Cancer Chemother Pharmacol (2007) 59:671–679
DOI 10.1007/s00280-006-0323-5

ORIGINAL ARTICLE

Pharmacodynamic and toxicokinetic evaluation of the novel MEK inhibitor, PD0325901, in the rat following oral and intravenous administration

Alan P. Brown · Tage C. G. Carlson · Cho-Ming Loi · Michael J. Graziano

Received: 9 May 2006 / Accepted: 7 August 2006 / Published online: 31 August 2006

Abstract The MEK–mitogen-activated protein kinase (MAPK) signal transduction pathway is involved with numerous cellular processes including cell growth and differentiation. Phosphorylation of MAPK (pMAPK) by MEK results in activation of this pathway. In various solid tumors, the MEK–MAPK pathway is constitutively active; therefore inhibition of this pathway may provide a therapeutic strategy for treating cancer. The objective of this study was to determine the extent and duration of inhibition of pMAPK in selected normal tissues in rats following single oral or intravenous (IV) doses of the novel MEK inhibitor, PD0325901. Male Sprague–Dawley rats (9/group) received either single oral (PO) or IV doses of PD0325901 at 10, 30, or 100 mg/kg (60, 180, and 600 mg/m^2, respectively). Controls received vehicle alone which was aqueous 0.5% hydroxypropylmethyl-cellulose/0.2% Tween 80 for PO dosing and 20% beta-cyclodextran sulfobutyl ether in water (w:v) for IV dosing. Animals (3/group/day) were euthanized on Days 2, 3, and 4, at approximately 24, 48, and 72 h after dosing, respectively. The effects on pMAPK in liver and lung were determined by Western blot analysis and compared with plasma PD0325901 levels. Satellite animals (6/dose/route) received single PO or IV doses and serial blood samples were collected for determination of toxicokinetic parameters of PD0325901 and its major metabolite. In general, systemic exposure to PD0325901 was comparable between routes of administration due to high PO bioavailability (56–109%). Plasma area under the concentration–time curve values of the pharmacologically inactive carboxylic acid metabolite ranged from 18 to 40% of PD0325901. Clinical signs of toxicity occurred at 100 mg/kg PO or IV, indicating the maximum-tolerated dose had been achieved. On Day 2, pMAPK was inhibited 57–95% in liver and 86–99% in lung at all doses, irrespective of route of administration. On Day 3, lung pMAPK remained inhibited 75–91% at all IV doses and by 88% after the 100-mg/kg PO dose. Liver pMAPK remained inhibited 79 and 91% on Day 3 after 100 mg/kg by IV and PO doses, respectively. On Day 4, liver pMAPK was still inhibited 66% after the 100-mg/kg PO dose. The EC_{50} and EC_{90} plasma drug levels for inhibition of lung pMAPK were calculated to be 20 and 99 ng/ml, respectively. Liver pMAPK levels were inhibited at least 50% at plasma PD0325901 concentrations $\geq$50 ng/ml. In conclusion, single PO or IV doses of PD0325901 resulted in dose-dependent inhibition of pMAPK in liver and lung. Inhibition of pMAPK in liver was comparable between routes of administration at $\leq$30 mg/kg, whereas inhibition of pMAPK in lung occurred for a longer duration following IV administration. Measurement of pMAPK in normal tissues served as a means for assessing the pharmacologic activity of PD0325901 and should be included in toxicity studies to evaluate toxicity–pharmacology relationships.

Keywords Anticancer · Biomarkers · MAPK

A. P. Brown (✉) · T. C. G. Carlson · M. J. Graziano
Drug Safety Research and Development,
Pfizer Global Research and Development,
2800 Plymouth Road, Ann Arbor, MI 48105, USA
e-mail: alan.brown@pfizer.com

C.-M. Loi
Pharmacokinetics, Dynamics, and Metabolism,
Pfizer Global Research and Development,
2800 Plymouth Road, Ann Arbor, MI 48105, USA

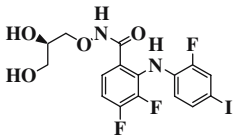
Introduction

The mitogen-activated protein kinase (MAPK) signal transduction pathways control key cellular processes such as growth, differentiation, and proliferation, and provide a means for transmission of signals from the cell surface to the nucleus. As a part of the RAS–RAF–MEK–MAPK pathway, MEK (MAP kinase kinase) phosphorylates the MAPK proteins ERK1 and ERK2 (extracellular signal-regulated kinases) [15]. Although MEK has not been identified as having oncogenic properties, this kinase serves as a focal point in the signal transduction pathway of known oncogenes (e.g., RAS) [10]. Aberrant activation of this pathway has been observed in a diverse group of solid tumors, and is believed to play a key role in neoplasia [5]. In addition to solid tumors, constitutive activation of the MEK/MAPK pathway appears to be involved with the pathogenesis of acute myelogenous leukemia [12].

Based on a significant amount of preclinical data, development of small molecule inhibitors of MEK appears to be a rational approach for treatment of various malignancies [3, 17]. The first MEK inhibitor to enter clinical trials was CI-1040 (also known as PD0184352), and was intended for oral administration. The Phase 1 study with CI-1040 demonstrated inhibition of phosphorylated ERK (pERK) in peripheral blood mononuclear cells and tumor samples, along with evidence of objective antitumor activity (partial response in pancreatic cancer) [1]. However, the level of antitumor activity in a multicenter Phase 2 study in patients with various solid tumors was not sufficient to warrant further development [14]. CI-1040 exhibited low oral bioavailability and high metabolism, which were primary factors resulting in insufficient plasma drug levels for antitumor activity.

PD0325901 (Fig. 1) is a highly potent and specific non-ATP competitive inhibitor of MEK (Ki of 1 nM against activated MEK1 and MEK2 in vitro), and demonstrates anticancer activity against a broad spectrum of human tumors in murine models [18]. Preclinical studies indicate that PD0325901 has the potential to impair growth of human tumors that rely on the MEK/MAPK pathway for growth and survival. Recent studies have indicated that tumors with BRAF mutations may be particularly sensitive for growth inhibition upon exposure to a MEK inhibitor such as PD0325901 [19]. This compound has greatly improved pharmacologic and pharmaceutical properties compared with CI-1040 (i.e., greater potency for MEK inhibition, higher bioavailability, and increased metabolic stability), and has significant promise for determining the therapeutic potential for treating cancer with an orally active MEK inhibitor [14, 18]. A Phase 1/2 trial has been initiated in breast, colon, non-small cell lung cancer, and melanoma patients in which PD0325901 is administered orally twice daily [8].

Fig. 1 Chemical structure of PD0325901



A key aspect in developing "targeted" therapies for cancer involves verifying biochemical activity of the drug against the putative target, and correlating pharmacologic activity with exposure and efficacy [4]. By demonstrating inhibition of the biochemical pathway of interest, pharmacodynamic relationships can be developed in which dose level, schedule, and route of drug administration can be selected to increase the likelihood of clinical response to therapy. By determining a dose and/or regimen that results in optimum activity against a molecular target rather than the maximum-tolerated dose, clinical toxicity may be decreased. Therefore, the development of analytical methods for measuring and/or evaluating the intended biochemical effects of drug exposure (biomarkers) is critical for achieving these goals.

This study was conducted to determine the extent and duration of inhibition of MAPK phosphorylation (pMAPK) in normal rat tissues (liver, lung) following either a single oral or intravenous (IV) dose of PD0325901. These tissues were selected for evaluation because they are highly perfused, provide sufficient sample size, and readily allow protein isolation. In addition, the MEK–MAPK pathway is present in both liver and lung [5, 6]. Plasma drug levels were examined along with inhibition of tissue pMAPK and comparisons were made between oral and IV dosing.

Materials and methods

Drug

PD0325901 was synthesized by Pfizer Global Research and Development, Ann Arbor, MI, USA. The chemical name (IUPAC) of PD0325901 is *N*-((*R*)-2,3-dihydroxy-propoxy)-3,4-difluoro-2-(2-fluoro-4-iodo-phenylamino)-benzamide.

PD0325901 was prepared for oral administration as a suspension in aqueous 0.5% hydroxypropylmethylcellulose/0.2% Tween 80 at concentrations of 2–20 mg/ml. For IV administration, PD0325901 was dissolved (sonication used) in 20% beta-cyclodextran sulfobutyl

ether (SBECD) in water (w:v) at concentrations of 2–20 mg/ml, and filter-sterilized through a 0.2-μm filter

Animals

Male Sprague–Dawley (Crl:CD[SD]IGS BR) rats obtained from Charles River Laboratories were assigned to this study and were approximately 8 weeks old and weighed approximately 230–285 g at initiation of dosing. Animals were housed individually in stainless steel wire mesh cages and acclimated to the laboratory environment for 6 or 7 days prior to dosing. Standard procedures/conditions applied for animal care, feeding, and maintenance of room, caging, and environment. Animals were fed powdered Lab Diet (5002) Certified Rodent Diet ad libitum and water was supplied ad libitum via an automatic system. This study was conducted in accordance with the current guidelines for animal welfare (National Research Council *Guide for the Care and Use of Laboratory Animals*, 1996). The procedures used in this study were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee.

Experimental design

The doses selected for this study were based upon results obtained from acute PD0325901 oral and IV toxicity studies in rats. In the acute IV study, lethality occurred at 400 mg/kg, clinical signs of toxicity (decreased skin turgor, fecal staining, soft feces, mucoid feces, hypoactivity, rough pelage, skin sore, and urine staining) and weight loss at 200 mg/kg, and minimal effects at 100 mg/kg. In the acute oral study, lethality occurred at ≥300 mg/kg and reversible toxicity (clinical signs of chromodacryorrhea, decreased skin turgor, diarrhea, soft feces, red staining of muzzle, rough pelage, and urine staining; and weight loss) occurred at 100 mg/kg. The mechanism(s) for lethality in these studies is unknown. Based upon these data, oral and IV doses of 10, 30, and 100 mg/kg were selected for the study. These doses were expected to be non-lethal, provide a range of plasma drug levels, and generate dose–response data with regards to inhibition of tissue pMAPK levels. In addition, 100 mg/kg was expected to provide exposure and pharmacodynamic data near the maximum-tolerated dose (MTD) by either the oral or IV routes of administration.

A total of 108 rats were randomly assigned by body weight (determined pretest) to treatment groups. Treatment groups were comprised of either 9 animals/group for controls (for both oral and IV vehicles) or 15 animals/group for PD0325901 administration (for both oral and IV dosing). All vehicle control animals and the first nine animals in each PD0325901 dose group were designated for pharmacodynamic assessment (i.e., evaluation of tissue pMAPK levels) as follows: three animals/group were necropsied approximately 24, 48, and 72 h after dosing (on Days 2, 3, and 4, respectively). The remaining six animals in each drug-treated group were designated for toxicokinetic assessment. Animals in the oral treatment groups received vehicle alone or PD0325901 by gavage in a dose volume of 5 ml/kg. Animals in the IV treatment groups received vehicle alone or PD0325901 in the tail vein in a dose volume of 5 ml/kg (at a rate of approximately 1 ml/min). The required volume of vehicle or drug formulation for each animal was based on individual body weight determined prior to dosing on Day 1. All animals were observed approximately 1 and 4 h postdosing, and once daily thereafter for clinical signs of toxicity.

Toxicokinetic parameters were assessed on Day 1 in animals designated for toxicokinetic assessment. Blood samples (approximately 1 ml) were collected at approximately 1, 2, 4, 7, 12, and 24 h after oral dosing. Each PO drug-treated animal was sampled at three time points. The same animals were sampled at the following time points: 1, 4, and 12 h; and 2, 7, and 24 h after oral dosing. In IV dosed animals, blood samples (approximately 1 ml) were collected at approximately 5 and 30 min, and 2, 6, 12, and 24 h after dosing. Each IV drug-treated animal was sampled at three time points. The same animals were sampled at the following time points: 5 min, 2 and 12 h; and 30 min, 6 and 24 h after IV dosing. Blood samples were collected into a tube containing EDTA as the anticoagulant, immediately placed on ice, and centrifuged (refrigerated). The plasma was separated and stored frozen at ≤−20°C prior to analysis. Following collection of the last blood sample, animals designated for toxicokinetic assessment were euthanized and discarded. Plasma concentrations of PD0325901 (R and S enantiomers combined; both pharmacologically active; achiral method) and the inactive carboxylic acid metabolite, PD315209, were determined using a validated liquid chromatographic procedure with tandem mass spectrometry detection (LC/MS/MS) with a lower limit of quantitation of 1 ng/ml. Composite concentration–time profiles were constructed and the toxicokinetic parameters derived included maximum concentration (C_{max}), time-to-C_{max} (T_{max}), and area under the concentration–time curve (AUC). Concentrations below the limit of quantitation were reported as zero (0) and used in evaluation of mean concentrations and estimation of AUC. For each dose level, oral bioavailability was

calculated by dividing AUC(0–24) after PO dosing by AUC(0–24) after IV dosing, and expressed as a percentage.

Three animals/group designated for pharmacodynamic evaluation were euthanized (carbon dioxide asphyxiation) for tissue collection at approximately 24, 48, and 72 h after dosing. Representative samples of liver and lung were collected from all animals. Tissue samples were frozen in liquid nitrogen and stored at −70°C prior to preparation of tissue lysates and measurement of total and pMAPK levels by Western blot analysis. Blots were probed for pMAPK using a polyclonal antibody that detects the phosphorylated forms of both ERK1 and ERK2 (Cell Signaling Technologies), which are two isoforms of MAPK. Blots were then stripped and re-probed using a pair of polyclonal antibodies that detect ERK1 and ERK2 (Santa Cruz) regardless of phosphorylation state (total MAPK). For both pMAPK and total MAPK, signal was generated via a secondary goat antirabbit horseradish peroxidase-conjugated IgG (Santa Cruz) in combination with chemiluminescent substrate (Pierce). Signal was detected and measured digitally using a Bio-Rad Fluor-S Max Imaging System. The ratio of pMAPK/total MAPK was determined for each sample to normalize the pMAPK signal to the total amount of MAPK protein analyzed.

Blood samples were collected from animals designated for pharmacodynamic evaluation at termination (24, 48, or 72 h postdose). Samples (approximately 3–5 ml) were collected into tubes containing EDTA as the anticoagulant, immediately placed on ice, and centrifuged (refrigerated). The plasma was separated and stored frozen at ≤−20°C prior to analysis of plasma concentrations of PD0325901 and PD315209 as described above. Blood samples collected from vehicle control animals were discarded. Mean percent inhibition of pMAPK in liver or lung was plotted against mean plasma PD0325901 concentration, at that respective time point, and analyzed using either simple E_{max} or sigmoid E_{max} models. The analysis was conducted irrespective of route of administration. In cases where the mean pMAPK/total MAPK ratio for a given dose group was greater than the concurrent control, it was considered that no inhibition occurred and a value of zero was used in the analysis.

Statistical analysis

Statistical analysis was conducted on individual animal pMAPK/total MAPK data. Treatment comparisons were performed on rank-transformed data using a dose-trend test sequentially applied at the two-tailed 1 and 5% significance levels within one-factor analysis of variance. Dunnett's test replaced the sequential trend test if the overall linear trend test was not significant at the 5% level and a quadratic trend was significant at the 1% level. All parameters were analyzed separately for each route of administration, time period, and tissue (liver or lung). The pMAPK/total MAPK ratio for liver or lung was averaged within each dose group and time point, and expressed as a percentage of the concurrent control ratio (mean percent of control). Data are presented as the mean percent of control for liver and lung pMAPK to indicate whether PD0325901 administration resulted in a decrease (inhibition), no change, or increase in pMAPK proteins.

Results

Clinical observations

No deaths occurred and clinical signs of toxicity were similar following oral or IV administration. Clinical signs were first observed on Day 2. Oral administration at 100 mg/kg resulted in ataxia, hypoactivity, red staining of the muzzle, and soft/reduced feces. Reduced feces were also seen after an oral dose of 30 mg/kg, and soft feces were observed at 10 and 30 mg/kg. IV administration at 100 mg/kg produced hypoactivity, red staining of the muzzle, and soft/reduced feces. Soft/reduced feces were observed at 30 mg/kg, and clinical signs were not seen following a 10 mg/kg IV dose. These clinical signs are relevant for rats and indicate that a MTD had been achieved at 100 mg/kg by both routes of administration. The MTD can be considered the dose at which further increases above it would likely result in morbidity, mortality, or inhumane conditions of stress in the test animal. Clinical signs occurred in a dose-dependent fashion, which was supported by increased exposures with increasing dose levels.

Toxicokinetics

Mean plasma concentration–time profiles of PD0325901 following IV and oral administration are shown in Fig. 2, and the associated toxicokinetic parameters of PD0325901 and its inactive carboxylic acid metabolite, PD315209, are presented in Tables 1 and 2. PD315209 was measured because it is the major metabolite of PD0325901. Plasma C_{max} and AUC values for PD0325901 and PD315209 increased with dose and confirmed drug exposure by both routes of administration. Drug exposures were similar between PO and IV routes as oral bioavailability of PD0325901 was

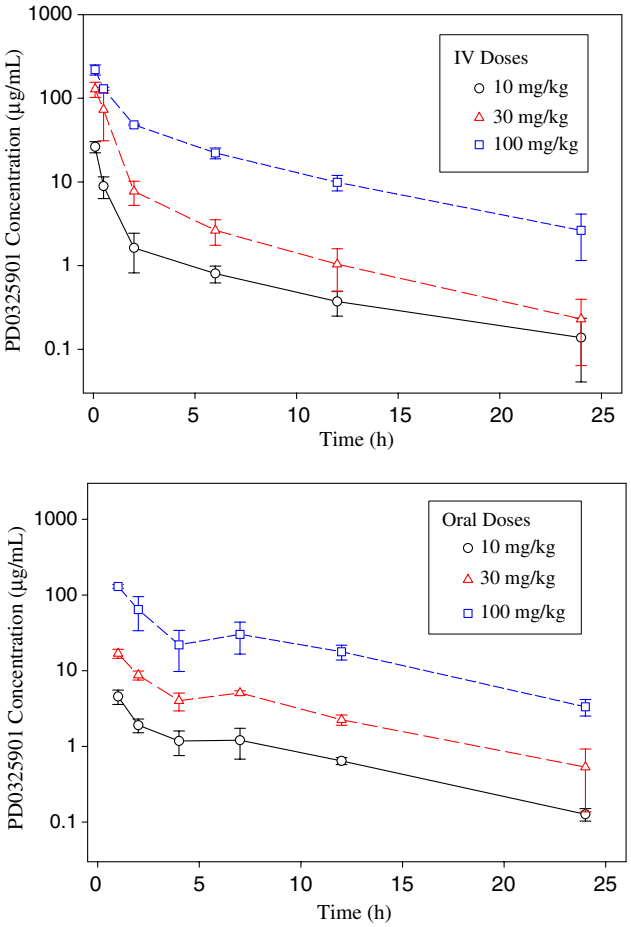


Fig. 2 Composite mean (±standard deviation) plasma PD0325901 concentrations over a 24-h period following IV (*upper panel*) or oral (*lower panel*) administration of PD0325901 to male Sprague–Dawley rats. Doses administered were 10, 30, or 100 mg/kg

Table 1 Plasma toxicokinetic parameters following oral administration of PD0325901

Dose (mg/kg)	Analyte	C_{max} (µg/ml)	T_{max} (h)	AUC(0–24) (µg h/ml)
10	PD0325901	4.57	1	21.5
	PD315209[a]	0.648	2	8.66
30	PD0325901	16.9	1	82.7
	PD315209	2.17	2	26.1
100	PD0325901	130	1	574
	PD315209	7.06	4	122

[a] Inactive carboxylic acid metabolite

77, 56, and 109% at 10, 30, and 100 mg/kg, respectively. Plasma AUC and C_{max} values of the carboxylic acid metabolite were similar between the two routes of administration. Plasma AUC values of the carboxylic acid metabolite ranged from 21 to 40% and 18 to 25% of the parent compound following oral and IV administration, respectively.

Table 2 Plasma toxicokinetic parameters following IV administration of PD0325901

Dose (mg/kg)	Analyte	C_{max} (µg/ml)	T_{max} (h)	AUC(0–24) (µg h/ml)
10	PD0325901	26.3	N/A	27.8
	PD315209[a]	0.768	2	6.83
30	PD0325901	129	N/A	148
	PD315209	2.71	0.5	26.6
100	PD0325901	220	N/A	528
	PD315209	8.43	2	108

N/A not applicable

[a] Inactive carboxylic acid metabolite

In vitro metabolism data have indicated that PD0325901 undergoes glucuronidation and oxidation of the parent molecule, with a similar metabolic profile between rat and human liver microsomes and hepatocytes (unpublished data). The carboxylic acid metabolite PD315209 is believed to be formed by enzymes other than cytochrome P450. Although PD315209 was comparably potent to the parent drug with inhibiting purified MEK1, the metabolite was significantly less potent in a cell-based assay compared with PD0325901 (IC_{50} values of 1.3 µM and 0.59 nM, respectively, for inhibiting pERK in tumor cells) and had decreased activity in vivo. The carboxylic acid metabolite may have decreased potential to enter cells and is considered to be pharmacologically inactive in vivo.

Pharmacodynamics

Summaries of pMAPK data in liver and lung are presented in Tables 3 and 4, respectively. Representative Western blots are presented in Figs. 3 and 4. Oral administration of PD0325901 produced dose-dependent inhibition of liver and lung pMAPK levels. On Day 2, pMAPK was inhibited 57, 84, and 95% in liver, and 92, 95, and 99% in lung at 10, 30, and 100 mg/kg, respectively. Inhibition of tissue pMAPK was not evident after Day 2 following oral doses at 10 or 30 mg/kg. However, pMAPK levels were inhibited on Days 3 and 4 in liver (66–91%), and on Day 3 in lung (88%) in the 100-mg/kg dose group. For each dose and route of administration, inhibition of tissue pMAPK declined with decreasing plasma drug levels, demonstrating the reversible nature of target inhibition by PD0325901.

Intravenous administration of PD0325901 also resulted in dose-dependent inhibition of pMAPK in liver and lung. On Day 2, pMAPK was inhibited 57, 74, and 70% in liver, and 86, 90, and 97% in lung at 10, 30, and 100 mg/kg, respectively. On Day 3,

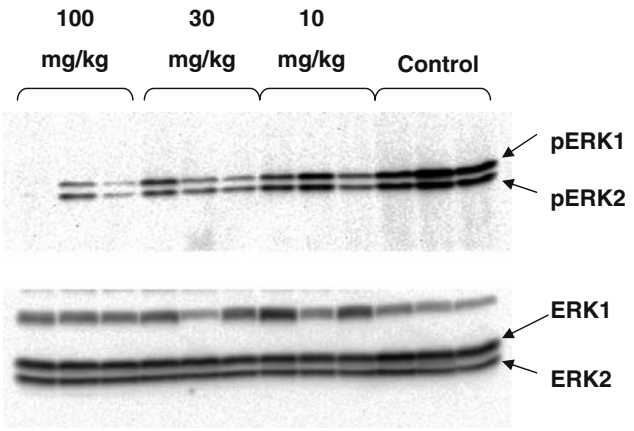


Fig. 3 Western blots of total and pMAPK in the liver 24 h (Day 2) following an oral dose of either PD0325901 or vehicle. *ERK1* and *ERK2*—isoforms of MAPK. *pERK1* and *pERK2*—phosphorylated isoforms of MAPK. *Each lane* represents a sample from an individual animal. Levels of *pERK1* and *pERK2* are decreased in a dose-dependent fashion

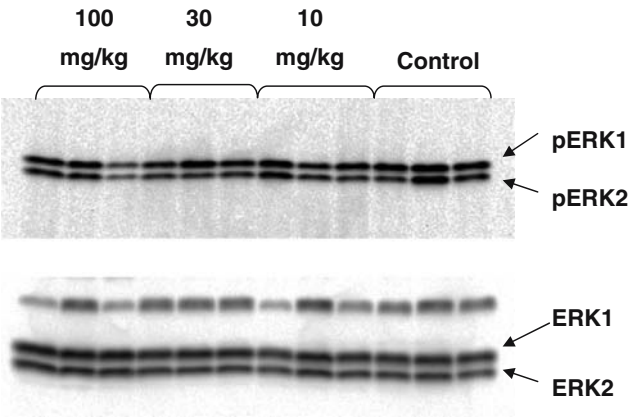


Fig. 4 Western blots of total and pMAPK in the liver 72 h (Day 4) following an oral dose of either PD0325901 or vehicle. *ERK1* and *ERK2*—isoforms of MAPK. *pERK1* and *pERK2*—phosphorylated isoforms of MAPK. *Each lane* represents a sample from an individual animal

Table 3 Liver pMAPK—mean percent of control

Study day[a]	Route	10 mg/kg	30 mg/kg	100 mg/kg
2	Oral	43	16**	5**
	IV	43	26*	30*
3	Oral	89	70	9**
	IV	55	71	21
4	Oral	70	53	34*
	IV	78	74	63

* $P < 0.05$ vs concurrent control

** $P < 0.01$ vs concurrent control

[a] Days 2, 3, and 4 represent approximately 24, 48, and 72 h after dosing, respectively

pMAPK levels were inhibited 75, 85, and 91% in lung at 10, 30, and 100 mg/kg, respectively. pMAPK was inhibited 79% in liver on Day 3 at 100 mg/kg although the change was not significant. Inhibition of tissue pMAPK was not evident on Day 4. On Day 4, lung pMAPK levels were increased 77 and 226% in the 10 and 30 mg/kg groups, respectively. Although the reason for this change is uncertain, it may have been due to low lung pMAPK values in the concurrent vehicle control group and/or represent experimental variability within the data set, as a clear dose–response relationship was not evident.

Table 4 Lung pMAPK—mean percent of control

Study day[a]	Route	10 mg/kg	30 mg/kg	100 mg/kg
2	Oral	8*	5**	1**
	IV	14*	10**	3**
3	Oral	87	124	12*
	IV	25*	15*	9**
4	Oral	138	177	109
	IV	177*	326**	74

* $P < 0.05$ vs concurrent control

** $P < 0.01$ vs concurrent control

[a] Days 2, 3, and 4 represent approximately 24, 48, and 72 h after dosing, respectively

Blood samples collected at approximately 48 and 72 h after dosing indicated prolonged exposure to PD0325901, irrespective of route of administration (Table 5). Plasma levels of PD315209 from 24 to 72 h after dosing are presented in Table 6. The relationship between inhibition of tissue pMAPK and plasma drug levels was examined. Plasma PD0325901 concentrations and inhibition of pMAPK in lung were fitted to a sigmoid E_{max} model (Fig. 5). Maximum inhibition (E_{max}) was determined to be 98% with associated EC_{50} and EC_{90} values of 20.4 and 99.1 ng/ml, respectively. The relationship between plasma PD0325901 concentrations and inhibition of liver pMAPK could not be adequately described with either simple E_{max} or sigmoid E_{max} models due to variability in the data. However, liver pMAPK was consistently inhibited by at least 50% at plasma PD0325901 concentrations ≥50 ng/ml (Fig. 6).

Table 5 Mean PD0325901 plasma concentrations (ng/ml)

Dose (mg/kg)	Route	Hours postdose		
		24	48	72
10	Oral	107	15.2	0.99
	IV	102	10.1	0.787
30	Oral	236	15.2	1.81
	IV	233	34.1	0.887
100	Oral	2,160	378	1.85
	IV	1,170	61.1	1.34

Plasma samples were collected at the time of liver and lung tissue collection

Table 6 Mean PD315209 (inactive carboxylic acid metabolite) plasma concentrations (ng/ml)

Dose (mg/kg)	Route	Hours postdose		
		24	48	72
10	Oral	61	11	0
	IV	36.5	30.8	0
30	Oral	114	12.5	2.08
	IV	66.1	31.2	0
100	Oral	1,490	320	4.86
	IV	487	82.8	1.41

Plasma samples were collected at the time of liver and lung tissue collection

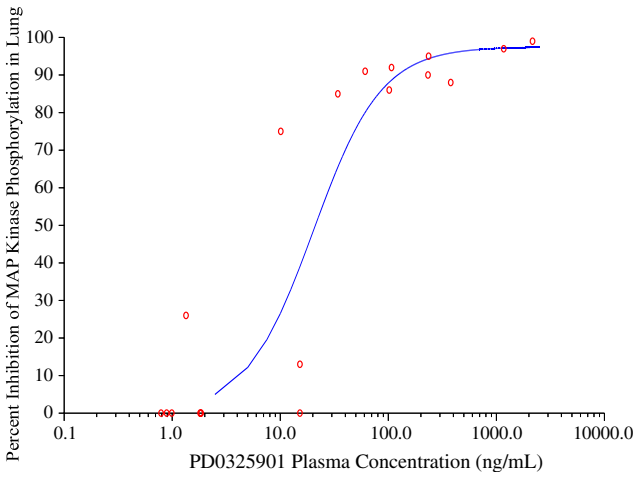


Fig. 5 Relationship of percent inhibition of lung pMAPK versus plasma PD0325901 concentration

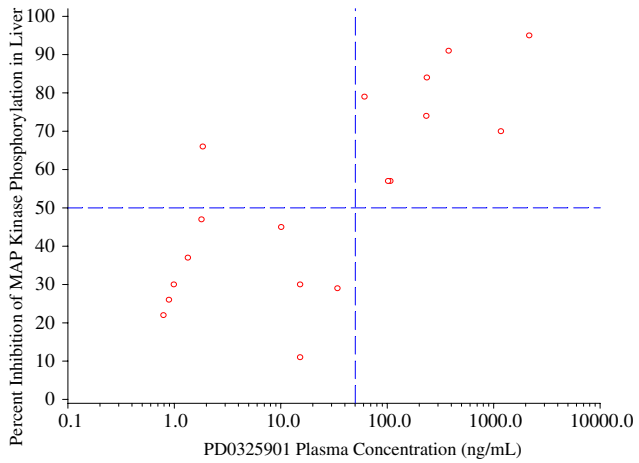


Fig. 6 Relationship of percent inhibition of liver pMAPK versus plasma PD0325901 concentration

Discussion

This study was conducted to determine whether a single oral or IV dose of PD0325901 inhibits pMAPK in liver and lung, and the duration of inhibition. Both routes of administration were initially evaluated for potential clinical development. Subsequently, an oral formulation of PD0325901 was selected for clinical development. Plasma drug levels were related to the pharmacologic activity of the drug and comparisons were made between oral and IV dosing. In general, systemic exposure to PD0325901, as determined by AUC(0–24), was comparable between both routes of administration due to high oral bioavailability (56–109%). This is noteworthy because many drug candidates fail in development due to inadequate pharmacokinetic properties. Inhibition of tissue pMAPK occurred at PD0325901 plasma AUC(0–24) $\geq$21.5 μg h/ml. Clinical signs of toxicity occurred at the high dose (100 mg/kg), irrespective of the route of administration, and indicated that the MTD had been achieved. The dose-related clinical observations were supported by the dose-related increase in systemic exposure.

Both oral and IV administration of PD0325901 inhibited pMAPK in liver and lung. These tissues were selected for evaluation because they are highly perfused, provide sufficient sample size, readily allow protein isolation, and have constitutive levels of pMAPK. These tissues were considered as surrogates for tumor tissue and provided a means for evaluating the pharmacologic activity of a MEK inhibitor in a naive animal. In the liver, inhibition of pMAPK was comparable between routes of administration at 10 and 30 mg/kg. However, inhibition of liver pMAPK occurred to a slightly greater extent and longer duration following an oral dose of 100 mg/kg, compared to the same dose by IV administration. This difference may have been due to the first-pass effect whereby drug delivery from the portal circulation after oral administration resulted in an initially high exposure of the liver to PD0325901. Plasma drug levels at the 24-, 48-, and 72-h time points were also higher at 100 mg/kg following oral dosing, which could have accounted for the greater extent of pMAPK inhibition.

In the lung, inhibition of pMAPK lasted longer following IV administration of PD0325901. An oral 10 mg/kg dose with C_{max} and AUC(0–24) values of 4.57 μg/ml and 21.5 μg h/ml, respectively, inhibited lung pMAPK levels for less than 48 h. Conversely, an IV dose of 10 mg/kg with C_{max} and AUC(0–24) values of 26.3 μg/ml and 27.8 μg h/ml, respectively, inhibited lung pMAPK levels through 48 h. Similar differences were also observed at the 30-mg/kg dose level. The longer duration of inhibition observed with IV dosing may be due to the higher C_{max} value.

The relationship between plasma PD0325901 levels and inhibition of pMAPK in lung was examined by use

of a sigmoid E_{max} model and the EC_{50} and EC_{90} values were 20.4 and 99.1 ng/ml, respectively. These values are very similar to the EC_{50} and EC_{90} values (16.5 and 86 ng/ml, respectively) for inhibition of pMAPK in colon 26 tumors (murine-derived tumor) implanted in mice receiving a single dose of PD0325901 [16]. In SCID mice implanted with various human tumors, PD0325901 was administered daily and tumor growth and pMAPK levels were evaluated in comparison to plasma drug levels. In that study, PD0325901 plasma drug concentrations of 100 ng/ml demonstrated significant pharmacologic activity [7]. PD0325901 inhibited pMAPK in rat liver at plasma concentrations ≥50 ng/ml. These data suggest that PD0325901 inhibits MEK in rat tissues at similar plasma concentrations that have demonstrated pharmacologic activity in various murine tumor models.

It is becoming more apparent that clinical utility of molecularly targeted therapies for the treatment of various cancers is dependent upon demonstrating biochemical activity of the candidate drug on the pathway intended for manipulation [4]. By demonstrating inhibition of the biochemical pathway of interest, pharmacodynamic relationships can be developed in which dose level, schedule, and route of drug administration can be selected to provide a rational means for non-clinical testing and clinical use of the drug [11, 13]. With molecularly targeted therapies, doses for clinical testing may be selected based upon maximum biological effect rather than MTD. This is especially applicable for those agents associated with tolerable toxicity in the clinical setting, an example of which is the monoclonal antibody against the epidermal growth factor receptor, cetuximab [9]. In the current study, Western blot analysis was used to demonstrate inhibition of MEK in two distinct normal tissues in the rat following PD0325901 administration. This technique provided a biomarker for measuring the degree of pharmacologic activity of PD0325901 following in vivo exposure, and was subsequently used in non-clinical toxicology studies of PD0325901 to demonstrate pharmacologic activity of the drug over a range of doses [2]. By including biomarkers such as these in toxicology studies, relationships between exposure, pharmacologic activity, and toxicity endpoints can be evaluated together. This provides a means for developing potential safety margins of targeted therapies. Western blot analysis of whole tissue homogenates provides a means for quantitating biochemical inhibition of targeted pathways. Alternative methodologies for biomarker evaluation are also available, including immunohistochemistry. A combination of Western blot analysis (peripheral blood mononuclear cells) and immunohistochemistry (tumor samples) was used to assess the pharmacologic activity of the predecessor MEK inhibitor, CI-1040, in a Phase 1 trial [1]. In a Phase 1 study of PD0325901 in patients with various solid tumors, inhibition of MEK was evaluated by using immunohistochemistry to measure pERK in tumor biopsies collected before and during drug treatment. Doses ≥2 mg bidaily (2.5 mg/m^2) consistently suppressed tumor pERK by an average of 84% relative to baseline, and doses ≥15 mg bidaily (18.5 mg/m^2) yielded average steady-state plasma concentrations >250 ng/ml which are predicted necessary for near maximal pERK suppression in xenograft models [8].

In conclusion, single oral or IV doses of PD0325901 resulted in dose-dependent decreases in pMAPK in liver and lung, due to inhibition of MEK. Inhibition of pMAPK in liver was generally comparable between routes of administration, whereas inhibition of pMAPK in lung occurred for a longer duration following IV administration of PD0325901, possibly due to higher PD0325901 plasma C_{max}.

References

1. Allen LF, Sebolt-Leopold J, Meyer MB (2003) CI-1040 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK). Semin Oncol 30(5 Suppl. 16):105–116
2. Brown AP, Courtney C, Carlson T, Graziano M (2005) Administration of a MEK inhibitor results in tissue mineralization in the rat due to dysregulation of phosphorus and calcium homeostasis. Toxicologist 84(S-1):108 (abstract 529)
3. Dent P, Grant S (2001) Pharmacologic interruption of the mitogen-activated extracellular-regulated kinase/mitogen-activated protein kinase signal transduction pathway: potential role in promoting cytotoxic drug action. Clin Cancer Res 7:775–783
4. Gelmon KA, Eisenhauer EA, Harris AL, Ratain MJ, Workman P (1999) Anticancer agents targeting signaling molecules and cancer cell environment: challenges for drug development? J Natl Cancer Inst 91(15):1281–1287
5. Hoshino R, Chatani Y, Yamori T, et al (1999) Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase pathway in human tumors. Oncogene 18:813–822
6. Jarvis WD, Auer KL, Spector M, et al (1997) Positive and negative regulation of JNK1 by protein kinase C and p42MAP kinase in adult rat hepatocytes. FEBS Lett 412:9–14
7. Koup JR, Liu J, Loi CM, et al (2004) PK/PD modeling of biomarker (p-ERK) response and tumor growth to PD0325901 in a human tumor xenograft mouse model. Proc Am Assoc Cancer Res 45:1248 (abstract 5409)
8. LoRusso P, Krishnamurthi S, Rinehart JR, et al (2005) A phase 1–2 clinical study of a second generation oral MEK inhibitor, PD0325901 in patients with advanced cancer. Proc Am Soc Clin Oncol (abstract 3011)
9. Luo FR, Yang Z, Dong H, et al (2005) Prediction of active drug plasma concentrations achieved in cancer patients by pharmacodynamic biomarkers identified from the Geo human carcinoma xenograft model. Clin Cancer Res 11(15):5558–5565

10. Mansour SJ, Matten WT, Hermann AS, et al (1994) Transformation of mammalian cells by constitutively active MAP kinase kinase. Science 265:966–970
11. Mendel DB, Laird AD, Xin X, et al (2003) In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 9:327–337
12. Milella M, Kornblau SM, Estrov Z, et al (2001) Therapeutic targeting of the MEK/MAPK signal transduction module in acute myeloid leukemia. J Clin Invest 108(6):851–859
13. Peralba JM, deGraffenried L, Friedrichs W, Fulcher L, Grunwald V, Weiss G, Hidalgo M (2003) Pharmacodynamic evaluation of CCI-779, an inhibitor of mTOR, in cancer patients. Clin Cancer Res 9:2887–2892
14. Rinehart J, Adjei AA, LoRusso PM, et al (2004) Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. J Clin Oncol 22(22):4456–4462
15. Sebolt-Leopold JS (2000) Development of anticancer drugs targeting the MAP kinase pathway. Oncogene 19:6594–6599
16. Sebolt-Leopold J (2003) Pfizer Global Research and Development internal publication. Summary of the preclinical pharmacology of PD0325901-0000. RR-REG 761-00063, October 28, 2003
17. Sebolt-Leopold JS, Dudley DT, Herrera R, et al (1999) Blockade of the MAP kinase pathway suppresses growth of colon tumors *in vivo*. Nat Med 5(7):810–816
18. Sebolt-Leopold JS, Merriman R, Omer C, et al (2004) The biological profile of PD0325901: a second generation analog of CI-1040 with improved pharmaceutical potential. Proc Am Assoc Cancer Res 45:925 (abstract 4003)
19. Solit DB, Garraway LA, Pratilas CA, et al (2006) BRAF mutation predicts sensitivity to MEK inhibition. Nature 439:358–362