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ORIGINAL ARTICLE

**Amrita V. Kamath · Saeho Chong
Ming Chang · Punit H. Marathe**

P-glycoprotein plays a role in the oral absorption of BMS-387032, a potent cyclin-dependent kinase 2 inhibitor, in rats

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Abstract *Purpose*: BMS-387032, a novel cyclin-dependent kinase 2 inhibitor, is currently in phase I clinical trials for anticancer therapy. The oral bioavailability of BMS-387032 has been found to be about 31% in rats. Absorption and first-pass metabolism were evaluated as possible reasons for the incomplete oral bioavailability in rats. *Methods*: Male Sprague-Dawley rats were given single doses of BMS-387032 intraarterially (9.1 mg/kg), orally (9.1 mg/kg), or intraportally (10 mg/kg). The routes of excretion of BMS-387032 after intravenous dosing were investigated in bile-duct-cannulated rats. The rate of metabolism of BMS-387032 was investigated in liver microsomes. The permeability of BMS-387032 was evaluated using Caco-2 cells, an in vitro model of the intestinal epithelium. To determine if BMS-387032 was a P-glycoprotein substrate, brain uptake studies were conducted in P-glycoprotein knockout versus wildtype mice. *Results*: The exposure in rats after an intraportal dose was similar to that after an intraarterial dose, indicating that absorption may play a greater role than liver first-pass metabolism in the low oral bioavailability seen in rats. After an intravenous dose, the percent of dose excreted unchanged in the urine and bile over a 9-h period was 28% and 11%, respectively. In vitro studies in rat liver microsomes showed low rates of metabolism of BMS-387032. The Caco-2 cell permeability of BMS-387032 was <15 nm/s in the apical to basolateral direction, and 161 nm/s in the basolateral to apical direction, indicating that it may be a substrate for an intestinal efflux transporter. A P-glycoprotein binding assay showed that BMS-387032 might be a P-glycoprotein modulator. Brain penetration studies in mice showed brain levels of BMS-387032 about 3.5-fold higher in P-glycoprotein knockout mice than in wildtype mice, providing evidence of BMS-387032 being a P-glycoprotein substrate. *Conclusions*: Poor absorption may be playing a greater role than extensive first-pass metabolism in the incomplete oral bioavailability of BMS-387032 seen in rats. The efflux transporter, P-glycoprotein, may be responsible for limiting absorption, as BMS-387032 appears to be a substrate of P-glycoprotein.

Keywords P-glycoprotein · Bioavailability · Absorption · CDK-2 inhibitor · Rat pharmacokinetics · Metabolism

A. V. Kamath (✉) · S. Chong · M. Chang · 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

Cyclin-dependent kinases (CDKs) are serine/threonine protein kinases that play a critical role in cell cycle regulation [7, 12]. Different CDK enzymes are involved in various phases of the cell cycle with the CDK2 enzyme necessary for progression from the G1 to the S phase of the cell cycle. The CDK2 pathway is altered in a wide variety of human cancers including breast, non-small-cell lung, colon, prostrate, ovarian, gastric and other carcinomas [13, 17, 22]. Aberrations leading to increased CDK2 activity have been associated with poor prognosis. An inhibitor of CDK2 would be a novel, mechanism-based anticancer drug with potential for broad utility in chemotherapy.

Several new chemotypes which are inhibitors of CDKs have been identified at Bristol-Myers Squibb [8, 11]. BMS-387032, an aminothiazole (Fig. 1), is a potent and selective CDK2 inhibitor that has been shown to induce G1 cell cycle arrest and apoptosis in a wide variety of tumor cells [11]. Studies in mice bearing human tumor xenografts have shown that BMS-387032 has antitumor activity which is superior to that of flavopiridol [11]. BMS-387032 is currently in phase I clinical trials as an intravenous agent. The potential for oral administration of BMS-387032 has been

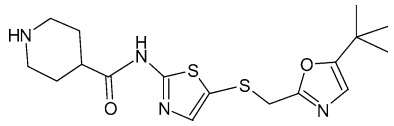

Fig. 1 Chemical structure of BMS-387032

investigated. Oral bioavailability of BMS-387032 is about 31% in rats. The purpose of this study was to determine the causes for the incomplete oral bioavailability seen in rats. Contributing mechanisms such as poor absorption or extensive first-pass metabolism were investigated.

Materials and methods

Chemicals

BMS-387032 was synthesized in the Oncology Chemistry Department at Bristol-Myers Squibb Pharmaceutical Research Institute. [^{125}I]-iodoarylazidoprazosin (specific activity 2200 Ci/mmol) was obtained from NEN Life Science Products (Boston, Mass.). 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.

Pharmacokinetics and oral bioavailability in rats

Adult male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, Mass.). All procedures were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee. Rats were surgically prepared with an indwelling jugular vein cannula. Rats that were given an intraarterial (i.a.) dose or an intraportal dose had an additional indwelling carotid artery cannula or an indwelling hepatic portal vein cannula, respectively. Rats were fasted overnight prior to dosing and fed approximately 6 h after dosing. Water was provided ad libitum throughout the study. The pharmacokinetics of BMS-387032 were investigated following a single dose of 9.1 mg/kg either i.a. as a 10-min infusion or orally by gavage ($n = 3$ rats per groups). A third group of rats ($n = 2$) were dosed intraportally at a dose of 10 mg/kg as a 10-min infusion. The dosing vehicle for all three routes of administration was ethanol/water (1:9 v/v). Blood samples were collected at 10, 20 and 40 min, and at 1, 2, 4, 6, 8 and 10 h after intraarterial and oral dosing. An additional 5-min sample was collected after intraarterial dosing. After intraportal dosing, samples were collected at 10, 15, 30, 45 min, and at 1, 2, 4, 6, 8, and 10 h after dosing. Approximately 0.25 ml blood was collected from the jugular vein catheter into heparinized tubes and plasma was obtained by centrifugation. Samples were analyzed for BMS-387032 by LC/MS/MS analysis.

Routes of excretion and metabolism in bile-duct-cannulated rats

Male Sprague-Dawley rats were surgically prepared with indwelling bile duct, duodenal, and jugular vein cannulae 2 days prior to drug administration. Rats were fasted overnight and for the duration of the study. Water was provided ad libitum throughout the study. During the study, control bile was infused into the duodenum at approximately 1 ml/h to avoid depletion of bile salts. BMS-387032 was administered as a 10-min intravenous infusion to two rats at a dose of 8.9 mg/kg in water. Urine and bile were quantitatively collected over a 9-h period and samples were analyzed for BMS-387032 by LC/MS/MS. The structures of any metabolites excreted in urine and bile were also determined.

Incubations with liver microsomes

BMS-387032 was incubated with mouse, rat, dog, and human liver microsomes. The mouse, rat and dog liver microsomes were prepared by standard methods [14], and were pooled from livers obtained from a group of animals of the same species (mouse, $n = 30$; rat, $n = 6$; dog, $n = 2$). The human liver microsomes were purchased from In Vitro Technologies (Baltimore, Md.) and were pooled from ten individual donors. The rates of oxidative metabolism were measured in duplicate under the following conditions: BMS-387032, 10 μ*M* final concentration (2 m*M* stock solution in water); final microsomal protein concentration approximately 1 mg/ml; NADPH 1 m*M*; pH 7.4 potassium phosphate buffer, 56 m *M*. Incubations were performed at 37°C and were initiated by the addition of the substrate. Incubations were quenched by the addition of one volume of acetonitrile after 10 min. Samples were analyzed by an LC/MS/MS assay and the percent metabolized was calculated based on the disappearance of the parent compound.

BMS-387032 was also incubated with mouse, rat, dog and human liver microsomes along with cofactors for glucuronidation. The experiment was conducted under the following conditions: BMS-387032, 20 μ*M* final concentration (2 m*M* stock solution in water); final microsomal protein concentration approximately 1 mg/ml; UDPGA 3 m*M*; pH 7.5 Tris buffer 100 m*M*, magnesium chloride 10 m*M*, alamethicin 25 ng/ml. Incubations were performed at 37°C and were initiated by the addition of the substrate. Incubations were quenched by the addition of one volume of acetonitrile at 10 min. An attempt was made to detect possible glucuronide(s) of BMS-387032 using an LC/MS/MS assay.

Permeability studies using Caco-2 cells

Caco-2 cells (American Type Culture Collection, Rockville, Md.) were seeded onto polycarbonate filter

membranes at a density of 80,000 cells/cm^2. The permeability studies were conducted with the monolayers cultured for approximately 21 days in culture. The transport medium buffer was modified HBSS containing 10 m*M* HEPES. The pH of the apical compartment was 5.5, 6.5 or 7.5, while the pH of the basolateral compartment was 7.4. Apical to basolateral permeability as well as basolateral to apical permeability studies were carried out with BMS-387032 at a concentration of 200 μ*M*. The bidirectional permeability studies were initiated by adding an appropriate volume of buffer containing BMS-387032 to either the apical (apical to basolateral transport) or basolateral (basolateral to apical transport) side of the monolayer. Samples were taken from both the apical and basolateral compartment at the end of a 4-h incubation period and concentrations of test compound were analyzed for BMS-387032 using a HPLC method with UV detection.

Photoaffinity labeling of P-glycoprotein

The P-glycoprotein binding assay was conducted using a photaffinity assay with [^{125}I]-iodoarylazidoprazosin as previously described [3]. Briefly, P-glycoprotein membrane proteins (Gentest) at a final concentration of 1 mg/ml were incubated in a 10 m*M* Tris-Mes buffer, pH 7.4, with 0, 0.5, 5 or 50 μ*M* BMS-387032 for 30 min at room temperature. [^{125}I]-iodoarylazidoprazosin at a final concentration of 4 n*M* was added and mixed and the samples were illuminated in a crosslinker (CL1000 ultraviolet crosslinker, UVP) at 365 nm for 15 min at room temperature. SDS-PAGE sample buffer (10% 2-mercaptoethanol and 90% 5× Laemmli sample buffer) was added and this was followed by incubation on a shaker for 30 min at room temperature. The sample was then loaded onto a 7.5% SDS gel and run at a constant voltage of 200 mV. Gels were then dried and exposed to a Kodak film for 16–24 h.

Brain uptake studies in P-glycoprotein knockout and wildtype mice

Brain uptake studies were conducted in wildtype and P-glycoprotein knockout mice. There were 12 mice per group. BMS-387032 was administered to both groups of mice intravenously at a dose of 30 mg/kg in an ethanol/water (1:9 v/v) vehicle. Brain and serum samples were collected at 0.25, 1, 2 and 6 h after dosing ($n = 3$ per time point). Brain samples were blotted dry and then homogenized with 3 ml water per 1 g brain. Brain and serum samples were analyzed for BMS-387032 by LC/MS/MS.

Sample analysis

Plasma samples from rat studies, and serum samples from mouse studies were treated with two volumes of acetonitrile containing 1.0 μg/ml of BMS-437854 as an internal standard. In vitro microsomal samples from the different species were treated with an equal volume of acetonitrile containing 1.0 μg/ml of the internal standard. The standard curve for the mouse brain samples was prepared by addition of standards into a PBS buffer homogenate of control brain followed by acetonitrile extraction. After centrifugation of the plasma, serum, and brain samples, a 5-μl portion of the clear supernatant 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 Hypersil BDS 2×20 mm, C18, 3 μm particles, maintained at 60°C, and a flow rate of 0.3 ml/min. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in 90% acetonitrile and 10% tetrahydofuran (THF) (B). The initial mobile phase composition was 95% A/5% B. After sample injection, the mobile phase was at the initial composition for 0.25 min before it was changed to 5% A/95% B over 0.75 min and held at that composition for an additional 1.0 min. The mobile phase was then returned to the initial conditions and the column reequilibrated for 0.49 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 by selected reaction monitoring. Ions representing the $(M+H)^+$ species for both BMS-387032 and the 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 381 → 112 for BMS-387032 and m/z 491.4 → 296.1 for the internal standard, BMS-437854. Cone voltage and collision energy were both optimized at 30 V for BMS-387032, and 40 V and 30 V, respectively, for BMS-437854. The standard curve ranged from 1.25 to 4000 ng/ml.

Bile and urine samples for metabolite elucidation were analyzed by LC/MS/MS. Bile samples were treated with one volume of acetonitrile and supernatant was analyzed after centrifugation. The HPLC was interfaced to a Finnagan's TSQ-7000 triple quadrupole mass spectrometer equipped with an electrospray interface.

Samples obtained from the Caco-2 cell permeability study were analyzed by HPLC-UV. The HPLC system consisted of the 2690 Waters separation module and a Waters 996 photodiode array detector (Waters, Milliford, Mass.). The column used was YMC ODA-AQ 4.6×150 mm, 3 μm particles, maintained at 25°C, and a flow rate of 1 ml/min. Mobile phase A consisted of 95% water, 5% acetonitrile, 0.115% trifluroacetic acid and mobile phase B consisted of 5% water, 95% acetonitrile, 0.115% trifluroacetic 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 followed by reverting back to 100% A and equilibrating for 5 min. Quantitation was based on the peak area of BMS-387032 in the samples.

Data analysis

The pharmacokinetic parameters for BMS-387032 were derived by noncompartmental methods [4] using the KINETICA software program. The C_{max} and T_{max} values were recorded directly from experimental observations. The AUC_{0n} and AUC_{tot} values were calculated using a combination of linear and log trapezoidal rules. The total body clearance (Cl), mean residence time (MRT), and the steady state volume of distribution (Vss) were also calculated after intravenous administration. The bioavailability (expressed as a percentage) was estimated by taking the ratio of dose-normalized AUC values after oral or intraportal doses to those after the intraarterial doses. Brain to serum ratio in mice was calculated by taking the ratio of $AUC_{(0–6\ h)}$ in brain and serum.

Metabolic stability data from liver microsomes was used to predict hepatic clearance values. The following equations [4] were used:

$$\text{Hepatic clearance} = (Q_H \times Cl_{int})/(Q_H + Cl_{int})$$

where Cl_{int} is the intrinsic clearance and Q_H is the hepatic blood flow. The intrinsic clearance was calculated from liver microsomal stability data, and scaled for amount of microsomal protein per gram liver.

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

The hepatic extraction ratio (ER_H) was obtained by the following equation:

$$ER_H = \text{Hepatic clearance}/Q_H$$

Oral bioavailability depends on absorption, metabolism in the walls of the gastrointestinal tract, and finally metabolism by the liver before the compound reaches the systemic circulation. Thus the oral bioavailability can be represented as:

$$\text{Oral bioavailability} = f_A \times f_G \times f_H$$

where f_A is the fraction of oral dose absorbed, f_G is the fraction of oral dose that is not metabolized in the gastrointestinal tract, and f_H is the fraction of oral dose escaping liver extraction during first pass

$$f_H = 1 - ER_H$$

Table 1 Pharmacokinetic parameters of BMS-387032 in rats

Parameter	Intraarterial ($n=3$)	Oral ($n=3$)	Intraportal ($n=2$)
Dose (mg/kg)	9.1	9.1	10
C_{max} (μg/ml)	4.33 ± 1.42	0.2 ± 0.13	9.24
T_{max} (h)	–	4.7 ± 1.2	–
AUC_{tot} (μg×h/ml)	2.38 ± 0.28	0.74 ± 0.33	3.91
Cl (ml/min/kg)	64.4 ± 7.8	–	–
V_{ss} (l/kg)	14.7 ± 5.3	–	–
$t_{1/2}$ (h)	5.3 ± 1.3	1.7 ± 0.4	4.0
MRT (h)	3.7 ± 1.0	4.9 ± 0.3	2.3
Bioavailability (%)	–	31	150

Assuming $f_G = 1$ (i.e., no metabolism in the gastrointestinal tract), and using the calculated f_H and oral bioavailaibility values, the value of f_A was calculated.

Results

Pharmacokinetics and oral bioavailability in rats

Pharmacokinetic parameters in the rat after intraarterial, oral and intraportal administration are summarized in Table 1 and the plasma concentration–time profiles are presented in Fig. 2. The systemic plasma clearance of BMS-387032 in rats was rapid (64.4 ml/min/kg), and greater than the hepatic blood flow of 55 ml/min/kg in rats [2]. The steady-state volume of distribution (Vss) was high (14.7 l/kg), greater than the blood volume of 0.054 l/kg [2], indicating significant extravascular distribution. The estimated elimination half-life was 5.3 h and the MRT was 3.7 h. The oral bioavailability of BMS-387032 in rats was 31%. The time to reach peak concentrations (T_{max}) after an oral dose was 4.7 h in the rat. In order to understand the mechanism behind the incomplete bioavailability in the rat, BMS-387032 was dosed intraportally. The bioavailability after an intraportal dose was greater than 100%. This indicates that

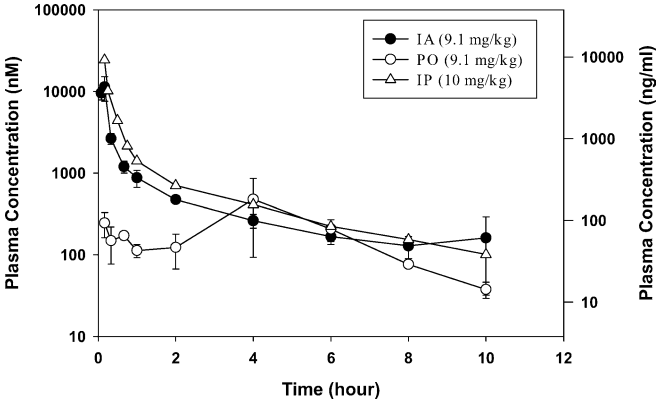

Fig. 2 Plasma concentration versus time profiles of BMS-387032 in rats after intraarterial (*IA*), oral (*PO*), or intraportal (*IP*) administration (means ± SD)

Table 2 Rate of oxidative biotransformation of BMS-387032 in liver microsomes

Species	Rate of metabolism (nmol/min/mg protein)	Intrinsic clearance ml/min/kg)[a]	Hepatic clearance (ml/min/kg)[b]
Mouse	0.08	31.5	23.3
Rat	0.19	34.2	21.1
Dog	0.12	17.3	11.1
Human	0.09	8.5	6.0

[a]Intrinsic clearance: (rate of metabolism/concentration)×(mg microsomal protein/g liver)×(g liver/kg body weight); where concentration = 10 μ*M*, (mg microsomal protein/g liver) = 45, and (g liver/kg body weight) = 87.5 (mouse), 40 (rat), 32 (dog), and 21 (human)
[b]Hepatic clearance: (Q_H×intrinsic clearance)/(Q_H + intrinsic clearance), where Q_H is the hepatic blood flow

absorption plays a greater role than first-pass metabolism in the lower oral bioavailability seen in rats.

Routes of excretion and metabolism in bile-duct-cannulated rats

After an intravenous dose of 8.9 mg/kg in bile-duct-cannulated rats, the percentages of dose excreted unchanged as BMS-387032 in the urine and bile over a 9-h period were 28% and 11%, respectively. In addition to BMS-387032, minor metabolites were found resulting from cleavage of the amide bond, oxidation of the thioether linkage and hydroxylation of the *t*-butyl group [11]. Four metabolites (two M + 16, one M + 32, and *t*-butyl oxazolic acid) were found in the urine and five metabolites (two M + 16 and three M + 32) were found in the bile.

In vitro metabolism in microsomes

The in vitro rate of oxidation of BMS-387032 tested at a concentration of 10 μ*M* was low in mouse, rat, dog, and human microsomes (range 0.08–0.19 nmol/min/mg protein). The data are shown in Table 2. The hepatic clearance values predicted from liver microsomal studies in mouse, rat, dog, and human were about 26–38% of the hepatic blood flow values in the respective species. The hepatic clearance in rats was predicted to be 21 ml/min/kg which is only about 33% of the total clearance observed in rats (64.4 ml/min/kg). In addition, there was no glucuronidation of BMS-387032 seen in liver microsomes fortified with cofactors necessary for glucuronidation in mouse, rat, dog, or human. These results indicate that BMS-387032 is not extensively metabolized by either oxidation or glucuronidation pathways in any of the species tested. Using the predicted hepatic clearance value in rats, the hepatic extraction ratio (ER_H) and the fraction of dose that escapes liver extraction during first pass (f_H) were found to be 0.38 and 0.62, respectively. Using these values and the oral bioavailability of 31% in rats, the fraction of dose absorbed in the rats (f_A) was calculated to be only 51%. Hence poor absorption appears to play a significant part in the incomplete oral bioavailability observed in rats.

Table 3 Permeability of BMS-387032 (at a concentration of 200 μ*M*) across Caco-2 cell monolayers

pH values	Pc (nm/s)	
	Apical to basolateral	Basolateral to apical
5.5	18 ± 8	–
6.5	14 ± 7	161 ± 15
7.5	33 ± 12	–

Caco-2 cell permeability

The permeability coefficient (Pc) of BMS-387032 in Caco-2 cells at different apical pH values was low and pH-independent (Table 3). The permeability was comparable to that of mannitol which is poorly absorbed in humans [9]. The permeability of mannitol typically ranges from 15 to 30 nm/s. The permeability of BMS-387032 in the opposite direction, i.e., basolateral to apical, was much higher (161 nm/s compared to 14 nm/s in the apical to basolateral direction). A large difference of more than tenfold in the bidirectional transport suggests that BMS-387032 might be a substrate of P-glycoprotein.

P-glycoprotein photoaffinity assay

The effect of BMS-387032 on the photoaffinity labeling of P-glycoprotein was evaluated using an analog of prazosin, which is a P-glycoprotein substrate [1]. The P-glycoprotein photoaffinity assay showed that the labeling of P-glycoprotein decreased in a concentration-dependent manner (Fig. 3). At a concentration of

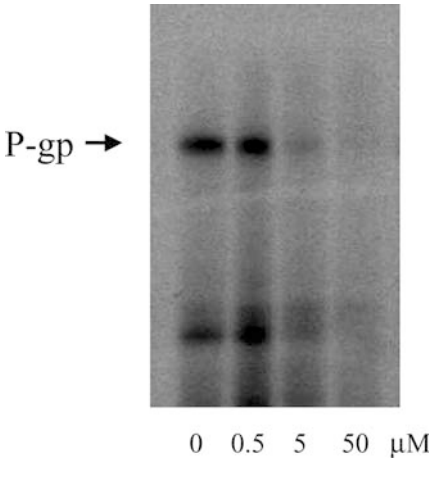

Fig. 3 Photoaffinity labeling of P-glycoprotein by [^{125}I]-iodoarylazidoprazosin in the presence of various concentrations (0–50 μ*M*) of BMS-387032

Table 4 Brain and serum levels of BMS-387032 in wildtype and P-glycoprotein knockout mice

Parameter	Wildtype	P-glycoprotein knockout
Dose (mg/kg)	30	30
Brain $AUC_{(0-6\ h)}$ (μg×h/ml)	2.58	9.06
Serum $AUC_{(0-6\ h)}$ (μg×h/ml)	9.63	9.21
Brain/serum AUC ratio	0.27	0.98

50 μ*M*, the binding of [^{125}I]-iodoarylazidoprazosin to P-glycoprotein was completely inhibited. These results suggest that BMS-387032 may be a P-glycoprotein modulator.

Brain uptake studies in P-glycoprotein knockout and wildtype mice

The results of the brain uptake studies are shown in Table 4 and the concentration-time profile is presented in Fig. 4. After an intravenous dose of 30 mg/kg, the brain to serum $AUC_{(0-6\ h)}$ ratio was 0.27 in the wildtype mice and 0.98 in the P-glycoprotein knockout mice. The serum levels were comparable in the two groups while the brain levels in the P-glycoprotein knockout mice increased 3.5-fold compared to the levels in the wildtype mice. In addition to the Caco-2 cell permeability data, these studies provide further evidence of BMS-387032 being a P-glycoprotein substrate.

Discussion

The oral bioavailability of BMS-387032 in rats was found to be about 31% [11]. This incomplete oral bioavailability could be due to poor absorption and/or extensive first-pass metabolism. On administration of an intraportal dose, where the intestinal absorption step is bypassed and the dose is given directly into the hepatic portal vein, the bioavailability increased to more than 100%, some of which may have been due to saturation of liver metabolism. This increase in exposure after an intraportal dose indicated that absorption rather than liver first-pass metabolism was responsible for the low oral bioavailability seen in rats. This was corroborated by in vitro studies in rat liver microsomes which showed low rates of metabolism, both oxidative and glucuronidation. From in vitro rat liver microsomal stability data, the hepatic clearance in rats was predicted to be 21 ml/min/kg which is only about 33% of the total clearance observed in rats. Using in vitro liver microsomal data, and in vivo oral bioavailability values in the rat, the fraction of dose absorbed in rats was found to be only 51%. Hence the incomplete bioavailability in rats appears to be largely due to poor absorption of BMS-387032. A significant proportion of BMS-387032 appears to be excreted unchanged in the urine and bile in rats. There were some minor metabolites detected in urine and bile in rats over a 9-h period after intravenous administration. The major clearance pathways in rats may be urinary and biliary excretion, with metabolism accounting for a smaller proportion of the high clearance observed in rats.

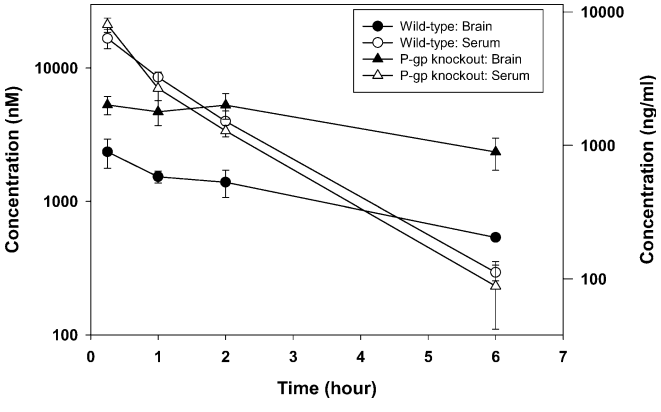

Fig. 4 Brain and serum concentration versus time profiles of BMS-387032 in wildtype and P-glycoprotein knockout mice

Caco-2 cells are derived from a human colon carcinoma and grow to become a confluent monolayer on a semipermeable membrane [6]. They become polarized and form tight intercellular junctions, and thereby strongly resemble the intestinal epithelium. The rate of passage of compounds through the barrier is used to determine a permeability coefficient, which can be related to the in vivo absorption of the compound [9, 10]. The Caco-2 cell permeability of BMS-387032 was low (<15 nm/s) in the apical to basolateral direction, and 161 nm/s in the basolateral to apical direction, indicating that it may be a substrate for an intestinal efflux transporter.

P-glycoprotein is an ATP-dependent efflux transporter that is widely distributed in the body; in the intestinal mucosa, canalicular membranes of the liver, proximal tubules of the kidney, and endothelial cells of the blood–brain barrier [5, 18], and appears to play an important role in drug absorption as well as disposition [19, 21]. P-glycoprotein has been implicated in restricting the oral absorption of various compounds thereby decreasing their bioavailability. A P-glycoprotein binding assay showed that BMS-387032 may be a P-glycoprotein modulator. The absorption as well as disposition of BMS-387032 may be affected by P-glycoprotein activity. P-glycoprotein knockout mice are a good model to identify P-glycoprotein substrates [16]. P-glycoprotein is expressed at the blood–brain barrier and lack of this transporter in P-glycoprotein knockout mice leads to increased brain penetration of compounds that are P-glycoprotein substrates [15, 16, 20]. Brain penetration studies in mice showed brain levels of BMS-387032 about 3.5-fold higher in P-glycoprotein knockout mice than in wildtype mice, providing further evidence of BMS-387032 being a P-glycoprotein substrate. The incomplete absorption in rats is most likely due to the

efflux of BMS-387032 by P-glycoprotein in the intestine. In addition P-glycoprotein may also be playing a role in the excretion of BMS-387032 into urine and bile.

In summary, the incomplete bioavailability of BMS-387032 in rats appears to be due to poor absorption rather than extensive first-pass metabolism. The efflux transporter, P-glycoprotein, may be responsible for limiting the absorption, as BMS-387032 appears to be a substrate of P-glycoprotein.

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