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Inhibition of Angiogenesis and Metastasis in Two Murine Models by the Matrix Metalloproteinase Inhibitor, BMS-275291

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ABSTRACT

BMS-275291 is an p.o. bioavailable, sulfhydryl-based matrix metalloproteinase (MMP) inhibitor currently in clinical development for the treatment of cancer. This inhibitor was designed to potently inhibit MMP activities while minimally affecting those of other metalloproteases (*e.g.*, sheddases) involved in the release of cell-associated molecules such as tumor necrosis factor-α, tumor necrosis factor-α receptor, interleukin-6 receptor, or L-selectin. *In vitro*, BMS-275291 is a potent inhibitor (nM) of the activities of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-14. BMS-275291 inhibits tumor growth in a B16BL6 model of experimental metastasis, and in this model, BMS-275291 treatment results in a dose-dependent reduction in the number of lung metastases compared with vehicle controls. BMS-275291 also inhibits angiogenesis in a murine angiogenesis model, where once daily treatment with BMS-275291 results in a dose-dependent inhibition of endothelial cell migration into s.c. implanted Matrigel plugs. Pharmacokinetic studies demonstrated that the plasma concentrations of parent BMS-275291 in mice exceeds the *in vitro* IC_{50} values for MMP-1, MMP-2, MMP-7, MMP-9, and MMP-14 for at least 4 h after the administration of a therapeutic dose of BMS-275291. Taken together, these data demonstrate that BMS-275291 inhibits MMP activities that contribute to tumor metastasis and angiogenesis.

INTRODUCTION

MMPs[2] are a family of zinc-dependent endopeptidases that are responsible for the degradation of ECM proteins during normal tissue remodeling processes such as embryonic growth, wound healing, and angiogenesis (1–3). Members of this family include collagenases, stromelysins, gelatinases, MT-MMPs and Matrilysin. MMPs are secreted by a wide variety of cell types, and their enzymatic activity is directed at the ECM, including basement membranes. During normal physiological processes, endogenous inhibitors regulate the proteolytic activities of these proteinases. If, however, this balance is disrupted and MMP levels increase, their activities directly contribute to the pathology of cancer and other disease states. Elevated levels of MMPs have been shown for many histological types of cancer, including breast, lung, prostate, colorectal, ovarian, and gastric (4, 5), and elevated levels of certain MMPs correlate with more rapid cancer progression in many cancer types (4).

The growth of a solid tumor and its ability to metastasize are dependent on angiogenesis (6, 7). The formation of new capillaries requires the migration of endothelial cells and extensive tissue remodeling. Proteolytic enzymes, such as MMPs, must degrade the various compartments of the ECM to facilitate the remodeling of the ECM required for tumor growth, tumor angiogenesis, and metastasis. Consequently, controlling MMP activity(s) using synthetic small-molecule inhibitors (MMPIs) is attractive as a potential means to intervene in several of the rate-limiting steps in this pathway. In this study, the pharmacokinetic profile and the antimetastatic and antiangiogenic activities of BMS-275291, a novel p.o. active broad-spectrum MMPI, are described.

MATERIALS AND METHODS

Compound Preparation. Clinical grade BMS-275291 (8) was manufactured at Bristol-Myers Squibb Co. (New Brunswick, NJ) and stored in sealed glass opaque vials at ambient temperature. For *in vitro* assays, BMS-275291 was suspended in DMSO containing 1.4 mM 2-mercaptoethanol and stored at −30°C. For *in vivo* studies, BMS-275291 was prepared daily in sterile water using sonication (15 min) at ambient temperature. BMS-275291 was administered to mice p.o. on various schedules using clean, autoclaved, stainless steel gavage needles.

Enzymatic Assays. Human recombinant MMP-1 (collagenase 1), MMP-2 (gelatinase A), MMP-3 (stromelysin 1), MMP-7 (Matrilysin), and MMP-14 (MT1-MMP) were expressed in *Escherichia coli* as inclusion bodies and purified as described (9). Native human MMP-9 (gelatinase B) was purchased from Boehringer Mannheim (Indianapolis, IN). MMP activities were determined in the presence of 2-mercaptoethanol (140 μM) in proximity-based substrate peptide assays (10, 11). MMP-2, MMP-7, MMP-9, or MT1-MMP activity was determined in the linear range using the substrate peptide, Mca-P-L-G-L-Dnp-A-R-NH_2 (Peptide International, Louisville, KY); MMP-1 activity was determined using Mca-P-L-G-L-Dpa-A-R-NH_2 (Bachem, King of Prussia, PA); and MMP-3 activity was determined using Mca-R-P-K-P-V-E-Nva-W-R-K(Dnp)-NH_2 (Peptide International). Substrate hydrolysis was measured using a fluorometer (Fluoroscan Ascent; Labsystems, Franklin, MA). The optimal MMP concentration for each proximity-based substrate peptide assay was determined empirically under reducing conditions.

Cell-based assays were used to test the effects of BMS-275291 on the shedding of TNF-α, TNF-RII, L-selectin, and IL-1-RII from the cell surface. Human peripheral blood mononuclear cells were obtained from normal blood donors. Cells (5×10^5 for the TNFα and TNF-RII assays; 2×10^6 for the L-selectin assays; and 1×10^6 for the IL-1-RII assays) were concurrently treated with different concentrations of BMS-275291 and stimulated with mitogen [lipopolysaccharide (100 ng/ml for 22 h; *E. coli* serotype B8:0127; Sigma Chemical Co., St. Louis, MO) for TNFα and TNF RII assays; phorbol-13-myristate-12-acetate (250 nM for 20 min., Sigma Chemical Co.) for L-selectin assays; and IL-13 (10ng/ml for 18 h) for IL-1-RII assays]. After treatment (37°C; 5% CO_2), culture supernatants were collected and transferred to individual wells of a 96-well plate. Levels of the cell-associated molecules or receptors released in the medium were subsequently measured by ELISA (R&D Systems; Minneapolis, MN) following the manufacturer's instructions.

The effects of BMS-275291 treatment (24 h; 37°C; 5% CO_2) on the release of the IL-6-R from the cell surface was determined as described above, except that HL-60 cells (5×10^5) stimulated with phorbol-13-myristate-12-acetate (8 nM) were used. ELISA (R&D Systems) measured levels of soluble IL-6-R contained in culture supernatant.

Mouse Strains. Female athymic (BALB/c-*nu/nu*) mice obtained from Harlan (Indianapolis, IN) were used in the Matrigel plug angiogenesis model. Immunocompetent C57BL/6 mice (Harlan) were used in the B16BL6 exper-

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[2] The abbreviations used are: MMP, matrix metalloproteinase; ECM, extracellular matrix; MT-MMP, membrane-type MMP; MMPI, MMP inhibitor; BMS-275291, (S)-*N*-[2-mercapto-1-oxo-4-(3,4,4-trimethyl-2,5-dioxo-1-imidazolidinyl)butyl]-L-leucyl-*N*,3-dimethyl-L-valinamide; MT1-MMP, membrane type-1 MMP; TNF-α, tumor necrosis factor-α; TNF-RII, TNF-α receptor II; IL, interleukin; IL-1-RII, interleukin-1 receptor II; IL-6-R, interleukin 6-receptor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MA, methyl acrylate; AA, acrylic acid; AUC, area under the curve.

imental lung metastasis model and pharmacokinetic studies. Mice received food and water *ad libitum* and were maintained in a controlled environment according to American Association of Laboratory Animal Care regulations.

Murine B16BL6 Experimental Lung Metastasis Model. The murine B16BL6 melanoma cells used in these studies were a metastatic variant of the B16 melanoma (12) obtained from Dr. William E. Fogler (Entremed, Rockville, MD). For each assay, B16BL6 cells were harvested from low passage ($\leq$3) cultures grown *in vitro* in DMEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with heat-inactivated 10% fetal bovine serum (Life Technologies, Inc.) at 37°C under a humidified 95%:5% (v/v) mixture of air and CO_2. Cells were washed twice using sterile HBSS and suspended in HBSS to a concentration of 3×10^5 viable cells/ml. Cells routinely were >95% viable at the time of inoculation. Cells were administered i.v. (6×10^4 cells/mouse) into the tail vein (day 0) of syngeneic C57BL/6 mice. Compounds or vehicle were administered p.o. (9–10 mice/group) 2 h before and 2, 24, 48, and 72 h after cell injection (time 0). The health status of the mice was monitored daily. Mice were killed 10 days after tumor cell injection, and their lungs were removed and fixed in neutral-buffered formalin (10%). The number of B16BL6 colonies present on the surface of each set of lungs was determined by visual inspection using a stereoscopic dissecting microscope. Data are presented as the percentage of inhibition, defined as: 1 − (number of B16BL6 metastases in treated groups/number of B16BL6 metastases in vehicle controls) × 100. A result of ≥35% inhibition was considered active.

Murine Matrigel Plug Angiogenesis Model. Ice-cold Matrigel (250 μl; Becton Dickinson, Bedford, MA) containing VEGF (75 ng/ml; Pepro Tech, Inc., Rocky Hill, NJ) and bFGF (300 ng/ml; Pepro Tech, Inc.) was implanted s.c. in athymic mice on day 0. The Matrigel polymerized immediately after implantation into mice, forming a gel plug. Beginning on day 0, BMS-275291 was administered p.o. once daily for 7 days at the indicated dose levels. One day after the last dose, Matrigel plugs were harvested, fixed overnight in 10% buffered formalin, embedded in paraffin, and sectioned (5-μm sections). To confirm that the migrating cells were endothelial cells, individual sections from control groups were stained with antimurine CD34 (BD PharMingen, San Diego, CA) or anti-von Willebrand factor (Dako, Carpinteria, CA) antibodies using standard avidin/biotin-based immunohistochemical techniques. All sample sections were stained with H&E, and the number of endothelial cells present in each plug section was measured using a video imaging system (Image Pro-Plus; Empire Imaging, Princeton, NJ). Fifty fields (at ×20) per plug section were randomly counted. Data are presented as the percentage of inhibition of cell migration, defined as: 1 − (number of migrating cells in treated groups/number of migrating cells in vehicle controls) × 100. A result of ≥35% inhibition was considered active.

Pharmacokinetic Studies. BMS-275291 contains a free sulfhydryl group that *in vivo* has the potential to form disulfide linkages with other sulfhydryl-containing molecules. Consequently, blood samples were collected in Vacutainer tubes that contained MA:K_3EDTA. MA was used because it stabilizes the free sulfhydryl group of parent BMS-275291 to form BMS-275291-MA derivatives. These BMS-275291-MA derivatives in mouse plasma are subsequently converted (by hydrolysis) to AA derivatives, BMS-275291-AA, which can then measured directly to provide parent (active) drug concentrations.

A single dose of BMS-275291 in water was administered p.o. to 54 C57BL/6 mice of each sex at doses of 30 and 90 mg/kg. Blood samples were taken from three mice/sex/dose level at 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 h after dosing. At each sampling time point, individual blood samples were collected in Vacutainer tubes containing MA:K_3EDTA. Samples were placed on ice for ~30 min before incubation in a water bath at 35°C for 30 min to convert BMS-275291-MA to BMS-275291-AA. Samples were subsequently extracted with 0.1 N HCl and methyl tert-butyl ether and analyzed for parent BMS-275291 using an Liquid chromatography/ tandem mass spectrometry method validated by Bristol-Myers Squibb. The standard curves were linear over the concentration range of 1–100 ng/ml and had R^2 values of ≥0.992. The average deviation from nominal was no more than ±6%, and the between-run precision and the within-run precision were no more than 8% relative SD. Results indicate that the assay was accurate, precise, and reproducible.

Plasma concentrations *versus* time profiles were analyzed using noncompartmental analysis by the MENU/PKMENU software system. The data for parent BMS-275291 was considered adequate to give approximations of the apparent peak plasma concentration (C_{max}) and the time to peak concentration (T_{max}), which were recorded directly from the experimental observations. The 24-h area under the plasma concentration-time profile, (AUC, 24 h), was calculated by a combination of the trapezoidal and log trapezoidal rules (13).

RESULTS

***In Vitro* Activities of BMS-275291.** BMS-275291, a small synthetic MMPI which contains a free mercaptoacyl group as the zinc-binding group (Fig. 1), was rationally designed to inhibit a broad range of MMP activities while sparing those of the sheddases (14, 15). The IC_{50} values (the concentration at which the activity of an enzyme is inhibited by 50%) were determined *in vitro* under reducing conditions using purified human MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-14 and quenched fluorescent peptide substrates. These assay conditions were used to maximize the potential of the mercaptoacyl zinc-binding group to inhibit MMP activities. Consequently, the IC_{50} values for MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, and MMP-14 are 9, 39, 157, 23, 27, and 40 nM, respectively. Additionally, BMS-275291 does not inhibit (IC_{50} values >30,000 nM) the *in vitro* activity of serine or cysteine proteases, which include chymotrypsin, elastase, plasmin, cathepsin B, trypsin, thrombin, and urokinase plasminogen activator (data not shown). In cell-based *in vitro* assays, BMS-275291 does not inhibit (IC_{50} values >100,000 nM) the metalloproteases responsible for the release of TNF-α, TNF-RII, L-selectin, IL-1-RII and IL-6-R. These results therefore demonstrate that BMS 275291 is a selective, potent nanomolar inhibitor of MMP *in vitro* activities. Importantly, it does not inhibit closely related sheddase activities responsible for the release of cell-associated molecules.

Effects of BMS-275291 in the Murine B16BL6 Experimental Lung Metastases Model. The *in vivo* efficacy of BMS-275291 was examined in a murine B16BL6 experimental lung metastases model where B16BL6 cells injected i.v. form tumor foci in the lungs. Mice were treated p.o. with BMS-275291 (10–90 mg/kg) 2 h before and 2, 24, 48, and 72 h after tumor injection. Mice were killed 10 days after tumor cell injection, and the number of clearly visible B16BL6 metastases was counted. The average number of B16BL6 metastases in the untreated mouse controls was 286 ± 45 (Study A; Table 1) and 186 ± 25 (Study B; Table 1). As shown in Table 1, a dose-dependent inhibition of tumor metastases was observed, as indicated by the reduced number of lung metastases observed in MMPI-treated mice relative to the untreated controls. In particular, treatment with BMS-275291 at 60 or 90 mg/kg resulted in ~40% inhibition of lung metastases (Table 1). At these dose levels, there was no effect on body weight or any other clinical signs of toxicity. These results demonstrate that p.o.-administered BMS-275291 has antimetastatic activity that is dose dependent.

Effects of BMS-275291 in a Tumor-Independent Murine Matrigel Plug Angiogenesis Model. BMS 275291 was examined for its ability to inhibit angiogenesis in the tumor-independent Matrigel plug angiogenesis model. Typically, in control mice, an angiogenic response is shown by the migration of a large number of endothelial

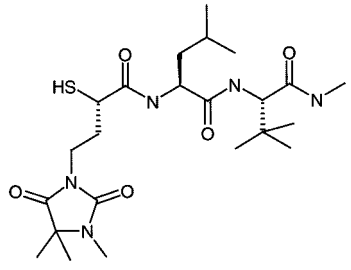

Fig. 1. Structure of the broad-spectrum, sulfhydryl-based MMPI, BMS-275291.

Table 1 *Antimetastatic effects of BMS-275291 in the murine B16BL6 experimental lung metastases model*

Mice received B16BL6 melanoma cells intravenously (6×10^4 mouse) at time 0. BMS-275291 or vehicle (water) were administered p.o. (9–10 mice/group) at −2 h, +2 h, +24 h, +48 h and +72 h relative to time of cell injection (time 0). Mice were killed 10 days after tumor cell injection, and the number of B16BL6 metastases was counted. All sets of lungs had more than one colony. Results are expressed as the percentage of inhibition (% relative to untreated control), and a result of ≥35% inhibition was considered active.

Study	Treatment	Dose (mg/kg)	B16BL6 metastases (Mean ± SE)	% inhibition
A	Control (water)		286 ± 45	
	BMS-275291	90	168 ± 25	41[a]
		30	233 ± 20	19
		10	269 ± 26	6
B	Control (water)		186 ± 24	
	BMS-275291	90	112 ± 11	40[a]
		60	115 ± 9	38[a]
		45	141 ± 12	24[a]
		30	168 ± 17	10

[a] $P < 0.05$; P determined using the two-tailed Student t-test.

cells in representative Matrigel plug sections (Table 2) as well as by the presence of incipient blood vessels (Fig. 2*a*). As shown in Table 2, treatment with BMS-275291 resulted in a significant ($P < 0.05$) reduction in the number of endothelial cells that migrated into Matrigel plugs and a disorganized endothelial cell architecture (Fig. 2*b*) relative to control animals. Treatments with BMS-275291 at doses of 30–90 mg/kg decreased endothelial cell migration by 40 and 58%, respectively (Table 2). These data show that once-daily BMS-275291 oral treatment for 7 days in this model results in a dose-dependent antiangiogenic effect.

Pharmacokinetics of BMS-275291. BMS-275291 contains a free sulfhydryl group that is capable of forming disulfide linkages with itself or other sulfhydryl-containing compounds *in vivo*. Because parent BMS-275291 (BMS-275291 containing a free sulfhydryl group) is believed to be the physiologically active form, an analytical method was developed, using MA, to measure parent BMS-275291. The composite plasma concentration *versus* time profiles for parent BMS 275291 after a single oral dose of 30 or 90 mg/kg are shown in Fig. 3, and the pharmacokinetic parameter values are summarized in Table 3. On the basis of composite profiles, T_{max} occurred at 0.5 h after dosing. For doses increasing in the ratio of 1:3, the mean C_{max} values increased in ratios of 1:30 (male) and 1:27 (female), and mean AUC, 24-h values increased in ratios of 1:40 (male) and 1:31 (female). The C_{max} and AUC, 24-h values were similar in male and female mice, indicating no gender differences in the pharmacokinetics of BMS-275291 in mice. Overall, the results demonstrated that mice were exposed to parent BMS-275291 after a single oral BMS-275291 dose and the exposure increased in a ratio greater than the dose increment. In addition, at a dose of 90 mg/kg (an active dose in the efficacy studies), the plasma concentrations of parent BMS-275291 exceeded the *in vitro* IC_{50} values for MMP-1 (4.5 ng/ml), MMP-2 (20.5 ng/ml), MMP-7 (11.5 ng/ml), MMP-9 (12.5 ng/ml), and MMP-14 (20.0 ng/ml) for at least 4 h after oral administration (Fig. 3). These results demonstrate that mice were exposed to unchanged, biologically active BMS-275291 after a single oral administration, and that the parent BMS-275291 plasma levels exceeded the IC_{50} values for several MMP enzymes.

DISCUSSION

The MMPs are a family of enzymes involved in degrading and remodeling the ECM. MMP activity is tightly regulated in normal physiological states, but loss of this regulation, in diseases such as cancer, results in destruction of the ECM and subsequent tumor progression. Destruction of the ECM promotes tumor metastasis and angiogenesis, both through the direct effect of eliminating a barrier to tumor cell invasion and indirectly through the release of growth factors sequestered in the ECM that induce migration (16, 17). A variety of MMPIs have been shown to have activity in preclinical models of cancer (18–26). Typically, in preclinical models the compounds are administered early in disease progression, and inhibition of the number and/or size of experimental metastases are observed. Several MMPIs of varying selectivity have been evaluated clinically (19, 27–29). To date, MMPIs have shown little clinical benefit when used as monotherapy in patients with advanced disease, and treatment with MMPIs has typically resulted in musculoskeletal side effects, including joint pain and stiffness, that limit both the dose level administered and the duration of therapy (2, 18, 19, 23, 27–29). In the studies described here we demonstrate the preclinical antimetastatic and antiangiogenic activity of BMS-275291, a novel, broad-spectrum MMPI currently in Phase II clinical trials.

Murine B16BL6 melanoma cells, which are aggressive variants of the B16 cell line (12), are reported to express gelatinase A (MMP-2) and MT1-MMP (MMP-14). When these cells are injected i.v. into mice, they extravasate from the bloodstream and subsequently colonize the lung (experimental metastasis). Direct injection of these cells into the circulation bypasses many of the earlier steps of the metastatic process and allows for the determination of their ability to traverse capillaries and grow in a secondary site. In this experimental metastasis model, BMS-275291 was shown to markedly inhibit the number of lung-surface metastases that developed after injection of these cells (Table 1). These findings are consistent with the growing body of evidence that implicates MMP activities as significant contributors to both tumor cell and endothelial cell migration. In these studies, the maximum dose level evaluated was 90 mg/kg. At this dose level there was no evidence of toxicity, suggesting that it would be possible to treat the mice with increasing dose levels. However, additional escalation of the dose was limited by the solubility of BMS-275291. Therefore, it is not clear whether increasing the dose would result in increased efficacy, or whether the 40% inhibition seen represents the maximum efficacy that can be achieved with BMS-275291 in this aggressive metastatic model.

Endothelial cells are reported to express MMPs and tissue inhibitors of metalloproteinases, which together play important roles in tissue remodeling, particularly ECM degradation. During physiological angiogenesis these inhibitors tightly regulate MMP activities. In addition, angiogenic mitogens such as VEGF and bFGF are known to modulate MMP and tissue inhibitors of metalloproteinase expression (30–34). Treatment with BMS-275291 resulted in the dose-dependent inhibition of endothelial cell migration in the Matrigel plug model (Fig. 2, Table 2). As shown in Fig. 2, treatment with BMS-275291

Table 2 *Effect of BMS-275291 at various dose levels in the Matrigel plug assay where mice were treated once daily for 7 days*

Matrigel containing VEGF and bFGF was implanted subcutaneously into athymic mice on day 0. Daily BMS-275291 p.o. treatments began on day 0 and continued to day 6 (9–10 mice/group). Matrigel plugs were harvested on day 7 and processed for histochemical analyses. A result of ≥35% inhibition was considered active.

Treatment	Mean no. of cells migrating into the Matrigel plugs ± SE	% inhibition of cell migration ± SE (relative to control)
Untreated control	6106 ± 163	
BMS-275291		
90 mg/kg	2577 ± 150	58 ± 2.5
60 mg/kg	3272 ± 268	46 ± 4.4
30 mg/kg	3640 ± 104	40 ± 1.7
10 mg/kg	4280 ± 244	29 ± 4.0
1.1 mg/kg	5113 ± 235	16 ± 3.8

$P < 0.05$; P determined using the two-tailed Student t-test.

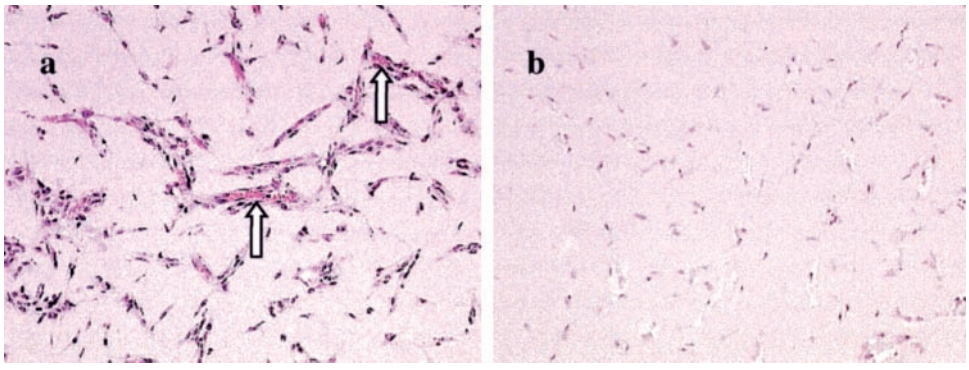

Fig. 2. Effect of BMS-275291 in the Matrigel plug assay. Matrigel containing VEGF and bFGF was implanted s.c. into athymic mice on day 0. Oral BMS-275291 treatments began on day 0 and continued daily to day 6. Matrigel plugs were harvested on day 7 and processed for histochemical analyses. *a*, representative Matrigel plug section from an untreated control athymic mouse. *b*, plug section from an athymic mouse treated with BMS-275291 (90 mg/kg). *Arrows*, presence of RBCs inside the vessels.

also resulted in a reduction in the number of functional blood vessels seen in the Matrigel plugs. In these studies, the once-daily BMS-275291 treatments began on the day of Matrigel implantation. In mice, after a single administration, the plasma levels of parent BMS-275291 exceeded the *in vitro* IC_{50} values for MMP-1, -2, -7, -9, and -14 at least 4 h after administration at the active dose of 90 mg/kg (Table 3, Fig. 3). The inhibition of endothelial cell migration achieved in mice at these drug exposures suggests that although the plasma levels achieved were sufficient to inhibit migration, they were not sufficient to effect complete suppression of endothelial cell migration. It is not clear whether increasing the dose level or the frequency would increase the time that the plasma concentration was above the *in vitro* IC_{50} and would increase further the antiangiogenic activity observed. The dose-related inhibition of endothelial cell migration observed between 1 and 90 mg/kg suggests that greater antiangiogenic activity may be observed at higher doses. However, the limited solubility of BMS-275291 prevented additional dose escalation.

In both the B16BL6 experimental lung metastases and the Matrigel plug models, ~50% inhibition of metastatic foci or endothelial cell migration (Tables 1 and 2) was achieved at the maximum dose level that could be administered to mice. These results may be attributable to the fact that effective drug levels in the plasma were achieved only for a few hours each day. Interestingly, the plasma exposures of BMS-275291 in patients are substantially higher than those achieved in rodent models (35). It is also likely that other proteases, including serine, cysteine, aspartyl, and metalloproteases which are not inhibited by BMS-275291, contribute to tumor metastasis and angiogenesis (7, 16, 33). These other proteolytic activities combined with those of the MMPs can directly contribute to matrix degradation and also have the potential to release sequestered growth factors present in the ECM or uncover and modify cryptic sites that modulate cellular responses (7, 16, 17). Examples of these factors include the release of VEGF, bFGF, epidermal growth factor, and transforming growth factor-β from degraded matrix (5, 7, 16, 17, 36) and the exposure of cryptic sites in laminin-5 and plasminogen by proteolysis (37–39). Additionally, MMPs can proteolytically modify nonmatrix substrate components critically involved in these processes. These include, MT1-MMP involvement in the activation of proMMP-2 (40), posttranslational modification of fibroblast growth factor receptor 1 by MMP-2 (36), or IL-1β (2) and heparin binding-epidermal growth factor by MMP-3 (41). Additional clarification of the relationship of these protease activities to tumor angiogenesis and metastasis may be achieved by studies that evaluate combination therapy using different classes of proteolytic inhibitors.

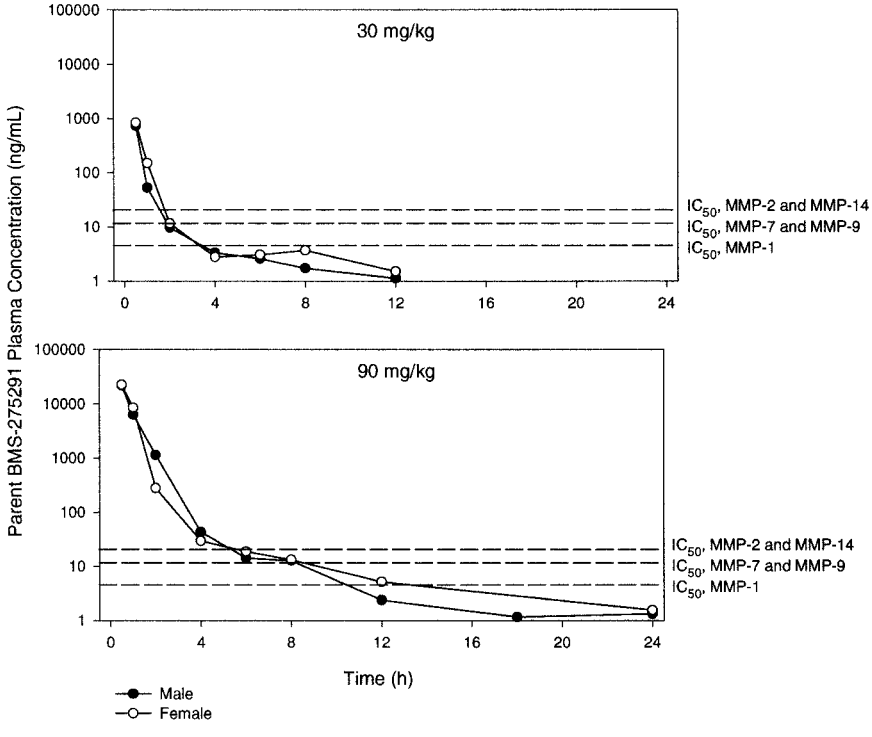

Fig. 3. Composite plasma concentration-time profiles of parent BMS-275291 in male and female C57BL/6 mice.

Table 3 *Pharmacokinetic parameter values of parent BMS-275291 in C57BL/6 mice*

Dose (mg/kg)	C_{max} (ng/ml)		T_{max} (h)		AUC, 24 h (ng·h/ml)	
	Male	Female	Male	Female	Male	Female
30	722	834	0.5	0.5	440	582
90	21943	22287	0.5	0.5	17555	18102

Synthetic MMPIs have been reported to inhibit tumor progression and angiogenesis in a variety of *in vivo* models (19–26). These compounds inhibit the *in vitro* activities of many MMPs, and most are synthetic analogues that use hydroxamic acid as the zinc-binding group (4, 15, 27, 28). Several of these hydroxamate-based inhibitors have demonstrated a dose-limiting arthralgia/myalgia in clinical trials (27–29), and it has been suggested that these dose-limiting side effects may result from the inhibition of MMP-1 (18). This toxicity may also be mediated by the inhibition of a class of closely related metalloproteases referred to as the sheddases (8), which are known to regulate the shedding of cell-surface molecules that mediate inflammatory processes such as TNF-α and TNF-RII. Clinical toxicity may also result from inhibiting the proteolytic processing of other cell-surface molecules, such as L-selectin, IL-1-RII, and IL-6R (8). It is known that certain MMPIs affect the activities of these enzymes (42–44). BMS-275291 was designed to inhibit a subset of MMP activities that include gelatinases, stromelysins, collagenases, and MT- MMPs and not inhibit those of the sheddases. The results presented here demonstrate that BMS-275291 is a potent broad-spectrum inhibitor of MMP activities (including MMP-1, -2, -3, -7, -9, and -14) that does not affect the activities of sheddases responsible for the *in vitro* release of cell-associated molecules (TNF-α and L-selectin) and receptors (TNF RII, IL-1RII, and IL-6R). The importance of sparing the activity of the sheddases was evaluated in a 3-month marmoset toxicology study. In this study, daily p.o. treatment with BMS-275291 did not result in histopathological changes in joint or tendon tissues (8). In contrast, these adverse changes were observed in marmosets treated with MMPIs known to inhibit sheddases. Together these data support the hypothesis that inhibiting sheddase activities may contribute to the arthralgia/myalgia observed in patients treated with MMPIs that inhibit sheddases.

The results described here demonstrate that oral BMS-275291 treatment results in the inhibition of angiogenesis and tumor metastasis using two murine models, a B16BL6 experimental metastasis model and a tumor-independent Matrigel plug model. These data support the potential of this MMPI to inhibit MMP activities critical in angiogenic processes that underlie tumor cell growth and metastasis. The clinical relevance of MMP and metalloprotease (sheddase) inhibition to efficacy and toxicity should be clarified from the ongoing clinical trials of BMS-275291.

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