SB-239063, a Potent and Selective Inhibitor of p38 Map Kinase: Preclinical Pharmacokinetics and Species-Specific Reversible Isomerization

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Purpose. A series of studies was conducted to evaluate the preclinical pharmacokinetics of SB-239063 (*trans*-1-(4-hydroxycyclohexyl) -4-(4-fluorophenyl) -5- [(2-methoxy) pyrimidin-4-yl] imidazole), a potent and selective p38 MAP kinase inhibitor.

Methods. SB-239063 was administered both i.v. and p.o. in the rat, dog, cynomolgus monkey, and rhesus monkey, with standard pharmacokinetic parameters generated from the concentration vs. time data.

Results. Initial rat studies suggested possible nonlinear disposition; however, assay refinement revealed an *in vivo trans-cis* isomerization of SB-239063 to a metabolite with nearly identical chromatographic and mass spectral properties. SB-239063 exhibited low to moderate clearance and good bioavailability in the rat and dog, but poor bioavailability in the cynomolgus monkey. Substantial *in vivo trans-cis* isomerization occurred in the rat and cynomolgus monkey, but occurred to a far lesser extent in the dog. The isomerization reaction was reversible, with a recycled fraction of 0.20 and 0.0003 in the rat and cynomolgus monkey, respectively. In the rhesus monkey, bioavailability was also poor, but no *in vivo* isomerization was observed.

Conclusions. These studies demonstrate the necessity of exercising vigilance in conducting high-throughput analytical method development, and the importance of using a variety of preclinical species when evaluating the disposition of new drug candidates.

KEY WORDS: pharmacokinetics; SB-239063; p38 MAP kinase; isomerization; reversible metabolism.

INTRODUCTION

The mitogen-activated protein (MAP) kinases regulate a wide variety of cellular signal transduction processes. Pharmacological inhibition of MAP kinases, particularly of p38 MAP kinase, has been investigated extensively as a potential therapeutic approach to a number of human diseases, including cancer, autoimmune diseases, stroke, and chronic inflammatory conditions (1). Many compounds that inhibit p38 MAP kinase have been synthesized and their effects in both *in vitro* and *in vivo* disease models characterized; many of these compounds are members of the pyridinyl aryl imidazole class (2). Recently, a new member of this chemical series with improved potency and biological activity has been described. SB-239063 (*trans*-1-(4-hydroxycyclohexyl) -4- (4-fluorophenyl) -5- [(2-methoxy) pyrimidin-4-yl] imidazole; Fig. 1) potently inhibits p38α and p38β (IC_{50} = ~40 nM for both isoforms) and is more than 2000-fold selective against various other kinases, including p38 γ and δ, ERK, JNK1, c-Raf, EGF, and PKCα (3). SB-239063 also is active *in vivo,* affording protection against ovalbumin-induced pulmonary eosinophil influx in the mouse and guinea pig, as well as reducing neutrophilia and fibrosis in the rat and guinea pig (4). SB-239063 also reduces brain injury and neurological deficits *in vivo* in a rat model of cerebral focal ischemia (5). SB-239063 is thus a candidate for development for the treatment of various diseases including rheumatoid arthritis, pulmonary disease, and stroke.

Although the metabolism and pharmacokinetics of other imidazole-containing p38 inhibitors, including SB-203580 and SB-242235, have been described (6), no prior studies had been conducted to evaluate the disposition of SB-239063. Thus, the objective of this set of studies was to evaluate the pharmacokinetics of SB-239063 in the rat, dog, and monkey. In this work, preliminary rat studies suggested the possibility of nonlinear elimination kinetics (>100% oral bioavailability). However, experiments designed to elucidate this phenomenon in the rat revealed no convincing evidence for deviation from linearity for SB-239063. As an alternate explanation, it was proposed that SB-239063 may undergo *in vivo* transformation to produce a metabolite that confounds the analytical assay. Indeed, assay refinement revealed an *in vivo trans-cis* isomerization of SB-239063 to a metabolite of identical molecular mass with nearly identical chromatographic and mass spectral properties (SB-249547). Determination of bioavailability using the discriminating assay resulted in approximately 58% bioavailability in the rat. This refined analytical methodology was subsequently used to characterize the pharmacokinetics and reversible metabolism of SB-239063 and SB-249547 in the rat, dog, and monkey.

MATERIALS AND METHODS

Materials

SB-239063, its *cis*- isomer (SB-249547), the related ketone (SB-239061), and various additional analogs were synthesized by the Department of Medicinal Chemistry at SmithKline Beecham Pharmaceuticals (King of Prussia, PA) and were determined to be at least 99% pure (see Fig. 1 for relevant structures). All other materials were purchased from standard vendors, and were of the highest available purity.

Animals

Male outbred rats (Crl:CD(SD)IGS BR; Charles River, Raleigh, NC) weighing 270 to 380 g; male purebred beagle dogs (Marshall Research, North Rose, NY) weighing 10.3 to 12.2 kg; male cynomolgus monkeys (*Macaca fascicularis*) weighing 3.7 to 7.3 kg; and male rhesus monkeys (*Macaca*

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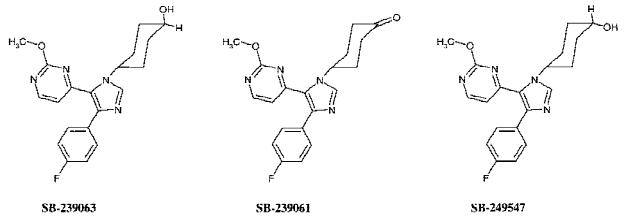

Fig. 1. Chemical structures of SB-239063 (*trans*-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl)-5-[(2-methoxy)pyrimidin-4-yl] imidazole), SB-239061 (5-[4-(2-methoxy) pyrimidinyl]-4-(4-fluorophenyl)-1-(4-oxocyclohexyl) imidazole), and SB-249547 (*cis*-1-(4-hydroxycyclohexyl)-4-(4-fluorophenyl)-5-[(2-methoxy) pyrimidin-4-yl] imidazole).

mulatta) weighing 5.4 to 6.2 kg (all monkeys from Charles River, Houston, TX) were used in these studies. All animals were housed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* in individual cages in unidirectional airflow rooms with controlled temperature (22 ± 2°C) and relative humidity (50 ± 10%) and 12-h light/dark cycles (0600–1800). Filtered tap water and a standard animal diet (Purina Mills, St. Louis, MO) was available *ad libitum*. Whenever overnight fasting was employed prior to dosing, food was provided after the 240-min blood sample was obtained on the study day. All animal use was conducted according to protocols reviewed and approved by the Institutional Animal Care and Use Committee prior to the study, and all surgical procedures were conducted using aseptic techniques in special-purpose operating suites.

In Vivo Pharmacokinetic Studies

Dosages were administered in isotonic saline (intravenous solutions), analytical-grade water (oral solutions), or 1% aqueous methylcellulose (oral suspensions). All suspensions were triturated in a glass mortar and pestle to yield a homogeneous mixture and were shaken well immediately prior to use. The concentration of test compound in each dose was verified by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS). For crossover studies, the same animals ($N = 3$ per group unless otherwise noted) received the intravenous and oral dosages at least 2 (rats) or 7 (nonrodents) days apart. A complete blood chemistry panel was performed on all dogs and monkeys prior to each study day to obtain baseline values and to ensure maintenance within the normal range during the study. Blood samples were collected from the study animals at various times during and after drug administration, as noted in the figures; plasma (50 μL) was isolated by centrifugation and stored at −70°C until analysis.

Rats

Rats received surgically implanted femoral vein catheters for drug administration 4 to 5 days prior to the experiment; unless otherwise noted, all blood sampling was via a lateral tail vein. For intravenous dosages, compound was administered at the indicated dose as a 30-min intravenous infusion (4 mL/kg). Oral dosages were delivered as a bolus gavage (16 mL/kg). To investigate pharmacokinetic linearity, additional rats received both femoral vein (for dosing) and contralateral femoral artery (for blood sampling) catheters. These dual-cannulated rats received SB-239063 either as an overnight constant-rate infusion or as an accelerated intravenous infusion (15 mg/kg total dose) as described previously (7).

Nonrodents

For dog studies, a cephalic vein catheter was temporarily placed in each dog for blood sampling; for intravenous doses, a catheter was also placed in the saphenous vein for dosing. For monkeys, on each study day, a catheter was temporarily placed in a cephalic vein (for blood sampling) and a contralateral saphenous vein (intravenous study days only, for dosing). Monkeys were fasted overnight prior to each study day. All intravenous doses were delivered as 60-min infusions (4 mL/kg) and oral doses were administered as a bolus gavage (4 mL/kg) or capsule.

Analytical Procedures

For initial quantitative analysis of SB-239063, analyte was isolated from 50 μL of sample by protein precipitation with acetonitrile. Analysis was performed by HPLC/MS/MS using a Sciex API triple quadrupole mass spectrometer with a TurboIonSpray interface. A Hypersil aminopropyl (APS-2; 2 × 50 mm, 3 μm internal diameter) column was used with a mobile phase of an 85/15 (v/v) mixture of acetonitrile/10 mM ammonium acetate (pH 4.0). The lower limit of quantitation was 10.0 ng/mL.

To explore the hypothesis of the formation of an interfering metabolite *in vivo,* the quantitative analytical method was altered. The refined analytical method employed complete chromatographic separation of SB-239063, SB-249547, and SB-239061 on a 2 × 50 mm Inertsil ODS3 column (3 u packing) under isocratic conditions (60/40, 10 mM ammonium acetate [pH 5.0]/acetonitrile) at a flow rate of 300 μL/min. Positive-ion multiple reaction monitoring was employed for the MS/MS detection of all three analytes on a Sciex API triple quadrupole mass spectrometer. The lower limit of quantitation for all analytes with the revised analytical methodology ranged from 2.0 to 10.0 ng/mL.

Pharmacokinetic Analysis

Concentration versus time profiles after intravenous and oral administration were obtained for each animal. Noncompartmental pharmacokinetic analysis was performed using WinNonlin Professional Version 2.1 (Scientific Consultants, Inc., Mountain View, CA). Because of the presence of reversible metabolism in the rat and cynomolgus monkey, additional noncompartmental analysis was conducted to calculate pharmacokinetic and reversibility parameters according to standard techniques (8). Where appropriate, reported pharmacokinetic parameters have been adjusted for the reversible metabolism, otherwise, they are reported as apparent parameter estimates. Apparent oral bioavailability was estimated for each oral dosage by dividing the dose-normalized area under the concentration vs. time curve resulting from oral administration by that resulting from intravenous administration. Individual pharmacokinetic parameters were generated for each study animal; all data were averaged and reported as mean ± standard deviation.

Statistical Analysis

An unpaired or paired Student's *t*-test was used to assess the significance of differences between various parameters, as described in the Results. One-way analysis of variance (ANOVA) with a Bonferroni post hoc comparison was used to assess the significance of differences in parameters across groups, where appropriate. In all cases, a probability level of $p < 0.05$ was predetermined as the criterion of significance.

RESULTS

Initial pharmacokinetic parameter estimates in the rat derived from an analytical method that did not discriminate between isomers suggested that SB-239063 displayed favorable properties in the rat, including low clearance (9 mL/min/kg) and apparent oral bioavailability of 152%. There was no evidence of formation of either the corresponding ketone (SB-239061), O-desmethyl SB-239063, or O-desmethyl SB-239061. The high apparent bioavailability, as well as the shape of the intravenous concentration-time profile, suggested the possibility of nonlinear elimination of SB-239063. To further evaluate this possibility, a second, fourfold lower, oral dose was given to the same rats (data not shown). Exposure following the second oral administration was dose-proportional; apparent bioavailability was not different between the two oral doses ($p = 0.92$, Student's two-tailed paired *t*-test) and remained in excess of 100%. Next, an attempt was made to define the range of pharmacokinetic linearity for SB-239063 in the rat using an accelerated infusion experiment (7) over a concentration range of 0 to 3500 ng/mL. No nonlinearities were detected over this concentration range (data not shown). To refine the data set at low concentrations where an accelerated infusion may demonstrate poor resolution, a second linearity experiment was conducted by infusing SB-239063 to three steady-state concentrations (375, 1840, and 2330 ng/mL). Apparent clearance did not differ between these steady-state conditions (10.5 ± 2.0, 8.43 ± 1.17, and 12.4 ± 1.1 mL/min/kg, respectively; $p = 0.08$, one-way ANOVA), nor did the average clearance (10.3 ± 1.9 mL/min/kg) differ from that previously measured (9.19 ± 2.85 mL/min/kg; $p = 0.45$, unpaired two-tailed Student's *t*-test), suggesting that nonlinear elimination did not account for the observed high rat bioavailability.

Next, it was proposed that SB-239063 may undergo a metabolic transformation to produce an analytically interfering metabolite *in vivo*. This hypothesis was based in part on *in vitro* experiments using [^{3}H]SB-239063 in rat, cynomolgus monkey, and human liver microsomes and slices as described elsewhere (11; full manuscript in preparation). To address this possibility, an assay was developed capable of chromatographically separating and selectively quantifying SB-239063, SB-239061 (the ketone derivative of SB-239063), and SB-249547 (the *cis*-isomer of SB-239063). Rat pharmacokinetic data for SB-239063 derived using the discriminating assay and appropriate correction for reversible metabolism are displayed in Table I, with concentration versus time profiles shown in Fig. 2. In the rat, apparent distributional volume was similar with the selective assay to that previously observed. However, the actual half-life was significantly shorter, and clearance significantly higher, for SB-239063 measured using the selective analytical method. Apparent oral bioavailability of SB-293063 in the rat was approximately 58% when dosed as either a solution or a suspension (suspension data not shown). Substantial *in vivo* formation of SB-249547 was observed in the rat following administration of SB-239063 (Table I, Fig. 2). Isomerization was more extensive following oral administration than after the intravenous dose; the area under the control (AUC) ratio of *cis*- to *trans*- isomers was approximately 1.26 and 2.04 after i.v. and oral administration, respectively.

In separate follow-up studies, rats also were dosed with SB-249547 (the *cis*-isomer) and SB-239061 (the ketone). SB-249547 demonstrated moderate clearance with a long but variable terminal half-life, and approximately 60% apparent oral bioavailability (Table II). *In vivo* isomerization of SB-249547 to SB-239063 did occur in the rat; as with the reverse reaction, the *cis*- form was favored *in vivo* (AUC ratio of *cis*- to *trans*- isomers was approximately 4.80 and 5.17 after i.v. and oral administration, respectively). The *cis*- to *trans*- conversion occurred to a somewhat greater extent after oral than

Table I. Pharmacokinetic Parameters Defining Disposition of SB-239063 and Formation of SB-249547 in the Rat, Cynomolgus Monkey, Rhesus Monkey, and Dog Using the Selective Analytical Assay

	Rat		Cynomolgus monkey		Rhesus monkey		Beagle dog	
Parameter	I.V.	P.O. solution	I.V.	P.O. solution	I.V.	P.O. suspension	I.V.	P.O. solution
Dose (mg/kg)	0.90 ± 0.01	0.95 ± 0.02	2.95 ± 0.01	3.84 ± 0.04	2.99 ± 0.01	9.63 ± 1.55	1.97 ± 0.00	4.15 ± 0.09
Cmax (ng/mL)[a]	246 ± 12	177 ± 42	4340 ± 1295	35.5 ± 6.9	2378 ± 281	57.3 ± 43.9	2900 ± 1236	3790 ± 304
AUC (min · μg/mL)[a]	28.8 ± 10.5	18.8 ± 8.0	287 ± 65	6.12 ± 0.64	177 ± 34	13.1 ± 3.0	829 ± 583	1410 ± 737
Half-life (min)[a]	74.4 ± 31.5	[b]	73.8 ± 15.9	—	170 ± 114	—	373 ± 211	—
CL (mL/min/kg)[a]	44.3 ± 4.7	—	19.2 ± 3.5	—	17.3 ± 3.1	—	3.18 ± 1.80	—
Vss (L/kg)	3.02 ± 0.57[c]	—	0.701 ± 0.395[c]	—	1.01 ± 0.28	—	1.11 ± 0.77	—
Bioavailability (%)[a]	—	57.6 ± 5.9[c]	[c]	3.02 ± 0.35[c]	—	1.97 ± 0.14	—	89.9 ± 8.9
SB-249547 Cmax	121 ± 72	205 ± 104	173 ± 21	12.2 ± 3.6	Not measurable		22.8, 51.4	44.6, 44.1
SB-249547 AUC	36.3 ± 19.1	38.4 ± 29.5	13.0 ± 3.7	1.06 ± 0.46	Not measurable		5.86, 9.23	13.0, 15.0

Data presented as mean ± SD ($N = 3$).

[a] SB-239063 parameter significantly different in the rat ($p < 0.05$, Student's unpaired two-tailed t-test) from that determined using the nonselective analytical method.

[b] Parameter not determined.

[c] Apparent parameter estimate.

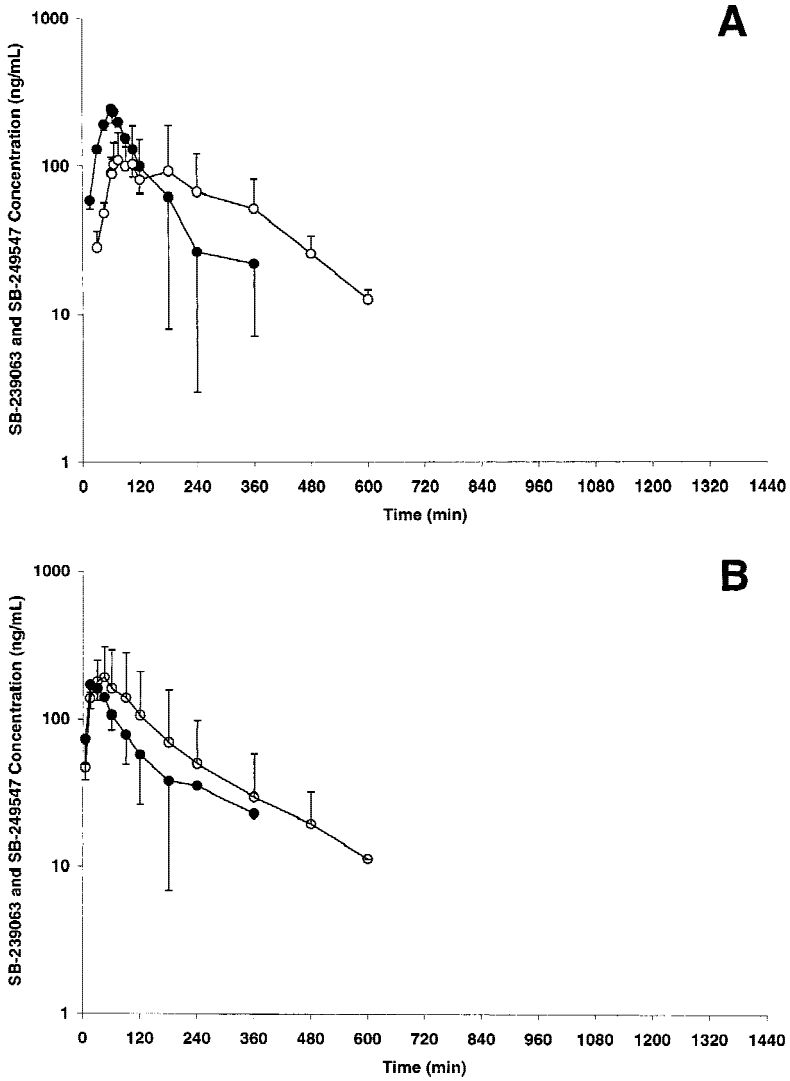

Fig. 2. Plasma concentration-time profiles for SB-239063 (closed symbols) and SB-249547 (open symbols) in the rat following administration of SB-239063 measured using the selective analytical method. Symbols represent average (± SD) measured plasma concentrations after (**A**) intravenous or (**B**) oral administration; lines are straight-line interpolation between data points. Error bars are unidirectional for clarity.

Table II. Apparent Pharmacokinetic Parameters Defining Disposition of SB-249547 and Formation of SB-239063 in the Rat and Cynomolgus Monkey Using the Selective Analytical Assay

	Rat		Cynomolgus monkey	
Parameter	I.V.	P.O. solution	I.V.	P.O. suspension
Dose (mg/kg)	0.923 ± 0.013	4.61 ± 0.13	2.99 ± 0.00	8.86 ± 0.14
Cmax (ng/mL)	348 ± 49	864 ± 400	8547 ± 6089	607 ± 88
AUC (min · μg/mL)	44.2 ± 14.2	138 ± 64	486 ± 285	68.1 ± 25.6
Half-life (min)	398 ± 307	—[a]	1337 ± 615	—
CL (mL/min/kg)	32.1 ± 6.0	—	8.10 ± 4.05	—
Vss (L/kg)[b]	9.78 ± 7.09	—	1.94 ± 0.849	—
Bioavailability (%)[b]	—	61.2 ± 10.4	—	6.01 ± 3.45
SB-239063 Cmax	38.9 ± 20.4	144 ± 80	96.1 ± 75.8	155 ± 37
SB-239063 AUC	9.18 ± 6.75	26.7 ± 10.4	9.28 ± 8.00	13.1 ± 3.5

Data are presented as mean ± SD ($N = 3$).
[a] Parameter not determined.
[b] Apparent parameter estimate.

Table III. Formation of SB-239063 and SB-249547 in the Rat and Cynomolgus Monkey following Intravenous and Oral Administration of SB-239061 Using the Selective Analytical Assay

Parameter	Rat I.V. (solution)	Rat P.O. (solution)	Rat P.O. (suspension)	Monkey I.V. (solution)	Monkey P.O. (solution)
SB-239061 dose (mg/kg)	1.7	3.6	31	1.9	8.0
SB-239061 Cmax (ng/mL)	21.5 ± 6.8	Not measurable	49.9 ± 31.7	Not measurable	Not measurable
SB-239061 AUC (min · μg/mL)	0.328 ± 0.191	Not measurable	3.54 ± 2.46	Not measurable	Not measurable
SB-239063 Cmax (ng/mL)	283 ± 61	452 ± 23	4330 ± 969	392 ± 143	403 ± 254
SB-239063 AUC (min · μg/mL)	31.6 ± 8.6	76.0 ± 19.4	734 ± 66	40.3 ± 12.4	51.7 ± 34.3
SB-249547 Cmax (ng/mL)	309 ± 178	1790 ± 37	10900 ± 2400	843 ± 292	128 ± 51
SB-249547 AUC (min · μg/mL)	75.4 ± 34.8	311 ± 108	2190 ± 703	80.3 ± 6.5	62.6 ± 37.8

Data are presented as mean ± SD ($N = 3$).

after i.v. administration; however, this route difference was not as great as that observed for the reverse isomerization. The data obtained after administration of SB-239061 are displayed in Table III. SB-239061 was rapidly and extensively converted to SB-239063 and SB-249547 after both intravenous and oral administration. As with separate administration of either of the two reduced isomers, the *cis*- isomer was favored over the *trans*- isomer (AUC ratio of *cis*- to *trans*- isomers was approximately 2.39 and 4.09 after i.v. and oral administration, respectively), and formation was more extensive after oral than iv administration. Furthermore, administration of a tenfold higher oral dosage produced dose-proportional formation of both isomers, suggesting that neither absorption nor reduction of the ketone was saturated at the higher dosage (Table III). Finally, the administration of both the parent and the metabolite allowed the calculation of standard reversibility parameters (8). Following intravenous administration, the recycled fraction of SB-239063 was 0.20 ± 16, and the exposure enhancement afforded by the metabolic recycling was 1.29 ± 0.28.

To evaluate the performance of SB-239063 in a nonhuman primate, a study was conducted in the male cynomolgus monkey, also using the discriminating analytical assay; these concentration versus time profiles are displayed in Fig. 3. As shown in Table I, SB-239063 demonstrated a half-life of 73.8 ± 15.9 min in the cynomolgus monkey, with moderate total plasma clearance and an apparent distributional volume that approximated total body water. In contrast to the rat, SB-239063 demonstrated poor apparent oral bioavailability when administered as either a solution (3%) or suspension (<3%; suspension data not shown) in the cynomolgus monkey. Also in contrast to the observations in the rat, the *trans*- to *cis*-isomerization following intravenous administration was not extensive in the cynomolgus monkey, with the AUC ratio of *cis*- to *trans*- isomers being approximately 0.05 and 0.84 after i.v. and oral administration, respectively.

As with the rat, cynomolgus monkeys also were dosed with SB-249547 and SB-239061 in separate follow-up studies. As shown in Table II, SB-249547 demonstrated lower clearance than SB-239063, a somewhat larger apparent distributional volume, and a substantially longer terminal elimination half-life. Apparent oral bioavailability of SB-249547 was low, but was somewhat improved compared to that of SB-239063 (6.0 ± 3.5% for SB-249547 compared with 2.4 ± 1.2% for SB-239063). As with the rat, *cis*- to *trans*- isomerization also occurred in the cynomolgus monkey, though the reaction was not extensive; the AUC ratio of *cis*- to *trans*- isomers was approximately 52 and 5.23 after i.v. and oral administration, respectively. The data obtained after administration of SB-239061 are displayed in Table III. As in the rat, the cynomolgus monkey rapidly and extensively converted SB-239061 to SB-239063 and SB-249547 after both i.v. and oral administration. The *cis*- isomer was more extensively formed than the *trans*- isomer, although not to the same degree as in the rat. The estimated reversibility parameters in the cynomolgus monkey also demonstrated the low degree of reversible metabolism in this species compared with the rat. Following intravenous administration, the recycled fraction of SB-239063 was 0.00035 ± 0.00030, and the exposure enhancement afforded by the metabolic recycling was only 1.0003 ± 0.0003.

In an attempt to elucidate whether the poor oral performance of SB-239063 was limited to the cynomolgus monkey, a study also was conducted in male rhesus monkeys; these data are displayed in Table I and Fig. 4. The pharmacokinetic parameters of SB-239063 in the rhesus monkey were similar to those in the cynomolgus monkey, including moderate clearance and poor oral bioavailability. Interestingly, however, the *trans*- to *cis*- conversion detected in the cynomolgus monkey was absent in the rhesus monkey; neither SB-239061 nor SB-249547 were detected in the rhesus monkey after either i.v. or oral administration.

Finally, SB-239063 was evaluated in the male beagle dog using the selective analytical assay; the results of these experiments are summarized in Table I and Fig. 5. As with the rat and monkey, SB-239063 demonstrated low clearance with a distributional volume somewhat greater than total body water and a relatively long terminal elimination half-life. In contrast to the monkey, however, excellent oral bioavailability was observed in the dog (89.9 ± 8.9%). *In vivo trans*- to *cis*-conversion was observed in the dog; however, the extent of conversion was much less extensive than that observed in the rat or the cynomolgus monkey (AUC ratio of *cis*- to *trans*- isomers was approximately 0.02 after i.v. and oral administration).

DISCUSSION

The initial objective of the studies described here was to characterize the preclinical pharmacokinetic properties of SB-239063, a potent and selective inhibitor of p38 MAP kinase under consideration for clinical use in the treatment of a variety of disease states. These studies have demonstrated that in the rat and the dog, SB-239063 displays pharmacokinetic properties desirable in a clinical development candidate, including low to moderate clearance, a reasonably long ter-

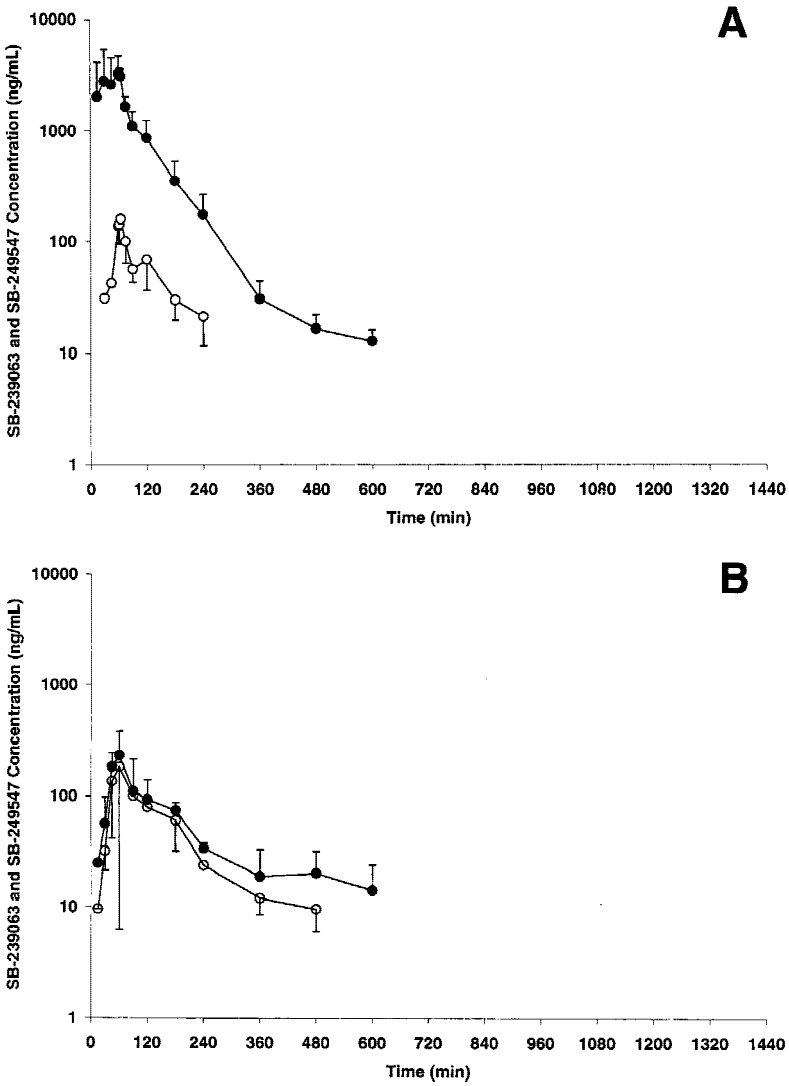

Fig. 3. Plasma concentration-time profiles for SB-239063 (closed symbols) and SB-249547 (open symbols) in the cynomolgus monkey following administration of SB-239063 measured using the selective analytical method. Symbols represent average (± SD) measured plasma concentrations after (**A**) intravenous or (**B**) oral administration; lines are straight-line interpolation between data points. Error bars are unidirectional for clarity.

minal elimination half-life, and good apparent oral bioavailability. These observations are consistent with those previously observed for SB-242235, a structurally related pyrimidinyl imidazole-containing p38 inhibitor. In contrast, less favorable properties were observed in both the cynomolgus and rhesus monkey, particularly poor oral bioavailability. From the present data, the mechanism underlying this profound species difference in bioavailability is unclear; extrapolation of the predicted pharmacokinetic performance of SB-239063 in humans will thus require further investigation.

Although the definitive dispositional parameters for the rat were indeed favorable, the initial pharmacokinetic parameter estimates for SB-239063 were substantially different. The *in vivo trans-* to *cis-* isomerization of SB-239063 to generate the mass-equivalent isomer SB-249547 resulted in an apparent clearance that was significantly lower than the actual value, and apparent oral bioavailability well in excess of 100%, as a consequence of more substantial isomerization after oral administration. Prior to the identification of the isomerization reaction, the available data in the rat appeared to suggest the possibility of nonlinear elimination of SB-239063, and studies were conducted to characterize the range of linearity for the compound. Nonlinear elimination was not completely unanticipated, because the structural analogs SB-203580 and SB-242235 are known to demonstrate nonlinear disposition in the rat (6). However, unlike SB-203580, which is a potent inhibitor of cytochrome P450 (10), SB-239063 demonstrates no substantial inhibition of the major human cytochrome P450 isozymes *in vitro* (data not shown). Indeed, follow-up experiments indicated that SB-239063 elimination in the rat appears to be linear over the concentration range of interest, and *in vivo* reversible metabolism was identified as the mechanism for the apparent oral bioavailability in excess of 100%.

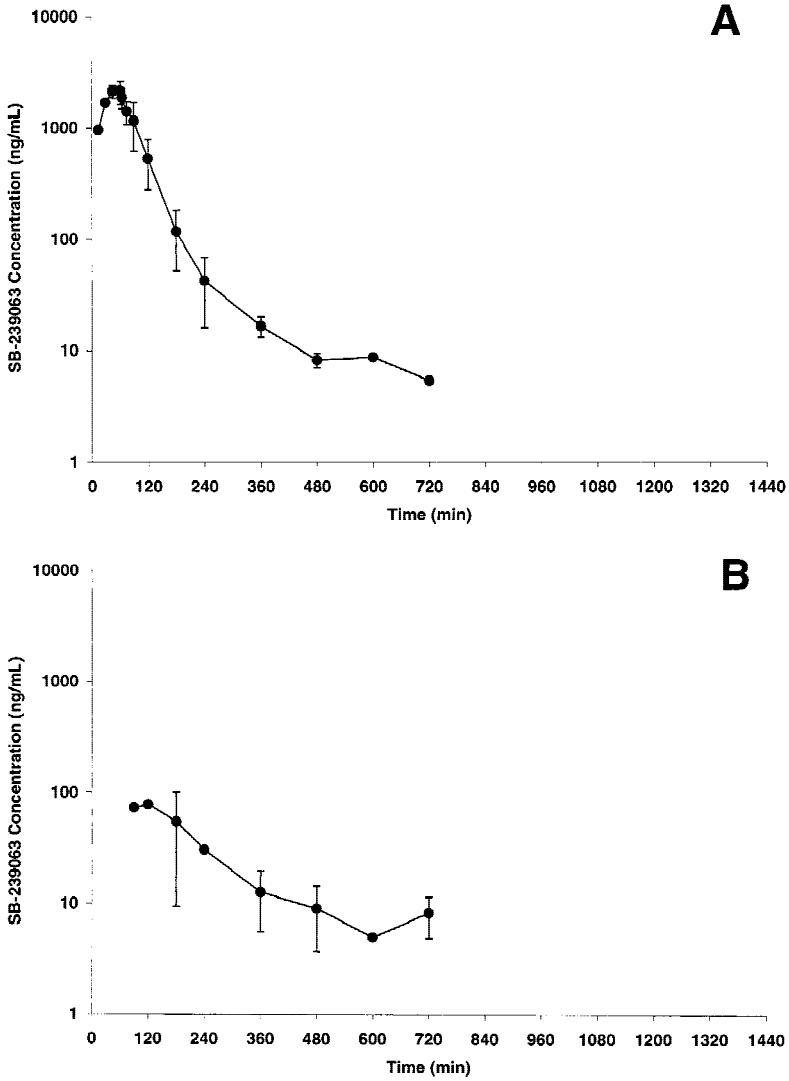

Fig. 4. Plasma concentration-time profiles for SB-239063 in the rhesus monkey following administration of SB-239063 measured using the selective analytical method. Symbols represent average (± SD) measured plasma concentrations after (**A**) intravenous or (**B**) oral administration; lines are straight-line interpolation between data points.

Although SB-239063 and SB-249547 were later determined to be chromatographically separable, for the purposes of rapid pharmacokinetic screening for evidence of favorable dispositional properties, the original (nonselective) assay employed an LC/MS/MS methodology using positive-ion electrospray ionization for quantitative analysis. The use of such techniques greatly accelerates method development and sample turnaround time, decreasing typical method development time to 1 to 2 days (11). Although LC/MS/MS techniques have undeniably revolutionized the application of pharmacokinetic screening in drug discovery, as with any analytical system, occasional failures will occur, and an apparently valid and robust assay may generate incomplete or misleading data. For SB-239063, the only indication of an undetected interference was the observation of oral bioavailability consistently above 100% in the rat. These data highlight the importance of maintaining due vigilance in ensuring that the analytical method of choice provides accurate results.

In the present investigation, it was determined that SB-239063 undergoes an *in vivo trans- to cis-* alcohol isomerization reaction in several species including rat, cynomolgus monkey, and human, but not in dog or rhesus monkey. Additional *in vitro* studies (9) have suggested that this reaction is primarily hepatic in nature, and likely occurs through an obligate ketone intermediate, SB-239061. Extensive reduction of SB-230961 has been demonstrated in both the present *in vivo* study and *in vitro;* the *in vivo* reductive stereopreference was not well predicted by the *in vitro* incubations. Taken together, these data are similar to those previously reported for *trans*-4′-(2-hydroxy-3,5-dibromo-benzylamino) cyclohexanol (HDBC; 14). HDBC was found to undergo efficient but species-specific *in vivo* first-pass *trans-* to *cis-* isomerization. This *trans-* to *cis-* reaction of the cyclohexanol occurred through an obligate cyclic ketone intermediate, as with SB-239063. Also as with SB-239063, the *in vitro* data did not accurately predict the *in vivo* isomerization for HDBC. With

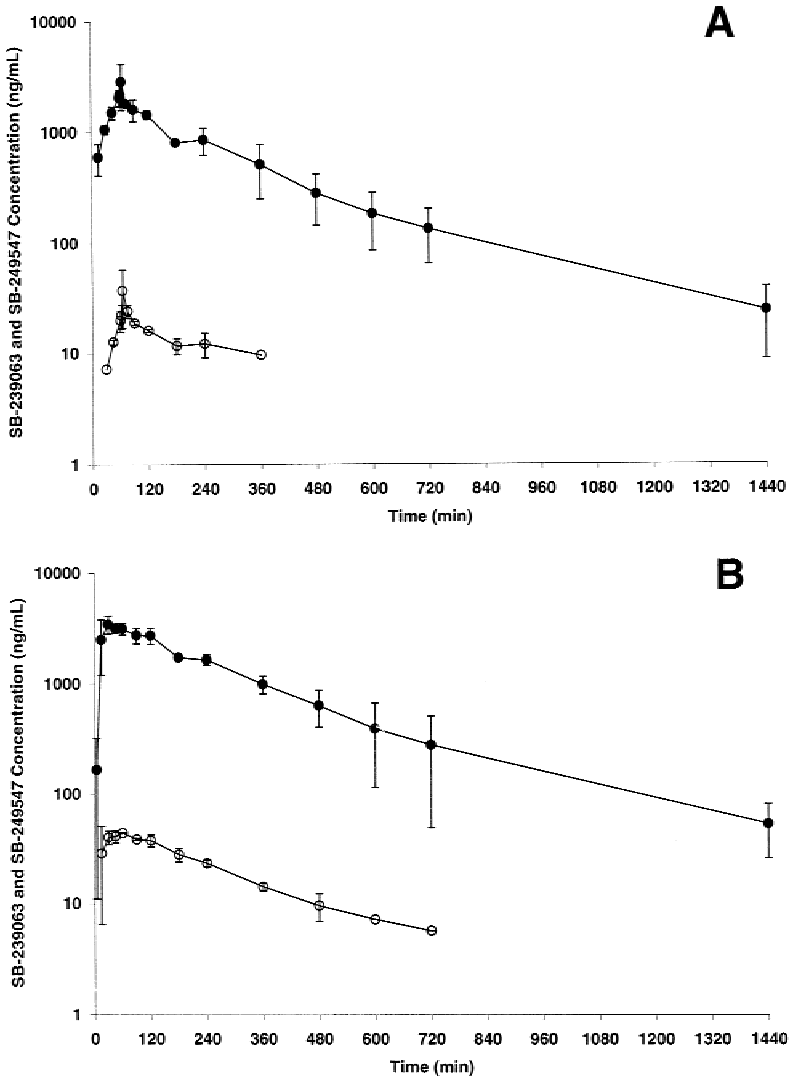

Fig. 5. Plasma concentration-time profiles for SB-239063 (closed symbols) and SB-249547 (open symbols) in the dog following administration of SB-239063 measured using the selective analytical method. Symbols represent average (± SD) measured plasma concentrations after (**A**) intravenous or (**B**) oral administration; lines are straight-line interpolation between data points.

both HDBC and SB-239063, the enzyme(s) responsible for this conversion have not been identified; further research will be required to more fully elucidate the conversion pathways.

Interspecies differences in rates and routes of metabolism are common observations, and consequently, the differences between the dog and other species examined were not surprising. However, the difference in the *trans- to cis-* isomerization in the two different monkey species evaluated was not anticipated. Although both types of animals are macaques, the cynomolgus monkey demonstrated *in vivo* isomerization of SB-239063 to SB-249547, whereas this reaction was negligible in the rhesus monkey. Published data indicate that several compounds, including rolipram (13), hydromorphone (14), and a series of aldose reductase inhibitors (15) demonstrate equivalent pharmacokinetics in the two species. For other molecules, including frusemide (16), isosorbide dinitrate (17), and sucralfate (18), elimination appears to be more rapid in the cynomolgus monkey, whereas opposite results have been observed for antipyrine (19), dexfenfluramine (20), and acivicin (21). Taken together with the present data for SB-239063, no clear interspecies structure-metabolic rate relationships are obvious for these compounds, and it appears that such differences are not *a priori* predictable.

In summary, these studies provide a characterization of the preclinical pharmacokinetics of SB-239063 in the rat, dog, rhesus monkey, and cynomolgus monkey. SB-239063 undergoes a species-specific *trans-* to *cis-* isomerization that results in interference with the original high-throughput analytical methodology. These data exemplify the need to carefully balance improved throughput in drug discovery with the requirement for accurate pharmacokinetic parameter estimates. Furthermore, it was also discovered that species differences exist for SB-239063 disposition, even between rhesus and cynomolgus monkeys. This observation suggests the importance of selecting a variety of preclinical species when evaluating the pharmacokinetics of potential new drug candidates.

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