Review

The importance of estimating the therapeutic index in the development of matrix metalloproteinase inhibitors

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Abstract

At least 56 matrix metalloproteinase (MMP) inhibitors have been pursued as clinical candidates since the late 1970’s when the first drug discovery program targeting this enzyme family began. Some of these clinical candidates were pursued for multiple indications. However, the two primary indications that have been targeted are cancer (24 drugs) and anti-arthritis (27 drugs). Cardiovascular disease was listed as an indication for 10 drugs. Forty-six MMP inhibitors have been discontinued, 7 remain in clinical development, and only 1 (Periostat® for periodontal disease) has been approved. Recently, negative phase II results were reported for the MMP inhibitor PG-116800, which was being evaluated as a treatment for post-ischemic myocardial remodeling to prevent heart failure. One major factor leading to the failure of PG-116800 and many of the other MMP inhibitors is the inadequate assessment of the therapeutic index, the ratio of dose required for efficacy vs. that for toxicology. This review describes the dose-limiting side effect that has hampered MMP inhibitor development (the musculoskeletal syndrome), cardiovascular clinical MMP inhibitor studies, a model of the therapeutic index using marimastat, and progress towards more selective MMP inhibitors not limited by the musculoskeletal syndrome.

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Keywords: Matrix metalloproteinase inhibitor; MMP; Collagenase; Drug discovery; Drug development; Therapeutic index; Attrition; Heart failure

1. Introduction

Abnormal expression and activity of matrix metalloproteases (MMPs) has been linked to the pathological processes underlying metastasis, angiogenesis, rheumatoid arthritis and osteoarthritis as well as cardiovascular disease. The potential utility of MMP inhibitors (MMPI) in the treatment of pathological cardiovascular remodeling has been underlined by preclinical observations that degradation of the extracellular matrix is critical for plaque destabilization [1–4], aneurysm formation [5–8], stent restenosis [9–11], post-ischemic myocardial remodeling [12–14], and the development of systolic heart failure [15,16]. Of the 56 MMPI's identified as clinical candidates, only 10 have listed potential cardiovascular indications (Table 1), only three of these compounds have published cardiovascular clinical data. The lack of MMPI cardiovascular clinical data can be attributed, in part, to an initial historic focus on developing MMPI's to treat pathological: (1) degradation of type II collagen in arthritis, and (2) degradation of extracellular matrix proteins involved in angiogenesis and metastasis promoting tumor growth [17,18]. The notable failure to demonstrate efficacy in arthritis and cancer clinical studies, or in the case of BAY-12-9566 which appears to facilitate tumor metastases in small cell lung cancer [19], has delayed additional MMPI cardiovascular development. Early clinical studies with MMPI's revealed a severe adverse side-effect frequently referred to as the musculoskeletal syndrome (MSS). The attempt of subse-
quent MMPi clinical trials to avoid MSS coupled with an inability to adequately assess the therapeutic index (i.e., the ratio between the dose required for efficacy vs. toxicology), may have resulted in dose selection beneath the minimal effective dose.

This review describes MSS, the tendonitis-like, dose-limiting side-effect which has hampered efficacy assessment, the preclinical data supporting MMPi treatment of plaque destabilization (MIDAS), stent restenosis (BRILLIANT), and post-ischemic myocardial remodeling (PREMIER). Because of the limited cardiovascular clinical data available, human data from marimastat cancer trials is related to an in vivo model of MMPi activity to show that marimastat doses employed in Phase III studies may have been below the minimal effective dose, thus explaining the lack of efficacy with this MMPi. Finally, progress toward the development of selective MMP inhibitors that does not interact with the catalytic zinc ion of MMPs is presented.

2. The musculoskeletal syndrome

Ironically, a class of drugs developed to treat arthritis, among other conditions, induces a tendonitis-like fibromyalgia or musculoskeletal syndrome (MSS) in humans. In a clinical study using marimastat, MSS events requiring dose modification were not observed during the first 28 days of dosing [20]. However, MSS occurred among a substantial number of patients who continued in the long-term continuation protocol. MSS events were dose related and consisted of joint pain, stiffness, edema, skin discoloration, and reduced mobility. The symptoms usually started in the small joints of the hand, as well as the shoulder girdle, typically on the dominant side. If dosing continues unchanged, these symptoms spread to involve other joints as well. Treatment with nonsteroidal anti-inflammatory agents does not alleviate symptoms. A total of 10/30 patients in the long-term continuation protocol developed

<table>
<thead>
<tr>
<th>Product or compound name</th>
<th>Structure</th>
<th>Originator/licensee</th>
<th>Indication</th>
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<tr>
<td>CP-471474</td>
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<td>Roche</td>
<td>Aortic aneurysm and arthritis.</td>
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<td>British Biotech</td>
<td>BRILLIANT-EU Restenosis</td>
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<td>RS-132908</td>
<td><img src="image5" alt="Structure" /></td>
<td>Roche</td>
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<tr>
<td>PG-166800</td>
<td><img src="image6" alt="Structure" /></td>
<td>Proctor and Gamble</td>
<td>PREMIER Post-ischemic myocardial remodeling</td>
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<tr>
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<td><img src="image7" alt="Structure" /></td>
<td>3-D Pharmaceuticals</td>
<td>Restenosis and atherosclerosis.</td>
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<tr>
<td>Doxycycline</td>
<td><img src="image10" alt="Structure" /></td>
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<td>Aortic aneurysm</td>
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<tr>
<td>Periostat® Doxycycline</td>
<td><img src="image11" alt="Structure" /></td>
<td>MIDAS Study Group</td>
<td>MIDAS Acute coronary syndrome</td>
</tr>
</tbody>
</table>

Of these 10, only three MMP inhibitors have published cardiovascular data: batimastat, PG-166800, and doxycycline.
MSS judged to be drug-related. Symptoms were severe enough in 5/10 of the patients exhibiting MSS that the dose was reduced. The time to onset of musculoskeletal toxicity for the five patients with severe events varied from 56 days (75 mg twice daily) to 199 days (25 mg daily). In another marimastat study, patients with gastric cancer developed arthralgia and joint stiffness. In addition, subcutaneous skin thickening of the palmar surface of the hands, associated with contracture of the digits was observed [21]. These changes are described as resembling Dupuytren's contracture, a thickening of the deep tissue that passes from the palm into the fingers, and eventually results in the fingers being pulled into the palm. The mean time to side-effect onset was 45 days, but was to a large extent reversible following the discontinuation of marimastat [21].

Other clinical studies have verified that MSS is dose and time-related; involves joints in the hands, arms and shoulders; is reversible following discontinuation of dosing; and unresponsive to analgesics and NSAIDs [22,23]. One group reported that MSS pain begins in the hands [20], however, another group reported that pain started in the shoulders and extends down to the hands which become edematous [24]. The exact sequence of events may depend on the drug used and the dosing regimen. The plasma drug concentrations necessary to produce efficacy for batimastat, marimastat, CGS-27023A and prinomastat also produced MSS [25,26]. Clinical studies in which MMPi treatment was not efficacious may have resulted because the therapeutic index was not clearly defined, and too low a dose was employed so as to avoid MSS. One study supporting this hypothesis reported that drug treated cancer patients with MSS showed a significant increase in survival time compared to those which did not [27]. However, increased survival in drug treated patients exhibiting more severe MSS may have also resulted from an increased sensitivity to an indirect (non-MMP) effect.

Several hypotheses based on a lack of selectivity have been advanced to explain MSS. An early hypothesis was that inhibition of MMP-1 (type 1 collagenase) activity induced MSS. Both marimastat and RS-130830 are hydroxamate MMP inhibitors (C[=O]NH2OH), and both produce MSS in humans. However, there is a wide separation in the IC50 against MMP-1 for these compounds (0.15 vs. 223 nM, respectively). The carboxylate inhibitors such as BAY 12-9566 and PG-116800 are even weaker inhibitors of MMP-1 (>5000 nM and 1080 nM, respectively) and also produce MSS in humans. The MSS observed following treatment with MMP-1 sparing inhibitors indicates that inhibition of this enzyme is not essential for this side-effect [28].

A more recent hypothesis is that inhibition of “sheddase” activity attributed to non-MMP “shallow pocket” metalloproteinases such as the adamalysins or tumor necrosis factor alpha converting enzyme is the molecular mediator of MSS [29]. The literature is unclear on this question. In a recently described rodent model of MSS [30], MMPi’s that are inactive against sheddases produces MSS like effect in rats. This model provides both a screen to assess MSS potential, and define the therapeutic index. MSS is quantified in this model by scoring the presence and magnitude of various clinical signs and histological changes such as: compromised ability to rest on their hind feet; high-stepping gait; reluctance or inability to move; and hind paw swelling. Histological changes such as soft tissue and bony changes, increased epiphyseal growth plate, synovial hyperplasia and increased cellularity in the joint capsule and extra capsular ligaments are also observed [30]. Marimastat treatment in rats produces a thickened growth plate, and synovial deterioration compared to the vehicle control. A moderate inflammatory cell infiltrate is also present in this model [30]. These changes are dose and time-dependent, and are reversible following the termination of dosing.

Identifying the mechanism of MSS has been complicated by the different functional role for a specific gene across species. For example, mutation of MMP-2 causes an arthritis-like syndrome in humans that involves carpal and tarsal osteolysis, osteoporosis, palmar and plantar nodules. This pathology is distinct from that of MMPi induced MSS [31]. The deletion of the MMP-2 gene in mice is not reported to result in similar joint defects. Deletion of MMP-2 has a different consequence in humans vs. mice.

Studies of MMP-9 and MMP-14 deficient mice suggest a role for these MMPs in one or more events associated with MSS (growth plate remodeling and endochondral bone formation, release of angiogenic factors, neo-vascularization, apoptosis, and ossification). Deletion of the MMP-9 gene in mice produces growth plate enlargement, due to a pronounced increase in the zone of chondrocyte maturation and hypertrophy [32]. MMP-14 (membrane type-1 MMP) gene deletion in mice produces joint defects including endochondral ossification defects, osteopenia, fibrosis of soft tissues and arthritis [33]. These changes are similar to those observed following chronic marimastat treatment in rats [30]. However, the soft tissue changes in both MMP-9 and MMP-14 knockout mice are characterized by fibrosis rather than the fibroblast hyperplasia observed following MMPi treatment. It is not clear what relevance the growth plate changes in mice have to MSS in humans given that epiphysis occurs at puberty while the average age of patients in MMPi trials is above 40 years.

The most convincing evidence that MSS is not due to MMP inhibition, per se, comes from experiments TIMP gene expression experiments. NMR studies indicate that the binding mode between TIMP homologs and MMPs are similar to zinc-chelating MMPi’s. The N-terminal side-chain (Thr2) of TIMP-1 57 and TIMP-2 [34] have both been shown to extend into the S1’pocket containing the catalytic Zn2+ of MMP-3. The direct catalytic inhibitors of MMPs also target the catalytic Zn2+ within the S1’ pocket, and presumably should share similar biological properties with the TIMPs. Overexpression of TIMP-1 and TIMP-3 is...
protective in mouse models of rheumatoid arthritis \[35,36\]. In one study, systemic treatment with significantly reduced paw swelling and increased grip strength compared to control groups. Radiographic assessment also demonstrated a significant reduction of joint destruction in the AdTIMP-1 group, which was confirmed by histologic analyses showing reduced formation of pannus and erosions \[36\]. Therefore, MSS appears to be the result of non-selectivity (i.e., the inhibition of some other metalloproteases), or the combined inhibition of a combination of several critical MMPs.

3. MMP inhibitors and atherosclerotic plaque

MIDAS (Metalloproteinase Inhibition with submicrobial doses of Doxycycline to prevent Acute coronary Syndromes), a 6-month prospective, randomized, double-blind study tested whether subantimicrobial doses of doxycycline hyclate (Periostat\textsuperscript{®}, 20 mg twice daily) reduced the incidence plaque rupture as measured by sudden death, myocardial infarction, and troponin-positive unstable angina in 26 patients with existing coronary artery disease vs. 24 patients on placebo \[37\]. MIDAS is supported by histological studies of atherosclerotic lesions that have shown that the plaque regions vulnerable to rupture are characterized by inflammatory infiltrate, MMP upregulation, and collagen degradation \[2,3\]. The mechanical forces at play on the vulnerable regions of atherosclerotic plaque may exacerbate the inflammatory response and proteolytic activity that ultimately results in vessel occlusion and a clinic event. Another rationale for evaluating the effect of Periostat in the setting of coronary heart disease originates from a report that periodontal inflammation is associated with an increased risk of heart disease and stroke \[38\]. The link between periodontal and coronary disease was strengthened in a subsequent study of 1147 men that revealed an association between periodontal disease, a chronic Gram-negative infection, and atherosclerotic mediated thromboembolic events \[39\]. This association has been hypothesized to result from an underlying inflammatory response trait predisposing individuals to develop both periodontal disease and atherosclerosis. In this scenario periodontal disease produces endotoxins and cytokines that initiate and exacerbate atherogenesis and thromboembolic events \[39\]. The relative risk for coronary heart disease, fatal coronary heart disease, and stroke are up to 2.8 times greater for those with periodontal disease vs. those without \[39\]. One potential mechanism linking periodontal and coronary disease involves periodontal bacteria gaining entry into the systemic circulation, and bacteremia causing changes within the arterial wall leading to atherosclerosis. A study of 50 human specimens removed from carotid arteries revealed periodontal pathogens in all specimens of which 26% were Porphyromonas gingivalis \[40\]. In a study using mice, oral exposure P. gingivalis resulted in the spread of bacteria into the bloodstream and ultimately the aorta, aortic inflammation ensued and accelerated atherosclerosis was evident by 17 weeks \[41\]. These results provide supporting evidence that oral infection can accelerate atherosclerotic lesion progression in the aorta.

Cytokines are involved in the destruction of periodontal tissue, and can stimulate increased production of C-reactive protein (CRP), an important marker of systemic inflammation. Patients with both coronary artery disease and periodontal disease have been observed to have significantly higher mean CRP levels (8-fold higher) compared to healthy control patients with neither disease \[42\]. When the disease group was provided periodontal treatment CRP levels dropped 65% by 3 months post-treatment. This effect persisted at 6 months post-treatment. In the MIDAS study, 50 patients were randomized to either a six-month subantimicrobial oral dose of Periostat (20 mg, bid) or placebo control \[37\]. At enrollment, the two treatment arms had similar demographic and clinical characteristics, including age, sex, and frequency of hypertension, diabetes, smoking, prior cardiac history, extent of coronary disease, presentation with acute myocardial infarction or unstable angina, and need for a percutaneous coronary intervention. Periostat significantly reduced CRP levels by 45.8% compared to baseline values at the six-month follow-up period. Periostat treatment was also associated with a 33.5% reduction in interleukin-6 and a 50% reduction in MMP-9 activity \((p < 0.05)\). Low-dose Periostat was safe with no discontinuations due to treatment-related side effects. However, there was no difference between the low-dose doxycycline and placebo groups in the composite endpoint of cardiovascular death, myocardial infarction, or troponin-positive unstable angina. Brown et al. suggested that their study may have been too short to permit adequate endpoint assessment. Finally, Brown et al. hypothesized that positive feedback loop may exist with systemic infection and inflammation accelerating underlying atherothrombosis, inducing myocardial injury, resulting in IL 6 elevation which stimulates hepatic CRP synthesis that in turn exacerbates atherothrombosis (in part through MMP upregulation) and thus ultimately predisposing to plaque destabilization and additional myocardial injury. It is not clear what mechanism(s) account for CRP and IL 6 reductions in MIDAS, or whether continued Periostat treatment would have a significant and substantial effect on cardiovascular morbidity and mortality. An additional complication in the interpretation of the MIDAS study is whether an adequate drug concentration was achieved within the arterial wall. The predominant mechanism by which Periostat decreases MMP activity does not appear to be through direct inhibition, but rather an indirect downregulation of MMPs. A comparable dose in another study has been reported to produce human plasma drug levels of 5–10 \(\mu\)M \[43\]. In an in vitro system, 50 \(\mu\)M doxycycline inhibited MMP-8 and MMP-13 degradation of collagen type II by 64% and 77%, respectively, and had no effect on MMP-1 \[44\]. The in vivo inhibition of the MMP catalytic site may require substantially higher concentrations.
of doxycycline than 50 μM, at least for the degradation of fibrillar collagen (types I and III). Doxycycline appears to decrease MMP activity by a variety of mechanisms: reduction of enzyme stability [45], reduction of RNA stability [46], and inhibition of transcription [47]. Some cell types and tissues may be more sensitive to doxycycline than others. For example, the dose of Periostat used to treat periodontal disease (20 mg twice daily) may be effective because of its reported ability to bind to the calcified surfaces of tooth roots [48]. Tissue drug concentration can be as much as 5-fold greater than that found in blood. The gradual release of doxycycline from teeth in active form also may contribute to increased exposure, and the prolonged protection that has been observed following drug discontinuation during the post-treatment period. Therefore, it is possible that gingival concentrations of Periostat are sufficient to directly inhibit MMPs, and these tissue concentrations were not achieved in the arterial wall of patients dosed with Periostat in the MIDAS study.

4. MMP inhibitors and stent restenosis

BRILLIANT-EU (Batimastat antiRestenosis trFaL utiLiz-Ing the BiodivYsio loc.c1 drug delivery PC steNT), tested whether an MMPi eluting stent (broad-spectrum inhibitor batimastat, 0.2 mcg/mm² of stent surface area) would inhibit smooth muscle cell migration without interfering with the re-endothelialization process [49]. The primary study endpoint was a composite of major adverse cardiac events (death/recurrent myocardial infarction (MI)/target lesion revascularization) at 30 days. Secondary endpoints included binary restenosis, subacute thrombosis at 30-day follow-up, MACE at 6 and 12 months, and quantitative coronary angiography at 6 months. Arterial injury, such as balloon angioplasty activates vascular smooth muscle cells to undergo a phenotypic change from a contractile state to a synthetic one producing proteolytic enzymes that degrade extracellular matrix proteins. Balloon injury induces the expression of MMPs [50,51] as well as MMP activators such as urokinase and tissue-type plasminogen activator [52]. Increased MMP activity facilitates SMC migration which, if too robust, can lead to restenosis [53]. Batimastat (30 mg/kg/day) treatment in a rat carotid artery balloon injury model significantly inhibited intimal thickening after arterial injury by decreasing SMC migration and proliferation [54]. BRILLIANT-EU study results showed that batimastat-eluting stent was safe, but had no beneficial effect on the rate of in-stent restenosis.

5. MMP inhibitors and post-ischemic myocardial remodeling

PREMIER (PREvention of MI Early Remodeling) trial was performed to evaluate the effect of the MMPi, PG-116800, reduced post-ischemic cardiac remodeling (i.e., left ventricular dilation). MMP activity increases within hours of ischemic injury in the heart, and changes in MMP and TIMP expression continue for months post-MI [14,55]. Chronic MMP upregulation has been proposed to mediate progressive cardiac remodeling and dilation that ultimately culminates in systolic heart failure. Inhibition of MMP activity by gene deletion or MMPi treatment ameliorates cardiac dilation [56–58]. In the PREMIER study, PG-116800 (also referred to as PGE-530742 and PGE-7113313), was administered at a dose of 200 mg bid, and the primary endpoint was post-ischemic remodeling as measured by increases in left ventricular end-diastolic volume (LVEDV). Drug treatment was initiated within 24–72 h following diagnosis of myocardial ischemia, and the duration of treatment was 90 days. LVEDV in the PG-116800 treated group was not significantly different than placebo (LVEDVI of 5.1 vs. 5.5 mL/m², respectively, p = 0.42), and their was a “trend towards an increase in musculoskeletal events” [59]. The rationale for developing PG-116800 was that it was an MMP-1 sparing compound that would avoid the MSS syndrome. As noted earlier, RS-130830 has a similar MMP-1 sparing profile to PG-116800, but was dropped from development because of MSS. In a preclinical study using infarcted pigs PGE-530742, a different formulation of PG-116800, was administered at a dose of 10mg/kg (tid), and reduced left ventricular dilation as measured by LVEDV by approximately 31% compared to placebo control [60]. Assuming equivalent pharmacokinetics between pigs and humans, the total clinical dose (2.5–3.0 mg/kg bid) was 3-fold to 6-fold less than that found effective in pigs. The lower human dose suggests a concern with triggering a side effect, presumably MSS. Therefore, the PREMIER trial may not have employed an adequate dose to test whether MMP inhibition reduces post-ischemic remodeling in humans.

6. The therapeutic index of marimastat

Marimastat was chosen as a reference agent in this review because it is the only MMPi with sufficient clinical information available to illustrate the therapeutic index and test the hypothesis that clinical dose selection may have been below the minimal effective dose. Marimastat works preclinically, improving median survival time and suppressing tumorigenesis in a variety of mouse cancer models [61–63]. Because MMPi’s are cytostatic rather than cytotoxic, conventional measures of efficacy such as reduction in tumor size could not be used to monitor drug activity. The rate of increase in serum tumor markers was used as a disease related biomarker strategy to guide dose selection based on both preclinical [62] and clinical [22] studies. This approach has been criticized because the rate of change in serum tumor marker levels does not necessarily reflect tumor regression [64]. As a consequence of these and other issues, phase I MMPi cancer trials were often followed...
immediately by phase II/III combination trials without the benefit of efficacy information from smaller studies. In addition to tumor markers, the use of circulating MMP levels as a biomarker has met with mixed success in phase II study. Analysis of circulating gelatinase levels by zymography is not predictive of cancer prognosis [65]. MMP-9 downregulation by col-3, a tetracycline derivative, does not correlate with efficacy [66].

An animal model used in our laboratory to study in vivo MMPi activity does provide an estimate of the concentration-effect relation of marimastat. The post-partum involuting rat uterus exhibits profound collagenolysis, a phenomenon initially described over a half-century ago [67,68]. This collagenolysis appears to be driven by matrix metalloprotease (MMP) upregulation and activity. Approximately 60% to 80% of uterine collagen is degraded and lost during the first 4 days postpartum in the rat [67,69]. MMP 13, or rat interstitial collagenase, has been proposed to mediate collagenolysis in the involuting rat uterus [70]. This phenomenon was used in our laboratory to screen MMPi anti-collagenolytic activity for triaging heart failure compounds. The MMPi, marimastat, was used as a positive control in this model. Rats were implanted with a subcutaneous osmotic minipump to administer marimastat because of the short half-life of this compound. Blood and uterine samples were collected on day 21 (i.e., the day prior to gestation) as a prepartum control. Samples from the other groups were collected on day 4 postpartum. Two represen-

![Picrosirius Red Stained Uterine Cross-Sections (Day 4 Post-Partum)](image)

Fig. 1. Representative uterine cross-sections harvested at day 4 postpartum and stained with picrosirius red to highlight fibrillar collagen. Little fibrillar collagen is evident in uterus from vehicle treated rat on left. The preservation of collagen in uterus from marimastat treated rat is obvious.

![Representative scanning electron micrographs from pre-partum control uterus, 4 day post-partum vehicle control, and 4 day post-partum marimastat treated uterus. This figure illustrates the dramatic degradation of collagen that occurs with uterine involution, and the preservation of collagen content and architecture with marimastat treatment.](image)

Fig. 2. Representative scanning electron micrographs from pre-partum control uterus, 4 day post-partum vehicle control, and 4 day post-partum marimastat treated uterus. This figure illustrates the dramatic degradation of collagen that occurs with uterine involution, and the preservation of collagen content and architecture with marimastat treatment.
tative uterine cross-sections harvested at day 4 postpartum and stained with picrosirius red to highlight fibrillar collagen (Fig. 1). The preservation of collagen in marimastat treated rat (right) compared to vehicle is obvious. Scanning electron microscopy of uterine cross-sections revealed that marimastat treatment did not alter the visual appearance of collagen deposition in the uterus (Fig. 2). Marimastat produced a dose-dependent inhibition of collagenolysis with a 56% inhibition of collagen degradation at 86 ng/mL (Fig. 3). Renkiewicz et al. in an experiment that tested the 3 highest doses used in the experiment above (3.25, 6.5, and 9.75 mg/kg/day) observed clinical symptoms and histological changes consistent with MSS in rat [30]. A 3.25 mg/kg/day dose of marimastat reduced uterine collagen degradation by 56% was shown to significantly reduce left ventricular dilation in the MI-rat model [71]. The results from this animal model may also provide a useful framework for modeling MMPi activity in other diseases. There are significant caveats in extrapolating the effect of marimastat in the involuting rat uterus to anti-tumor activity in humans given the difference in species, tissue types, MMPs (as well as TIMPs), and substrates involved. However, the concentration–effect relation of marimastat against fibrillar collagen degradation in the rat uterus, however, does provide a frame of reference for understanding the clinical dose-selection of marimastat.

Table 2 provides a synopsis of all the clinical studies in the literature for marimastat. In an initial phase I trial, the highest dose tested (800 mg) generated a mean maximal plasma concentration (Cmax) of 5.2 μM (2.0 μg/mL), and was reported as being well tolerated [72]. However, a subsequent multiple dose tolerance study reported that patients administered marimastat (100 mg, bid) for 2 weeks showed an arthritis like syndrome (i.e., MSS) which required the dose to be lowered [23]. Results from this study indicated that a 50 mg bid dose with plasma drug level less than or equal to 0.5 μM was tolerated. A subsequent meta-analysis of tumor biomarker data indicated that a dose of at least 10 mg with a plasma drug level of

<table>
<thead>
<tr>
<th>Target</th>
<th>Comparison</th>
<th>Phase</th>
<th>N</th>
<th>Dose(mg)</th>
<th>Dose freq.</th>
<th>MSS</th>
<th>Efficacy</th>
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<td>[22]</td>
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<td>64</td>
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<td>qd*, bid†</td>
<td>33% (&gt;28 days)</td>
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<td>bid</td>
<td>7%, 7%, 12%</td>
<td>Yes</td>
<td>[73]</td>
</tr>
<tr>
<td>Glioma</td>
<td>Placebo</td>
<td>III</td>
<td>162</td>
<td>20</td>
<td>bid</td>
<td>19%</td>
<td>No</td>
<td>[77]</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Placebo</td>
<td>III</td>
<td>369</td>
<td>10</td>
<td>bid</td>
<td>7%</td>
<td>Yes</td>
<td>[78]</td>
</tr>
<tr>
<td>SCLC</td>
<td>Placebo</td>
<td>III</td>
<td>555</td>
<td>10</td>
<td>bid</td>
<td>18%</td>
<td>No</td>
<td>[76]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Gemcitabine</td>
<td>III</td>
<td>239</td>
<td>10</td>
<td>bid</td>
<td>18% vs. 9%</td>
<td>No</td>
<td>[75]</td>
</tr>
<tr>
<td>Colorectal cancer (Liver mets)</td>
<td>Placebo</td>
<td>III</td>
<td>121</td>
<td>10</td>
<td>bid</td>
<td>35% vs. 2%</td>
<td>No</td>
<td>[27]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Placebo</td>
<td>III</td>
<td>180</td>
<td>10</td>
<td>bid</td>
<td>63%</td>
<td>No</td>
<td>[86]</td>
</tr>
</tbody>
</table>

CA 19/9 and CEA represent surrogate biomarkers of anti-tumor efficacy. Frequency of dosing is marked in studies which employed both qd (once daily) and bid (twice daily) dosing (* for qd and † for bid). If efficacy was based on a surrogate biomarker then the biomarker is identified in parenthesis. Studies with equivocal findings are denoted by “???.”
0.1 μM was necessary for efficacy based on a significant effect on surrogate biomarkers [22]. Therefore, marimastat appeared to have at least a 5-fold therapeutic index, and many of the phase III studies utilized a 10 mg bid dose based on this data. Because MMP inhibitors are cytostatic (cells are growth-arrested but viable) rather than cytotoxic (cells are killed), conventional measures of efficacy such as reduction in tumor size could not be used to monitor drug activity. The rate of increase of serum tumor markers was used as a disease related biomarker strategy to guide dose selection [22]. This approach was criticized because the rate of change in serum tumor marker levels does not necessarily reflect tumor regression [64]. Four out of nine phase II studies (Table 2) showed a beneficial effect based on tumor biomarkers for the treatment of pancreatic and colorectal cancer [20,22,73,74]. Phase III studies showed that marimastat did not significantly improve survival for neither pancreatic, colorectal, small cell lung cancer nor glioblastoma [27,75–77]. Marimastat did show a significant effect on survival in patients with gastric cancer; however, the increase in median survival was only 22 days [78]. MSS was evident in all phase II and III efficacy studies, and all these studies required some patients to go to a lower dose, or discontinue dosing all together. Fig. 4 shows all published marimastat clinical studies reporting plasma drug levels. The IC₅₀ (concentration required for 50% inhibition) for marimastat against a panel of human MMPs is shown on the far left (marimastat IC₅₀: MMP-1 0.15nM, MMP-2 0.25nM, MMP-3 20 nM, MMP-7 1.19 nM, MMP-8 0.14 nM, MMP-9 0.68 nM, MMP-12 0.26 nM, MMP-13 0.7 nM, MMP-14 1.5 nM, MMP-15 1.01 nM, MMP-16 0.5 nM, MMP-24 0.8 nM) and the plasma drug levels to the right. Dose(s) employed are shown above each efficacy study, and the incidence of MSS below. MSS has been reported to be dose-related within specific studies [20,22]. A concentration-MSS response is not evident across the studies shown in Fig. 4 for marimastat. Different MSS scoring systems, duration of treatment, and potentially different patient populations are potential explanations for the lack of a between study marimastat plasma concentration vs. MSS response relation. Another anomaly in Fig. 4 is that the 10 mg bid dose in both breast cancer studies fell far short of the desired 0.1 μM plasma concentration target level. The 10 mg/kg dose in the other cancer patient groups resulted in higher plasma drug levels. Marimastat appears to have different pharmacokinetics in breast cancer patients. The dashed line in Fig. 4 shows the plasma concentration of marimastat which reduced collagen degradation by 56% in the rat uterus in vivo efficacy model. Plasma levels in neither phase III shown in Fig. 4 exceeded this threshold, but both reported MSS. Although it is not clear what level of in vivo collagenolytic inhibition is required, Fig. 4 suggests that the assessment of marimastat clinical efficacy was limited by dose, and this has application to a recent phase II study of another MMPi, PG-116800.

Fig. 4. An illustration of the separation between the IC₅₀ (i.e., concentration required for 50% inhibition) for marimastat across different MMPs (closed circles on far left) and the plasma concentration required to produce a 56% inhibition of collagenolysis in a rat uterus model of in vivo efficacy (horizontal dashed line). The plasma drug levels achieved in marimastat phase I (P I), phase II (P II) and phase III (P III) clinical studies shown to right of marimastat IC₅₀’s. The dose employed in each study is shown above the plasma drug level symbols. The percent incidence of MSS in each study is shown below the plasma drug level symbols. The plasma drug level achieved in the 2 phase III studies were above the IC₅₀’s of all the MMPs tested, but below the EC₅₀ measured in the rat efficacy model.
7. Progress toward more selective MMP inhibitors

The search for selective MMP inhibitors has been driven by a lack of understanding regarding the molecular mechanism mediating MSS, the dose limiting side effect produced by MMPi’s. The affinity of most MMPi’s depends primarily upon two factors: a chelating moiety that interacts with the catalytic zinc ion, and hydrophobic extensions protruding from the catalytic site in the hydrophobic S1’ subsite, a deep pocket containing the catalytic zinc ion [79]. The specificity of MMPs results from the shape of the hydrophobic S1’ pocket. The S2’ subsite is a shallow pocket open to solvent with minimal protein-inhibitor interactions. Drug design has focused on utilizing S2’ interactions to build scaffolds of MMPi’s to enhance physicochemical and pharmacokinetic properties without affecting MMP binding affinity. The S3’ subsite is located on the periphery of the MMP active site, and drug design around this site typically produces little gain in inhibitor affinity. To date, MSS has appears to be intrinsic to MMP inhibitors which chelate zinc. Recently, micromolar inhibitors have been described which occupy the S1’ pocket of MMP-13 that do not interact with catalytic zinc [80]. Nanomolar pharmacophores have been described which occupy the S1’ pocket without interacting the catalytic zinc based on crystal structure analysis [81]. In addition, one of the MMP-13 inhibitors has 50% oral bioavailability thus indicating that it may be suitable for in vivo use [81].

8. Summary

To date, only three MMPi’s have been assessed clinically for cardiovascular indications: doxycycline for acute coronary syndrome, batimastat eluting stents for restenosis, and PG-116800 for the prevention of post-ischemic left ventricular dilation. In all three studies, there was no significant difference between the drug and non-drug treated groups. MMP inhibitors, which are potent zinc chelators such as batimastat and PG-116800, appear to induce the musculoskeletal syndrome, the major side-effect limiting dose-selection. A preclinical study of PG-116800, which demonstrated efficacy, had 3- to 6-fold higher plasma levels than that observed in the clinical study, and in the preclinical study dosing was three times daily rather than twice daily used for humans. Analysis of marimastat phase III data indicates that the dose employed may have been too low based on an in vivo rodent model of MMPi activity. The advent of selective MMP-13 inhibitors which do not interact with catalytic zinc represent a new MMPi pharmacophore. If this new class of selective MMPi’s does not cause MSS, then selective inhibitors against one of the approximately 26 other human MMPs may be developed if a compelling rationale for a single MMP mediating one or more cardiovascular diseases can be established.

References


