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


Pharmaceutical Research Institute, Bristol-Myers Squibb Co., Princeton, New Jersey 08543-4000. Phone: (609) 252-3794; Fax: (609) 252-6051.

ABSTRACT

BMS-275291 is an orally 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 α-selectin. In vitro, BMS-275291 is a potent inhibitor (IC50) 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 IC50 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 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 collagenses, stromelysins, gelatinases, MT-MMPs and Matrilysins. 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 antitumor 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 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-Dnp-A-R-NH2 (Peptide International, Louisville, KY); MMP-1 activity was determined using Mca-P-L-G-Dpa-A-R-NH2 (Bachem, King of Prussia, PA); and MMP-3 activity was determined using Mca-R-P-K-V-E-Nva-W-R-K(Dnp)-NH2 (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-R1, l-selectin, and IL-1-R1 from the cell surface. Human peripheral blood mononuclear cells were obtained from normal blood donors. Cells (5 × 106 for the TNFα, TNF-R1, l-selectin, and IL-1-R1 assays; and 2 × 106 for the l-selectin assays; and 1 × 106 for the IL-1-R1 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-R1 assays; phorbol-13-myristate-12-acetate (250 nM for 20 min., Sigma Chemical Co.) for l-selectin and IL-1-R1 assays; and IL-13 (100 ng/ml for 18 h) for IL-1-R1 assays. After treatment (37°C; 5% CO2), 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% CO2) on the release of the IL-6-R from the cell surface was determined as described above, except that IL-60 cells (5 × 105) 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-expe...
RESULTS

In Vitro Activities of BMS-275291. BMS-275291, a small synthetic MMPi which contains a free mercapoacyl 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 mercapoacyl 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-R1, -selectin, IL-1-R1 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 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...
sets of lungs had more than one colony. Results are expressed as the percentage of
10 days after tumor cell injection, and the number of B16BL6 metastases was counted. All
pered active.

antiangiogenic effect.

ger reduction in the number of endothelial cells that migrated into Matri-
gel plugs and a disorganized endothelial cell architecture (Fig. 2

Table 3. On the basis of composite profiles,
method was developed, using MA, to measure parent BMS-275291.
parent BMS-275291 (BMS-275291 containing a free sulfhydryl
itself or other sulfhydryl-containing compounds
sulfhydryl group that is capable of forming disulfide linkages with

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 com-
ounds 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 anti metastatic
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 colo-
nize 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 metas-
tasis 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 esca-
lation 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 inhibi-
tors of metalloproteinases, which together play important roles in
tissue remodeling, particularly ECM degradation. During physiologi-
cal 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>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>B16BL6 metastases (Mean ± SE)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control (water)</td>
<td>90</td>
<td>286 ± 45</td>
<td>16 ± 25</td>
</tr>
<tr>
<td>B</td>
<td>Control (water)</td>
<td>30</td>
<td>233 ± 20</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>269 ± 26</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>BMS-275291</td>
<td>90</td>
<td>112 ± 11</td>
<td>40a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>115 ± 9</td>
<td>38a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>141 ± 12</td>
<td>24a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>168 ± 17</td>
<td>10</td>
</tr>
</tbody>
</table>

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

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

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

* P < 0.05; P determined using the two-tailed Student t-test.
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 IC₅₀ 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 IC₅₀ 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 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.
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-R II. Clinical toxicity may also result from inhibiting the proteolytic processing of other cell-surface molecules, such as α-secretin, IL-1RII, 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 α-secretin) and receptors (TNF-R II, 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.

References


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


Cancer Res 2001;61:8480-8485.

Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/23/8480

Cited articles This article cites 36 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/23/8480.full#ref-list-1

Citing articles This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/23/8480.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.