Estimation of Oral Bioavailability of a Long Half-Life Drug in Healthy Subjects[1]

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Received April 13, 1998; accepted August 5, 1998

Purpose. To estimate and compare the oral bioavailability of a drug (BMS-187745) administered as single doses of oral solution of either the parent drug or its prodrug (BMS-188494).

Methods. A single-dose, two-period, three-treatment, control-balanced, residual-effect, incomplete block crossover study was completed in 16 healthy male subjects. All subjects received a 10 mg IV infusion of BMS-187745, and a single oral dose of either BMS-187745 (PO1) or BMS-188494 (PO2). A model is proposed to calculate the oral bioavailability of BMS-187745 which has a long half-life; incomplete data points were available to characterize its elimination phase. The plasma concentration-time data obtained following IV infusion of parent drug, and after administration of either PO1 or PO2 treatment were fitted simultaneously with systemic pharmacokinetic parameters shared by both the oral and IV routes of administration.

Results. The best simultaneous fittings of the plasma concentration-time data were obtained by using a biexponential pharmacokinetic model with a first-order absorption rate constant. The mean bioavailability (F) values of BMS-187745 estimated by the proposed model were 26.5% and 2.6% when given as oral solution of its prodrug and as the parent drug. The coefficient of variation (CV) of these F values are reasonable, ranging from 38–40%. In contrast, F calculated by the model-independent AUC method exhibited high CV, ranging from 111–120%.

Conclusions. The oral bioavailability values estimated by the proposed model were more reasonable compared to those calculated by the model-independent AUC method. The proposed approach may be useful for estimating bioavailability of long half-life drugs when incomplete data points are available to characterize their elimination phase.

KEY WORDS: bioavailability; pharmacokinetics; squalene synthase inhibitor; prodrug.

[1] Supported in part by a grant from Bristol-Myers Squibb Pharmaceutical Research Institute Princeton, New Jersey.

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ABBREVIATIONS: A_i, intercept coefficients; AUC, total area under the plasma concentration-time curve; AUMC, total area under the first moment curve; C_0, initial plasma drug concentration at time of dosing; C_{max}, maximum observed plasma concentration; CL, elimination clearance; CL_D, distribution clearance; D, oral dose of drug; F, bioavailability; k_a, first-order absorption rate constant; MRT, mean residence time; PO1, treatment = oral administration of 50 mg BMS-187745; PO2, treatment = oral administration of 80 mg BMS-188494; Q, zero-order infusion rate; T, duration of drug infusion; $t_{1/2}$, terminal phase disposition half-life; t_{max}, time of occurrence of C_{max}; V_c, central volume of distribution; V_{ss}, volume of distribution at steady-state; λ_i, exponential coefficients describing the distribution and elimination slopes.

INTRODUCTION

Bioavailability of a drug is defined as the fraction of extravascularly administered dose that reaches the systemic circulation. The area under the plasma drug concentration versus time curve (AUC) reflects the total amount of drug reaching the systemic circulation. Thus, for the drugs with linear pharmacokinetics, oral bioavailability (F) can be determined by comparing the dose normalized AUCs after oral and IV administration. The AUC up to the last concentration-time data point (C*, t*) can be determined by model-independent approaches such as the trapezoidal method, and AUC beyond the last sampling data point can be estimated as: C^*/λ_z where λ_z is the terminal slope. However, if incomplete data points were available to characterize λ_z, the use of model-independent methods may result in large variability in AUC values, which in turn make the F value difficult to estimate. In this report, a model is proposed to estimate the oral bioavailability in such cases. A drug (BMS-187745) that has a long half-life and had incomplete data point available to characterize its elimination phase was used as an example.

BMS-187745, a squalene synthase inhibitor, blocks a step exclusive to the cholesterol biosynthesis. Unlike inhibitors of HMG CoA reductase, BMS-187745 is expected to inhibit cholesterol biosynthesis without deleterious effects on the synthesis of other isoprenoids (1–4). The substrate for squalene synthase (farnesyl pyrophosphate) is readily metabolized by oxidation to a series of dicarboxylic acids which are freely excreted in urine (5). Therefore, BMS-187745 may be a potentially useful agent for safe and specific inhibition of cholesterol biosynthesis. Preclinical studies have demonstrated poor oral absorption of BMS-187745 which contributed to high variability in the oral bioavailability in animals. A bis-ester prodrug of the phosphate group of the parent drug was synthesized to improve the absorption. Both parent drug (BMS-187745) and its prodrug (BMS-188494) are S-enantiomers (Fig. 1). In preclinical studies, BMS-188494 produces inhibition of cholesterol synthesis in rats, and preferentially lowers plasma VLDL and LDL cholesterol in both hamsters and marmosets. The present study was designed to estimate and compare the oral bioavailability of BMS-187745 in healthy male subjects after a single oral administration of either parent drug or its prodrug.

EXPERIMENTAL METHODS

Subjects

Sixteen healthy male subjects who were normal by medical history, physical examination, electrocardiogram (ECG), and clinical laboratory screening criteria were enrolled in this study. The subjects had a mean age of 27 years (range, 19 to 33 years), a mean body weight of 73 kg (range, 50.3 to 94.5 kg), and a mean height of 176.3 cm (range, 162 to 189 cm). Females were not included in this study because fetotoxicity studies were not completed at that time. The subjects were assigned numbers 1 through 16 sequentially upon enrollment. Each subject was randomly assigned to one of the following treatment groups: PO1 = 10 mg IV infusion and 50 mg oral dose of parent drug (BMS-187745) (n = 8) or PO2 = 10 mg IV infusion of parent drug and 80 mg oral dose of its prodrug (BMS-188494) (n =

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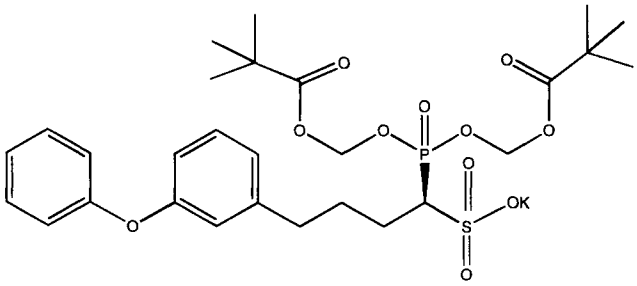
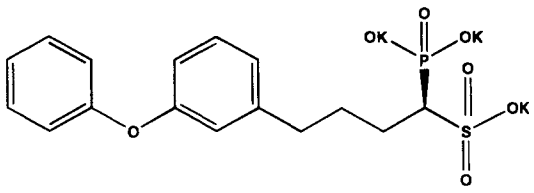


BMS-188494



BMS-187745

Fig. 1. Structures of prodrug (BMS-188494) and parent drug (BMS-187745).

8). The 80 mg dose of prodrug is molar equivalent to 50 mg dose of parent drug. In each treatment group, subjects were further randomized to receive either IV infusion followed by oral dose or vice versa. The 10 mg IV infusion of BMS-187745 was given over 30 min. The treatment periods were separated by a three week washout time. The subjects were not permitted to smoke, and did not take any medication, other than the study drug for the study duration. All subjects provided written informed consent.

Procedures

This was a single-dose, two-period, three-treatment, control balanced, residual effect, incomplete block crossover study. After an overnight (10 h) fast, blood and urine samples were obtained for clinical lab tests, and subjects received their dose of medication on the morning of Day 1. Subjects were fasted for 4 h following the dose. Blood samples (10 ml) were collected immediately before the IV infusion of drug and then at 0.08, 0.25, 0.5, 0.67, 0.83, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, 192, 264, 312, and 360 hours after the start of the infusion. Similarly, blood samples (10 ml) were also collected immediately before the oral dose and then at 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, 192, 264, 312, and 360 hours after oral dose.

This study was conducted and informed consent was obtained according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practice and the applicable laws and regulations of the country where the study was conducted.

Analytical Method

Plasma concentration of BMS-187745 was determined by a validated high performance liquid chromatographic/mass spectrometry (LC/MS) method. The internal standard, BMS-182549, was added to the plasma samples. BMS-187745 and internal standard were extracted from the samples by a protein precipitation procedure with use of methanol. After centrifuging, the supernatant was removed to a clean tube and further cleaned by adding toluene and shaking. After centrifuging, the methanol layer was removed and evaporated to dryness. The reconstituted extract was injected into a YMC Basic column with a HP 1090L HPLC system interfaced to a Hewlett-Packard 5989B mass spectrometer with an electrospray probe. The ions monitored were the $[M\text{-}H2)\text{-}H]^-$ anion at m/z 367 for BMS-187745, and the $[M\text{-}H\}^-$ anion and m/z 411 for BMS-182549. A gradient mobile phase system, consisting of water, methanol and ammonium acetate, was used. A 0.5 mL aliquot of each plasma sample was used for analysis. Analytical and shipping quality control samples were analyzed with the study samples.

Pharmacokinetics

BMS-187745 plasma concentration (C_p) versus time data were analyzed by both model-independent and model-dependent methods. The pharmacokinetic parameters were calculated by the former method using PKMENU, a validated proprietary main frame software program of Bristol-Myers Squibb. In PKMENU program, the area under the plasma concentration-time curve (AUC) was estimated by trapezoidal rule. The absolute bioavailability was calculated as:

$$F = \frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} \quad (1)$$

For model-dependent method, the plasma drug concentration-time profile during and after the IV infusion was described as:

$$Cp = \sum_{i=1}^{n} \frac{Ai_e^{-\lambda i.t}}{\lambda i} \{Q(e^{\lambda i.T} - 1)\} \quad (2)$$

where A_i and λ_i are intercept and slope parameters, t is time after starting the infusion, and T is the infusion period. Q is the zero-order infusion rate. During the infusion, i.e. when t = T, equation 1 simplifies to:

$$Cp = \sum_{i=1}^{n} \frac{Ai_e^{-\lambda i.t}}{\lambda i} Q(e_{\lambda i.t} - 1) \quad (3)$$

Equation 1 was applied for concentration-time profiles following the infusion. The plasma BMS-187745 concentration-time profile after oral doses of either parent drug or its prodrug (BMS-188494) was described as:

$$Cp = ka.F.D.\{\sum_{i=1}^{n} \frac{Ai}{ka - \lambda i} (e^{-\lambda i.(t-tlag)} - e^{-ka.(t-tlag)})\} \quad (4)$$

where k_a and F are the apparent first order absorption rate constant and bioavailability, D is the dose. A_i and λ_i are intercept and slope parameters as defined previously, and t_{lag} is the lag time. Residual drug (C_R), if present from the first phase of a crossover treatment, was added to these equations as:

$$C_R = \mathrm{Co}.e^{-\lambda z.t} \quad (5)$$

where C_0 is the measured concentration at the time of dosing and λ_z is the terminal slope.

The plasma BMS-187745 concentration-time data for each subject were fitted simultaneously with systemic pharmacokinetic parameters such as A_i and λ_i shared by both oral and IV routes of administration. The optimum fitting was obtained with a biexponential function (n = 2) using a non-linear regression program PCNONLIN (SCI Software Inc., Lexington, KY) to generate least-squares values of the intercept coefficients A_i (A_1, A_2,) and slopes λ_i (λ_1, λ_2). The generated values were used to calculate other pharmacokinetic parameters including clearance (CL), steady-state volume of distribution (V_{ss}), mean residence time (MRT), area under the concentration-time curve (AUC), area under the first moment curve (AUMC), and terminal half-life ($t_{1/2} = 0.693/\lambda_2$).

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed to compare k_a, F, CL, and V_{ss} values between treatment groups.

RESULTS

The two treatment groups included 8 male subjects each. The plasma concentration-time data for BMS-187745 were available over a 360 hr period after 10 mg IV infusion of parent drug, and a single oral dose of either 50 mg of parent drug (PO1) or 80 mg of its prodrug (PO2). The 80 mg dose of prodrug is molar equivalent to 50 mg dose of parent drug. One subject (#0012) had incomplete data following the infusion phase of the drug.

The observed mean values of concentration (C_0) at the time of dosing, maximum concentration (C_{max}) after dosing, and time (t_{max}) of occurrence of C_{max} were determined. The C_0 values reflect residual drug from the previous dose and averaged 500 ng/ml. Following oral dosing C_{max} was greater (2757 ng/ml) for PO2 than for PO1 (701 ng/ml) The mean t_{max} values were 6.3 and 4.9 hr for the PO1 and PO2 treatments. The difference in t_{max} values between treatment groups was not statistically significant (p = 0.23). However, the t_{max} values were more consistent for PO2 (range 3 to 8 hr) than for PO1 (range 2.5 to 12 hr). Examination of the plasma drug concentration-time data shows that significantly higher concentrations were achieved following the oral dose of the prodrug than the parent drug.

Model-Independent Method

The mean pharmacokinetic parameters of BMS-187745 estimated by the model-independent method are listed in Table 1. The mean AUC values of BMS-187745 were 3652, 377 and 1665 μg.hr/ml for the IV, PO1 and PO2 treatments. The coefficients of variation (CV) of these AUC values especially for IV treatment are very high, ranging from 64 to 250%. Although, the percent mean bioavailability (F) value for the PO2 treatment group (34%) was significantly higher than that for the PO1 treatment group (7%), the CV of these values are also very high, ranging from 111 to 120%. Thus, in this case, it was difficult to estimate the pharmacokinetic parameters by noncompartmental method. The high variability is mainly due to incomplete data available for characterization of elimination phase.

Table 1. Pharmacokinetic Parameters Estimated by the Model-Independent Method

	Treatment	Dose (mg)	AUC (μg.hr/ml)	$t_{1/2}$ (hr)	F (%)
Mean	IV	10	3652	2179	—
%CV			250	284	—
Mean	PO1	50[a]	377	389	7.30
%CV			83.2	44.6	120
Mean	PO2	80[a]	1665	557	34.3
%CV			64.3	43.5	111

[a] The 80 mg dose of prodrug is molar equivalent to 50 mg dose of parent drug.

Model-Dependent Method

Pharmacokinetic parameters including bioavailability were also calculated from the simultaneous biexponential fittings of the plasma BMS-187745 concentration-time data obtained from each subject following IV infusion and oral doses of either parent (PO1) or prodrug (PO2). The simultaneous fittings of plasma concentrations as a function of time following IV infusion and either treatment PO1 or PO2 are shown in Figure 2 for a typical subject from each treatment group. The oral and IV curves generally showed a parallel decline which facilitated the joint fitting of each subject's data.

The intercept coefficients A_1 and A_2 were consistent for both treatment groups (PO1 and PO2) (Table 2). The slope (λ_1 and λ_2) and half-life values were also consistent for both

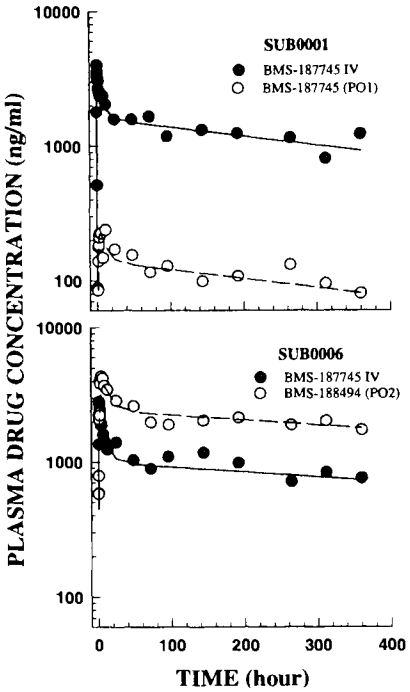


Fig. 2. Plasma BMS-187745 concentration versus time for a typical subject from each treatment group. Symbols are experimental data and lines represent the results of simultaneous least-squares regression fitting of the iv infusion and oral data to biexponential functions with first-order absorption rate constant.

Table 2. Pharmacokinetic Parameters for BMS-187745 Obtained from Biexponential Fitting of Plasma Data

Subject	Treatment sequence	Dose (mg)	A_1 (ml^{-1})	A_2 (ml^{-1}))	λ_1 (hr^{-1})	λ_2 (hr^{-1})	k_a (hr^{-1})	F (%)	t_{lag} (hr)
0001	PO1, IV	50, 10	166.8	153.9	0.139	0.00158	0.62	1.8	0.44
0002	IV, PO1	10, 50	160.2	90.2	0.184	0.00066	0.24	2.4	0.00
0005	IV, PO1	10, 50	163.7	105.2	0.340	0.00119	0.33	3.0	0.26
0007	PO1, IV	50, 10	115.8	104.5	0.316	0.00059	0.47	4.3	0.34
0009	IV, PO1	10, 50	135.3	142.8	0.202	0.00122	1.21	2.6	0.07
0012	PO1, IV	50, 10	257.1	91.4	0.112	0.00001[a]	0.41	2.2	0.00
0013	IV, PO1	10, 50	100.2	102.4	0.050	0.00116	0.89	3.5	0.09
0014	PO1, IV	50, 10	235.7	173.5	0.359	0.00056	0.76	0.9	0.36
0003	PO2, IV	80, 10	178.3	117.4	0.217	0.00093	0.37	17.7	0.45
0004	IV, PO2	10, 80	165.4	91.3	0.143	0.00073	0.12	31.8	0.51
0006	IV, PO2	10, 80	169.5	100.3	0.128	0.00088	0.29	37.2	0.09
0008	PO2, IV	80, 10	119.3	92.9	0.316	0.00152	0.48	39.4	0.34
0010	IV, PO2	10, 80	157.5	94.6	0.112	0.00034	0.29	31.2	0.29
0011	PO2, IV	80, 10	121.7	116.5	0.212	0.00098	0.54	16.1	0.44
0015	IV, PO2	10, 80	149.7	152.7	0.110	0.00133	0.18	26.1	0.39
0016	PO2, IV	80, 10	160.3	142.1	0.526	0.00124	0.49	12.7	0.39
Mean	IV/PO1		166.9	120.5	0.213	0.00099	0.62	2.6	0.19
%CV			32.8	26.3	53.7	39.5	52.6	40.1	89.8
Mean	IV/PO2		152.7	113.5	0.221	0.00099	0.34	26.5	0.36
%CV			14.1	20.6	64.4	37.3	44.6	37.9	35.7

[a] Value omitted from mean values.

treatment groups except for subject #0012, for whom λ_2 was significantly smaller (0.00001 hr^{-1}) and, therefore, half-life was larger (4012 days). For subject #0012, the elimination phase plasma concentrations were not available following IV infusion of drug which may be the reason for the significantly lower estimate of the λ_2 value.

The F values of BMS-187745 following oral dose of either parent drug or its prodrug are listed in Table 2. These values were significantly higher ($p < 0.0001$) for the PO2 treatment (F = 26.5%) group which received oral dose of prodrug than for the PO1 treatment group (F = 2.6%) which received parent drug. The CV of these F values are reasonable, ranging from 38–40%. The first-order absorption rate constant values (k_a) were significantly higher ($p = 0.05$) for the PO1 treatment group (0.62 hr^{-1}) than for the PO2 group (0.34 hr^{-1}).

The summary pharmacokinetic parameters are listed in Table 3. The AUC_{IV} values of BMS-187745 were similar ($p > 0.5$) with means of 1461 and 1321 μg.hr/ml for the PO1 and PO2 treatments. The mean clearance (CL) values were also similar ($p > 0.5$) for both PO1 and PO2 treatment groups with means of 0.0081 and 0.0088 L/hr. The CV of these CL values are reasonable, ranging from 37 to 40%. The mean residence time (MRT) remained constant with treatment and averaged 49 days. Similarly, the steady-state volume of distribution (V_{ss}) values of BMS-187745 were similar ($p = 0.5$) for both PO1 and PO2 treatment groups with means of 8.30 and 8.98 L (Table 3).

DISCUSSION

In healthy male subjects, BMS-187745 shows biexponential disposition after IV infusion of the parent drug, and first order absorption following an oral dose of either parent drug or its prodrug. There was no significant difference in the AUC_{IV}, MRT, CL, and V_{ss} of BMS-187745 between the PO1 and PO2 treatment groups (Table 3).

The disposition of BMS-187745 is biexponential with extremely long $t_{1/2}$ (820 hr) and MRT (1200 hr) values. This is

Table 3. Summary of Pharmacokinetic Parameters for BMS-187745

	Treatment	Dose (mg)	AUC_{IV} (μg.hr/ml)	CL (L/hr)	CL/BWT (ml/hr/kg)	CL_D (L/hr)	MRT (hr)	$t_{1/2}$ (hr)	Vc (L)	Vss (L)	Vss/BWT (ml/kg)
Mean	IV/PO1	10, 50	1461	0.0081	0.1085	0.449	1158	808	3.76	8.30	111.1
%CV			55	37	36	55	42	41	22	24	18
Mean	IV/PO2	10, 80	1321	0.0088	0.1222	0.463	1207	842	3.81	8.98	127.0
%CV			47	40	36	57	60	59	13	19	21
Mean	all subjects		1386	0.0085	0.1158	0.456	1184	826	3.79	8.66	119.6
%CV			49	37	35	54	51	50	17	21	20

caused by the very low clearance of the drug, viz. 0.116 ml/hr/kg compared to hepatic blood flow of 1150 ml/hr/kg. The volume of distribution of the drug is remarkably low, about 9L or 120 ml/kg suggesting that the drug is highly bound to plasma protein.

The prodrug (BMS-188494), a bis-ester of the phosphate group of the parent drug (BMS-187745), was synthesized to improve the bioavailability of the parent drug. Octanol-water partition coefficient (phosphate buffer; pH 7.0) for prodrug (log $P = 3.5$) was significantly higher than that of parent drug (log $P = -1.3$), indicating that the prodrug was significantly more lipophilic. This study shows that the prodrug has 10-fold higher bioavailability compared to the parent drug given as oral solution. The increase in bioavailability may, in part, be due to higher lipophilicity of prodrug compared to that of the parent drug.

The prolonged λ_2 phase creates a problem for assessing drug absorption kinetics and bioavailability because of the difficulty in characterizing λ_2 and waiting for washout of one drug dose before giving the cross-over formulation. A washout of 5 half-life values would require patient sampling for 172 days for this drug (BMS-187745).

These issues were largely overcome by joint fitting of the dual IV/PO profiles with inclusion of residual drug from previous dosing. Characterizing the terminal phase jointly for the IV and PO doses improves the precision of the λ_2 value and facilitates estimation of bioavailability parameters from these truncated profiles. However, there remains uncertainty in the true value of λ_2 or $t_{1/2}$ when data were collected over 360 hours for a drug with a $t_{1/2}$ which may be over 800 hours.

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