perkin Elmer Series 200 autosampler and a Hewlett Packard Series 1100 column compartment. The column used was a Keystone BDS-C18 2×20 mm, 3 μm particle size, maintained at 60°C and a flow rate of 0.3 ml/min. The mobile phase consisted of 10 m*M* ammonium acetate in 75:25 water/methanol mixture, pH 5.5 (A) and 10 m*M* ammonium acetate in methanol (B). The initial mobile phase composition was 95% A/5% B. After sample injection, the mobile phase was changed to 5% A/95% B over 1 min and held at that composition for an additional 1 min. The mobile phase was then returned to initial conditions and the column re-equilibrated for 0.5 min. Total analysis time was 2.5 min. The HPLC was interfaced to a Micromass Quattro LC tandem mass spectrometer equipped with an electrospray interface. UHP nitrogen was used as the

nebulizing and desolvation gas at flow rates of 100 l/h for nebulization and 900 l/h for desolvation. The desolvation temperature was 350°C and the source temperature was 150°C.

Data acquisition was via selected reaction monitoring. Ions representing the $(M+H)^+$ species for both the analyte and internal standard were selected in MS1 and collisionally dissociated with argon at a pressure of 1.5×10^{-3} T to form specific product ions which were subsequently monitored by MS2. The transitions monitored were *m*/*z* 523.2/335.0 for BMS-310705 and *m*/*z* 507.1/489.1 for BMS-247550 (internal standard). Cone voltage and collision energy were optimized at 45 V and 35 V, respectively, for BMS-310705 and 25 V and 15 V for the internal standard. The retention times for BMS-310705 and the internal standard were about 1.17 and 1.27 min, respectively. The standard curve for BMS-310705 ranged from 3.9 ng/ml to 2000 ng/ml. QC samples at three concentrations within the range of the calibration curve were also analyzed in duplicate with each analytical set. For BMS-310705, the predicted concentrations of at least two-thirds of the QC samples were generally within 20% of the nominal concentration, indicating acceptable assay performance.

Samples obtained from the Caco-2 permeability study were analyzed by HPLC-UV. The HPLC system consisted of a 2690 Waters separation module and a Waters 996 photodiode array detector (Waters, Milford, Mass.). The column used was YMC ODA-AQ 4.6×150 mm, 3 μm particle size, maintained at 25°C and a flow rate of 1 ml/min. Mobile phase A consisted of 95% water, 5% acetonitrile, 0.115% trifluoroacetic acid and mobile phase B consisted of 5% water, 95% acetonitrile, 0.115% trifluoroacetic acid. The initial mobile phase composition was 100% A. After injection the composition was changed to 95% A and 5% B over 1 min followed by 5% A and 95% B over the next 6 min. The system was maintained at this composition for an additional 7 min then returned to 100% A and equilibrating for 5 min. Quantitation was based on the peak area of BMS-310705 in the samples.

Data analysis

Plasma concentration data were analyzed with standard non-compartmental methods [8] using the KINETICA software program. Composite plasma concentration-time profiles were constructed for pharmacokinetic analysis in the mouse. The C_{max} and T_{max} values were recorded directly from experimental observations. The AUC_{tot} values were calculated using a combination of linear and log trapezoidal summations. The total body clearance (Cl), mean residence time (MRT), and the steady-state volume of distribution (Vss) were also calculated after IV or IA administration. The absolute oral bioavailability (expressed as a percentage) was estimated by taking the ratio of dose-normalized AUC values after oral doses to those after IV or IA doses.

The values for clearance of BMS-310705 found in mouse, rat, and dog, were used to predict these parameters in humans using allometric scaling techniques based on the body weight of each species [4]. Linear regression analysis was performed on clearance values versus body weight values obtained from mouse, rat, and dog, and clearance values in humans were predicted from the resulting equation: $Cl = a\times(\text{body weight})^b$ where a is the allometric coefficient, and b is the allometric exponent.

Metabolic stability data from human liver microsomes and Supersomes were also used to predict clearance values in humans, assuming that clearance was only via hepatic elimination. The following equations were used [8]:

$$Cl = (Q \times Cl_{int})/(Q + Cl_{int})$$

where Cl_{int} is the intrinsic clearance and Q is the hepatic blood flow.

The intrinsic clearance was calculated from microsomal as well as supersomal stability data, and scaled for amount of either microsomal protein or CYP protein per gram liver, respectively.

Using microsomal data, intrinsic clearance was calculated as follows:

$$\begin{aligned} Cl_{int} = {} & (\text{Rate of metabolism}/\text{Concentration of compound}) \\ & \times (\text{mg microsomal protein}/\text{gm liver}) \\ & \times (\text{gm liver}/\text{kg body weight}) \end{aligned}$$

where concentration = 20 μ*M*, milligrams microsomal protein/gram liver weight = 45, and gram liver weight/kilogram body weight = 87.5 (mouse), 40 (rat), 32 (dog), and 21 (humans).

In Supersome studies, clearance values for the individual CYPs were calculated in a similar manner as shown above in proportion to their relative activities in the liver (Table 2), and then added to get an estimate of the total clearance expected.

Results

p-Glycoprotein substrate assay in Caco-2 cells

Caco-2 cells are derived from a human colon carcinoma and undergo spontaneous differentiation in cell culture to resemble small intestinal epithelial cells. p-Glycoprotein is expressed on the apical membrane of the Caco-2 cells, and differences in bidirectional transport are used to determine whether a compound is likely to be a p-glycoprotein substrate. The permeability coefficient (Pc) of BMS-310705 in the apical to basolateral (A to B) direction in Caco-2 cells was 15 ± 1.2 nm/s at an initial concentration of 50 μ*M* at pH 7.4. The basolateral to apical (B to A) Caco-2 cell monolayer permeability was 401 ± 20 nm/s. The higher B to A permeability resulted in a high BA/AB ratio of 27. This large difference of

more than tenfold in the bidirectional transport suggests that BMS-310705 may be a substrate of the p-glycoprotein transporter.

Incubations with human liver microsomes and Supersomes

The in vitro rate of oxidation by human liver microsomes when tested at a concentration of 20 μ*M*, was found to be 0.21 nmol/min/mg protein. This corresponds to an intermediate clearance when compared to hepatic blood flow. Upon incubation of BMS-310705 in human liver microsomes along with compounds specific for the inhibition of individual cytochrome P450s, significant inhibition (>20%) was found only with the CYP3A4 inhibitors (Table 1). Upon incubation of BMS-310705 with human CYPs obtained from insect cells that expressed a single CYP enzyme, the enzymes that metabolized BMS-310705 were mainly CYP3A4 and to a lesser extent CYP1A2 and CYP2C9 (Table 2). Based on the strong inhibition of metabolism with specific CYP3A4 inhibitors in microsomes, and the metabolism rates with expressed enzymes, BMS-310705 appears to be a substrate for CYP3A4.

Plasma protein binding

The plasma protein binding was determined by ultrafiltration and was found to be 66%, 62%, 77%, and 69% in mouse, rat, dog, and human plasma, respectively. The nonspecific binding was found to be 25%. Due to the presence of nonspecific binding, the extent of protein binding was likely an overestimate of the true binding. BMS-310705 thus appears to show only low to moderate binding to plasma proteins.

Pharmacokinetics and oral bioavailability in mice

Pharmacokinetic parameters of BMS-310705 in mice obtained after single IV and oral doses of 5 and 15 mg/kg, respectively, are summarized in Table 3 and the plasma concentration-time profiles are presented in Fig. 2. The systemic plasma clearance of BMS-310705 in nude mice was rapid (152 ml/min/kg) and greater than the hepatic blood flow of 90 ml/min/kg in mice [5]. The steady-state volume of distribution was high (38 l/kg), greater than total body water [5], indicating significant extravascular distribution. The estimated half-life was 3.3 h and the mean residence time was 4.2 h. The oral bioavailability of BMS-310705 in mice was 21%.

Table 1 Inhibition of BMS-310705 metabolism in human liver microsomes by specific inhibitors of various CYPs

P450 inhibited	Inhibitor concentration (μ*M*)	Inhibitor	Inhibition (%)
1A2	20	Furafylline	12
2C9	20	Sulfaphenazole	<1
2C19	30	Tranylcypromine	7
2D6	15	Quinidine	<1
3A4	10	Ketoconazole	∽100
3A4	10	Troleandomycin	84

Table 2 Metabolism of BMS-310705 by human CYPs obtained from insect cells that express a single CYP enzyme (Supersomes) (*NS* not significant)

CYP examined	Rate of metabolism (pmol/min/pmol CYP)	Intrinsic clearance (ml/min/kg)[a]	Clearance (ml/min/kg)[b]
1A2	0.2	1.1	1.0
2C9	0.2	0.6	0.5
2C19	<0.001	NS	NS
2D6	<0.001	NS	NS
3A4	0.35	4.3	3.5

[a]Intrinsic clearance = (rate of metabolism/concentration)×(pmol CYP/g liver)×(g liver/kg body weight); where concentration = 10 μ*M*, pmol CYP/g liver = 2516 (1A1), 2632 (2C9), 52.2 (2C19), 387 (2D6), 5805 (3A4), and g liver/kg body weight = 21.
[b]Clearance = (Q × intrinsic clearance)/(Q + intrinsic clearance), where Q is the hepatic blood flow of 20.7 ml/min/kg.

Pharmacokinetics and oral bioavailability in rats

Pharmacokinetic parameters of BMS-310705 in rats obtained after single IA or oral doses are summarized in Table 4 and the plasma concentration time profiles are presented in Fig. 3. The systemic plasma clearance of BMS-310705 with the buffered formulation was rapid at 38.5 ± 15.3 ml/min/kg, representing about 70% of the hepatic blood flow of 55 ml/min/kg in rats [5]. The steady-state volume of distribution was high (54 ± 19 l/kg), greater than total body water [5], indicating significant extravascular distribution. Differences in exposure of BMS-310705 formulated in different dosing vehicles were seen after IA administration. The presence of Cremophor resulted in a fourfold increase in the AUC compared to the buffered formulation. The clearance and volume of distribution of BMS-310705 also showed a corresponding decrease of 4.8-fold and 2.7-fold,

Table 3 Pharmacokinetic parameters of BMS-310705 in mice (calculated using composite data)

Parameter[a]	IV	Oral
Dose (mg/kg)	5	15
C_{max} (n*M*)	–	345
T_{max} (h)	–	0.17
AUC_{tot} (n*M*h)	1047	656
$T_{1/2}$ (h)	3.3	3.3
MRT (h)	4.2	4.4
Clearance (ml/min/kg)	152	–
Vss (l/kg)	38	–
Bioavailability (%)	–	21

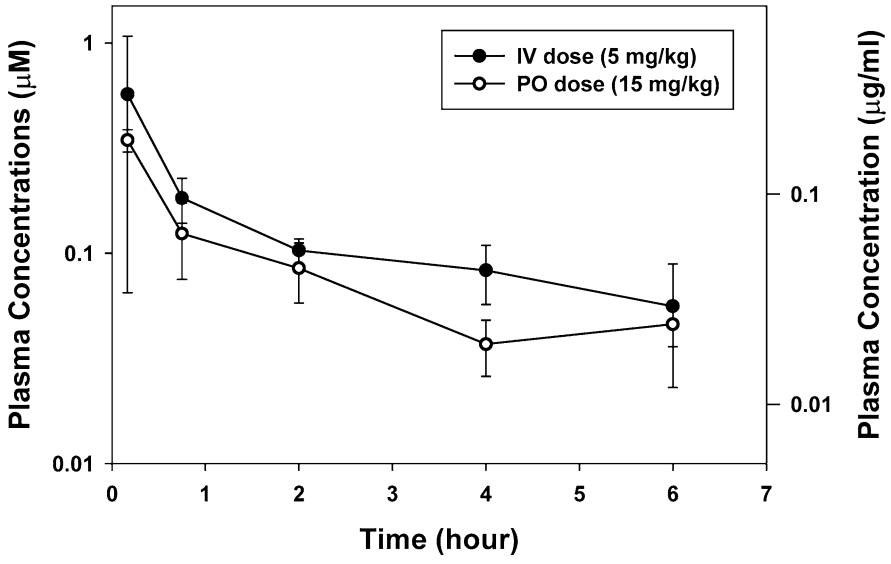

Fig. 2 Mean (±SD) plasma concentrations of BMS-310705 following IV (5 mg/kg) and oral (15 mg/kg) administration in mice

respectively, in the presence of Cremophor. Oral bioavailability in this study was 32% using the buffered formulation as the IA reference.

Pharmacokinetics and oral bioavailability in dogs

Pharmacokinetic parameters of BMS-310705 in dogs obtained after single IA or oral doses are summarized in Table 5 and the plasma concentration time profiles are presented in Fig. 4. The systemic plasma clearance of BMS-310705 in dogs was rapid (25.7 ml/min/kg), and represented about 83% of the hepatic blood flow of 31 ml/min/kg [5]. The steady-state volume of distribution was high (4.7 l/kg) and greater than total body water [5], indicating significant extravascular distribution. The estimated half-life was 3.9 h and the mean residence time was 3.3 h. The oral bioavailability of BMS-310705 in dogs was 40%.

Table 4 Pharmacokinetic parameters of BMS-310705 in rats. The data are presented as means ± SD

Parameter	IA vehicle 1[a] ($n=3$)	IA vehicle 2[a] ($n=3$)	Oral[a] ($n=3$)
Dose (mg/kg)	2	2	8
C_{max} (nM)	–	–	846 ± 275
T_{max} (h)	–	–	0.4 ± 0.2
AUC_{tot} (nMh)	8448 ± 2215	1900 ± 925	2407 ± 1279
$T_{1/2}$ (h)	32.4 ± 5.7	18.9 ± 7.4	21.4 ± 17.4
MRT (h)	44.2 ± 8.7	25.6 ± 10.0	24.0 ± 19.1
Clearance (ml/min/kg)	7.9 ± 1.8	38.5 ± 15.3	–
Vss (l/kg)	20.3 ± 2.7	54.1 ± 19.1	–
Bioavailability (%)[b]	–	–	31.7

[a]Vehicle 1 was Cremophor/ethanol/water (1:1:8 v/v); vehicle 2 was ethanol/phosphate buffer, 0.05 M, pH 7.4 (6:94 v/v); oral was ethanol/phosphate buffer, 0.3 M, pH 8 (18:82 v/v).
[b]The IA reference for bioavailability was vehicle 2

Prediction of clearance values in humans

Allometric scaling by body weight was used to predict systemic clearance in humans (Fig. 5). The linear regression of clearance versus body weight using data from mouse, rat, and dog, showed very good correlation, with a coefficient of correlation (R^2) of 0.98. The equation obtained after regression was $Cl = 42.3 \times (\text{body weight})^{0.73}$, giving a predicted systemic clearance in humans of 13.7 ml/min/kg (about 66% of the hepatic blood flow). Since the value of the allometric exponent fell between 0.7 and 0.8, no further correction factor was needed. Projected clearance values in humans were also calculated using data from human liver microsomes and Supersomes. The systemic clearance was predicted to be 6.7 ml/min/kg (about 32% of the hepatic blood flow) using human liver microsomal stability data. Studies in Supersomes showed that three CYPs were involved in the metabolism of BMS-310705. Addition of clearance values from the individual CYPs (Table 2) gave a predicted total clearance of 5.1 ml/min/kg which is similar to the value obtained from human liver microsomal studies.

Discussion

BMS-310705 was cleared rapidly in mice (152 ml/min/kg), rats (38.5 ± 15.3 ml/min/kg) and dogs (25.7 ml/min/kg). The volume of distribution in mice, rats, and dogs was 38, 54.1, and 4.7 l/kg, respectively (greater than total body water), signifying extensive extravascular distribution. The plasma protein binding was moderate (about 70% in all the species tested). The elimination half-life of BMS-310705 was similar in mice and dogs (3.3 h and 3.9 h, respectively) and longer in rats (18.9 ± 7.4 h). The systemic clearance in humans may be intermediate to high, ranging from 6.7 to 13.7 ml/min/kg, based on in vitro human liver microsomal stability data, and allometric scaling using clearance parameters

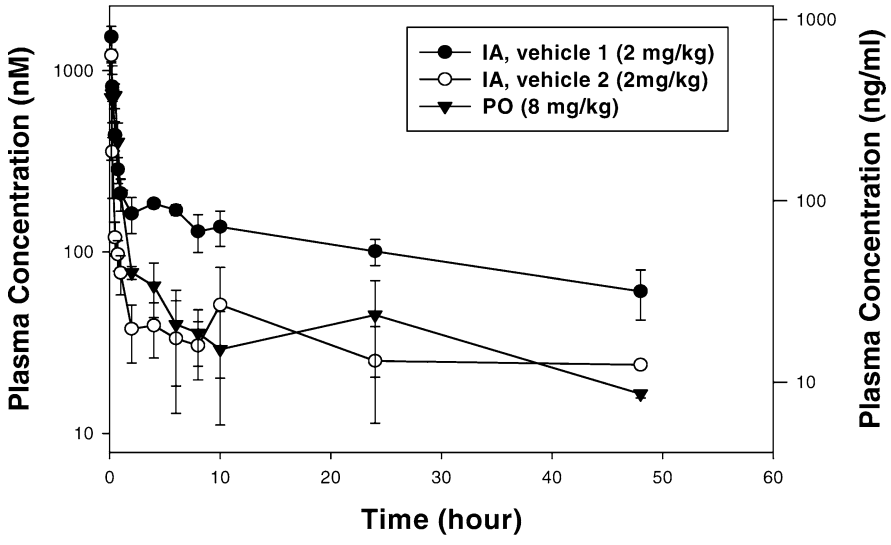

Fig. 3 Plasma concentrations (mean ± SD) of BMS-310705 following IA (2 mg/kg) administration using two different vehicles, and oral (8 mg/kg) administration in rats (*vehicle 1* Cremophor/ethanol/water, 1:1:8 v/v; *vehicle 2* ethanol/phosphate buffer, 0.05 *M*, pH 7.4, 6:94 v/v)

Table 5 Pharmacokinetic parameters of BMS-310705 in dogs. The data are presented as means ± SD

Parameter	IV ($n=2$)[a]	Oral ($n=3$)
Dose (mg/kg)	0.25	1
C_{max} (n*M*)	–	504 ± 120
T_{max} (h)	–	0.6 ± 0.1
AUC_{tot} (n*M*h)	320	523 ± 76.5
$T_{1/2}$ (h)	3.9	3.1 ± 0.8
MRT (h)	3.3	2.4 ± 0.3
Clearance (ml/min/kg)	25.7	–
Vss (l/kg)	4.7	–
Bioavailability (%)	–	40 (29, 51)[b]

[a]IV data were available for only two dogs.
[b]Individual bioavailability values for two dogs

obtained from three species, respectively. Both in vitro methods predicted very similar clearance values which were also reasonably close to that predicted by allometry. BMS-310705 was found to be a strong p-glycoprotein substrate, which could potentially decrease its intestinal absorption and hence its oral bioavailability. Despite this, the oral bioavailability of BMS-310705 in pH-buffered vehicles was found to be reasonably good at 21% in mice, 32% in rats, and 40% in dogs.

In rats, the presence of Cremophor in the IA formulation resulted in a significant increase in the AUC compared to the formulations not containing Cremophor. Studies, both in vitro and in vivo, have shown that Cremophor EL can affect the pharmacokinetics of many compounds [6, 14, 16, 19–21]. Several mechanisms have been proposed to explain these effects of Cremophor including inhibition of p-glycoprotein or other efflux transporters, inhibition of CYP3A4, and alteration of membrane fluidity. Sparreboom et al. [19] have suggested that the higher paclitaxel plasma levels observed when paclitaxel is coadministered with Cremophor are associated with encapsulation of drug within Cremophor micelles leading to changes in cellular partitioning and hence in the blood/plasma concentration ratios of paclitaxel. BMS-310705 appears to be a substrate of p-glycoprotein, and inhibition of p-glycoprotein-mediated

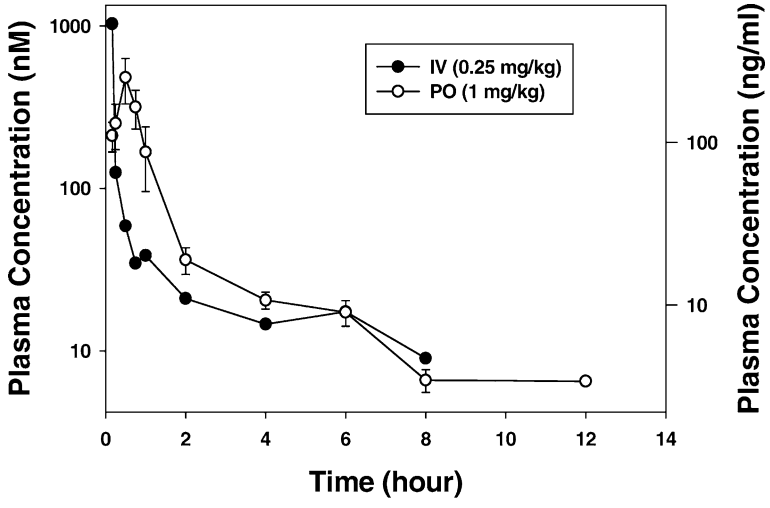

Fig. 4 Plasma concentrations (means ± SD) of BMS-310705 following IV (0.25 mg/kg) and oral (1 mg/kg) administration in dogs

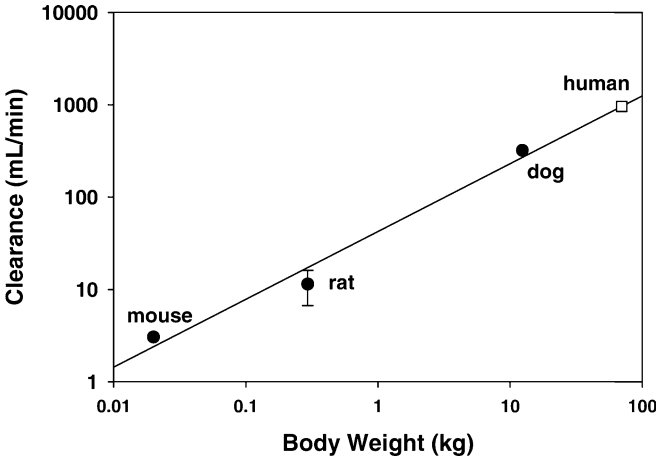

Fig. 5 Allometric scaling of clearance values

elimination pathways by Cremophor may be one of the causes of increased systemic exposure to BMS-310705 in the presence of Cremophor. In addition, inhibition of CYP3A4 by Cremophor decreasing the metabolism of BMS-310705 may also play a role in the increased exposure observed. Since pH-buffered formulations not containing Cremophor provided good exposure and bioavailability, Cremophor was not used in further development studies with BMS-310705 and the buffered vehicle was selected for further toxicological and clinical testing.

In summary, the preclinical pharmacokinetics of BMS-310705 were found to be acceptable for compound advancement and BMS-310705 is currently in phase I clinical trials [13, 18]. The presence of Cremophor in the formulation could affect BMS-310705 pharmacokinetics as evidenced from studies in rats, and should be avoided. Oral bioavailability using formulations not containing Cremophor were found to be adequate, suggesting potential for development of BMS-310705 as an oral anticancer drug.

Acknowledgements The assistance of the Technical Support Unit in animal maintenance, dosing, and sample collection, and Xiaohui Jiang in LC/MS/MS analysis is greatly appreciated.

References

1. Abraham J, Agrawal M, Bakke S, Rutt A, Edgerly M, Balis FM, Widemann B, Davis L, Damle B, Sonnichsen D, Lebwohl D, Bates S, Kotz H, Fojo T (2003) Phase I trial and pharmacokinetic study of BMS-247550, an epothilone B analog, administered intravenously on a daily schedule for five days. J Clin Oncol 21:1866–1873
2. Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, Goetz M, Lazarides E, Woods CM (1995) Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. Cancer Res 55:2325–2333
3. Borzilleri R, Zheng X, Schimdt RJ, Johnson JA, Kim SH, DiMarco JD, Fairchild CR, Gougoutas JZ, Lee F, Long B, Vite G (2000) A novel application of a Pd(O)-catalyzed nucleophilic substitution reaction to the regio- and stereoselective synthesis of lactam analogues of the epothilone natural products. J Am Chem Soc 122:8890–8897
4. Boxenbaum H (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J Pharmacokinet Biopharm 10:201–227
5. Davies B, Morris T (1993) Physiological parameters in laboratory animals and humans. Pharm Res 10:1093–1095
6. Gelderblom H, Verweij J, Nooter K, Sparreboom A (2001) Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. Eur J Cancer 37:1590–1598
7. Gerth K, Bedorf N, Hofle G, Irschik H, Reichenbach H (1996) Epothilones A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (Myxobacteria). Production, physico-chemical and biological properties. J Antibiot (Tokyo) 49:560–563
8. Gibaldi M, Perrier D (eds) (1982) Pharmacokinetics, 2nd edn. Dekker, New York
9. Johnson J, Kim SH, Bifano M, DiMarco J, Fairchild C, Gougoutas J, Lee F, Long B, Tokarski J, Vite G (2000) Synthesis, structure proof, and biological activity of epothilone cyclopropanes. Org Lett 2:1537–1540
10. Kowalski RJ, Giannakakou P, Hamel E (1997) Activities of the microtubule-stabilizing agents epothilones A and B with purified tubulin and in cells resistant to paclitaxel (Taxol). J Biol Chem 272:2534–2541
11. Lee FY, Borzilleri R, Fairchild CR, Kim SH, Long BH, Reventos-Suarez C, Vite GD, Rose WC, Kramer RA (2001) BMS-247550: a novel epothilone analog with a mode of action similar to paclitaxel but possessing superior antitumor efficacy. Clin Cancer Res 7:1429–1437
12. Lee FY, Vite G, Hofle G, Kim S, Clark J, Fager K, Kennedy K, Smykla R, Wen M, Leavitt K, Johnston K, Peterson R, Kamath A, Franchini M, Schulze G, Fairchild C, Raghavan K, Long B, Kramer R (2002) The discovery of BMS-310705: a water-soluble and chemically stable semi-synthetic epothilone possessing potent parenteral and oral antitumor activity against models of taxane-sensitive and -resistant human tumors in vivo. Proc Am Assoc Cancer Res 43:a3928
13. Mekhail T, Chung C, Holden S, Bukowski RM, Eckhardt SG, Cunningham M, Messina M, Cohen M, Peck R, Sikic B (2003) Abstract 515. Proc Am Soc Clin Oncol 22:129
14. Nerurkar MM, Burton PS, Borchardt RT (1996) The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm Res 13:528–534
15. Newton D, Wang R, Lu A (1995) Cytochrome P450 inhibitors: evaluation of specificities in the in vitro metabolism of therapeutic agents by human liver microsomes. Drug Metab Dispos 23:154–158
16. Rege BD, Kao JP, Polli JE (2002) Effects of nonionic surfactants on membrane transporters in Caco-2 cell monolayers. Eur J Pharm Sci 16:237–246
17. Regueiro-Ren A, Borzilleri RM, Zheng X, Kim SH, Johnson JA, Fairchild CR, Lee FY, Long BH, Vite GD (2001) Synthesis and biological activity of novel epothilone aziridines. Org Lett 3:2693–2696
18. Sessa C, Perotti A, Malossi A, Capri G, Cresta S, De Braud F, Dall'o' E, Voi M, Marsoni S, Gianni L (2003) Phase I and pharmacokinetic study of the novel epothilone BMS-310705 in patients with advanced solid cancer (abstract 519). Proc Am Soc Clin Oncol 22:130
19. Sparreboom A, van Zuylen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, Pillay M, Nooter K, Stoter G, Verweij J (1999) Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res 59:1454–1457

20. Tayrouz Y, Ding R, Burhenne J, Riedel KD, Weiss J, Hoppe-Tichy T, Haefeli WE, Mikus G (2003) Pharmacokinetic and pharmaceutic interaction between digoxin and Cremophor RH40. Clin Pharmacol Ther 73:397–405

21. Wandel C, Kim RB, Stein CM (2003) ''Inactive'' excipients such as Cremophor can affect in vivo drug disposition. Clin Pharmacol Ther 73:394–396