REVIEW

Inhibiting Metalloproteases with PD 166793 in Heart Failure: Impact on Cardiac Remodeling and Beyond

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Metalloproteinases (MMPs, also called matrixins) are extracellular proteolytic enzymes involved in the degradation of both matrix and nonmatrix proteins. Currently, 25 MMPs have been identified in humans, and the overexpression of one or more MMPs has been implicated in several pathologies, spanning from cancer to rheumathoid arthritis to cardiovascular disease. While research over the past 20 years has focused on understanding MMP biology and selectively inhibiting MMP activity, key issues that remain to be addressed include MMP roles in the context of normal versus pathological conditions and whether globally inhibiting MMPs improves or deteriorates overall organ function. In terms of cardiovascular disease, increased MMP expression has been demonstrated in the setting of myocardial ischemia, reperfusion injury, and during the progression to congestive heart failure. MMPs are also major contributors to the progression of atherosclerotic lesions. In this review, we focus on cardiovascular effects produced by PD 166793, a wide-broad spectrum MMP inhibitor, originally developed by Parke-Davis (now Pfizer). We will briefly review its structure, mechanism of action, and inhibitory capacity. Finally, we will illustrate the cardiac contexts, both *in vivo* and *in vitro*, in which PD166793 administration has proven beneficial.

Introduction

Matrix metalloproteinases (MMPs) are a large family of zinc-binding, calcium-dependent endopeptidases, involved in extracellular matrix (ECM) degradation and remodeling (ECM) (Fingleton 2007; Page-McCaw et al. 2007). MMPs proteolytically process collagen, elastin, fibronectin, matrix glycoproteins, and proteoglycans. In addition, MMPs also regulate cellular functions including the leukocyte role in the inflammatory response (McQuibban et al. 2000), the smooth muscle role in vascular tone control (Fernandez-Patron et al. 1999), and the platelet role in the blood clotting response (Sawicki et al. 1997). MMPs are involved in a plethora of physiological and pathological processes, from angiogenesis to metastasis, from cardiac and vascular remodeling to periodontal diseases (Fingleton 2007; Verma and Hansch 2007). They are produced and excreted by multiple connective tissues and proinflammatory cells, including fibroblasts, cardiomyocytes, endothelial cells, macrophages, neutrophils, and lymphocytes. MMPs are typically secreted as zymogens, a proactive status that requires proteolytic cleavage for activation. MMPs are activated by several different enzymes, including serine proteases, furin, plasmin, and other MMPs. Activation involves cleavage at the N-terminal prodomain to generate the active form. MMPs exhibit four relatively conserved domains: an N-terminal prodomain, a catalytic domain, hinge region, and a C-terminal hemopexin-like domain (Fig. 1). The hemopexin-like domain is present in all MMPs except MMP-7 and -28. The hemopexin domain is thought to be responsible for the macromolecular substrate recognition specificity and for the interaction with the tissue inhibitors of metalloproteinases (TIMPs). A subclass of MMPs includes the membrane-type MMPs (MT-MMPs) that have an additional transmembrane domain to anchor them to the cell surface. Calcium is always required for MMP full activity and calcium chelators are used for an *in vitro* demonstration for MMP-specific

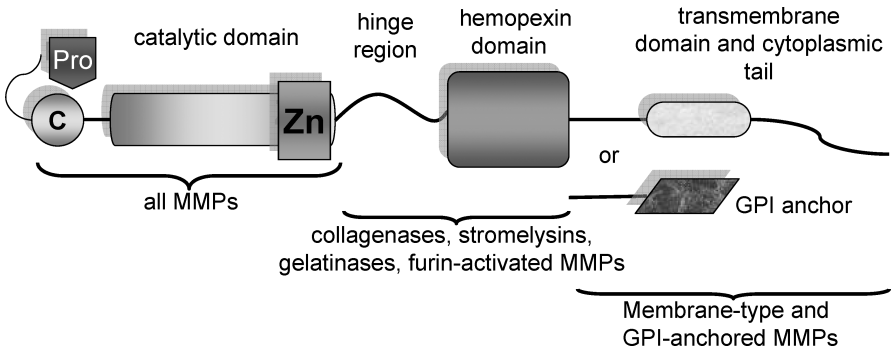

Figure 1 General structure of MMPs: basic domain structures and classification of the matrix metalloproteinases (MMPs) (modified from Lafleur et al. 2003). All MMPs have a signal peptide that targets the MMPs for secretion, a propeptide domain (containing a conserved Cys residue), and a catalytic domain. Some MMP subgroups also have a C-terminal hemopexin domain and a hinge region. Other MMP subgroups contain unique features such as: a transmembrane domain, a cytoplasmic tail, and a membrane-type (MT)-loop, a glycosylphosphatidylinositol (GPI) anchor or (not shown here) a furin recognition site (MT-MMPs, and MMP-11, -21, -23A/B, and -28), fibronectin type II repeats (MMP-2 and -9) and an N-terminal signal anchor, a cysteine array and an Ig-like domain (MMP-23).

involvement in an ECM-remodeling process (Tyagi et al. 1993).

MMPs are classified on the basis of domain structure but, more typically, they are listed by the *in vitro* ECM substrate preferences. However, it must be pointed out that MMPs are able to affect also several nonmatrix proteins such as cytokines, adhesion molecules, and receptors (Lindsey 2004). Further, while the substrate specificity for each MMP is far from being fully elucidated, it is well known that a substantial overlap exists among the different family members. Moreover, the overall function of a particular MMP is defined *in vivo* more by what substrates surround it and are cleaved by it than by which MMP is actually present (Lindsey 2004). This context-specificity of function determined by substrate availability explains why MMPs exert opposite effects, for example promote or inhibit growth in a given tissue at a given time (Egeblad and Werb 2002).

In this review we focus on the *in vivo* and *in vitro* cardiovascular effects produced by PD166793, a broad-spectrum inhibitor of MMPs. This compound, originally developed by Parke Davis, has been shown to be beneficial in the left ventricular remodeling process and in several animal models of congestive heart failure, regardless of the existence of a previous ischemic episode. Here, we illustrate aspects of PD166793 pharmacology and discuss studies in which MMPs have been inhibited by PD166793 to evaluate its impact particularly on the *in vivo* progression of congestive heart failure.

MPPs

Expression and Activation

MMP activity is generally negligible in steady-state basal conditions, but expression is rapidly induced by inflammatory cytokines, growth factors, and hormones (Nagase et al. 2006). Apparently, only MMP-2 is constitutively and substantially active in several tissues (Matrisian 1990; Saarialho-Kere et al. 1995) although this point has been recently reviewed (Schulz 2007). MMP expression is stimulated only when ECM remodeling is required, and this potentially explains why MMPs are regulated at multiple levels: (1) *transcription*; (2) *translation*; (3) *secretion;* and (4) *activation*. MMP gene expression is primarily regulated at the transcriptional level. Interestingly, Periostat (doxycycline hyclate), the only MMP inhibitor approved by the FDA to date, blocks MMP transcription and not MMP activity. However, several cytokines and growth factors modulate MMP mRNA stability and affect transcription rates of stromelysin (MMP-3), collagenases, and gelatinase A (MMP-2)(Overall et al. 1991; Delany and Brinckerhoff 1992; Birkedal-Hansen et al. 1993). The recent review by Chakraborti et al. offers an exhaustive overview of the possible transcription factors involved in MMP gene regulation (Chakraborti et al. 2003). In addition to the level of transcription, the activation of the pro-MMP precursor is another crucial regulatory step. Pro-MMPs remain quiescent until an activator intervenes. This "triggering" factor could be another MMP (e.g., MMP-3 can activate several MMPs) or different extracellular proteases (e.g., plasmin can activate MMP-3) (Murphy et al. 1999). However, some MMPs can be activated intracellularly by a pro-protein convertase furin (Stawowy and Fleck 2005).

Altering tissue redox conditions such as increased prooxidant levels or lowered NO bioavailability also activates latent MMPs (Henderson and Tyagi 2006). Although it seems unequivocal that reactive oxygen species may trigger MMP activation, whether an MMP inhibitor normalizes or only reduces excess in the

oxidative/nitrosative burden has not been determined yet. For instance, synthetic peroxynitrite ($ONOO^-$) can activate MMPs. In isolated rat hearts, the infusion of $ONOO^-$ into the coronary circulation leads to a rapid increase in the release of MMP-2 into the coronary effluent, along with a decline in contractile function (Gao et al. 2003). The use of PD166793 or trapping $ONOO^-$ with glutathione abrogated the effects of $ONOO^-$ on contractile function. Thus, $ONOO^-$-induced myocardial injury can be mediated by MMP-2. However, it remains unclear whether the MMP inhibitor PD166793 itself is able to scavenge peroxynitrite. PD166793 completely prevented angiotensin-II/tachypacing-induced diastolic dysfunction *in vivo*, but the extent of tissue redox imbalance (monitored by changes in GSH/GSSG ratio and a rise in malondialdehyde levels) were unchanged after MMPi in this subacute model of CHF (Paolocci et al. 2006).

Endogenous Inhibition

An imbalance between MMPs and the activity of their endogenous inhibitors, the TIMPs, may trigger ECM remodeling (Nagase et al. 2006). However, physiologic inhibitors of MMP not only include the TIMPs, but also more general inhibitors in the plasma such as $\alpha 2$ macroglobulin. TIMPs are low molecular weight proteins that collectively inhibit all activated MMPs. Some TIMPs are also able to bind pro-MMPs. Different TIMPs inhibit different MMPs with different specificities (Gomez et al. 1997). Binding of a TIMP to a MMP blocks the active site and prevents substrate access. TIMPs also have their own MMP-independent biological functions such as promoting cell proliferation (Corcoran and Stetler-Stevenson 1995), inhibiting angiogenesis (Chirco et al. 2006), and inducing cell death (Jourquin et al. 2005). These factors, coupled with a low plasma half-life for recombinant TIMPs, which would require high dosages and frequent dosing, limit the clinical use of recombinant TIMPs (Blavier et al. 1999). While TIMPs are the key MMP inhibitors in the tissue, plasma $\alpha 2$ macroglobulin thrombospondin-1, and thrombospondin-2 all bind MMPs and act as removal agents from the plasma environment (Baker et al. 2002). TIMPs are also sensitive to oxidative inactivation (Henderson and Tyagi 2006; Siwik et al. 2001), which contributes to the overall altered balance of proteinase/antiproteinase in LV remodeling.

Exogenous Inhibition of MMPs

Exogenous inhibition of MMPs can be achieved using inhibitors based on natural products or via synthetic inhibitors. Among the natural inhibitors are antibacterial drugs such as tetracyclines and actinonin and dietary polyphenols such as curcumin, cathecin, resveratrol, and genistein (Verma and Hansch, 2007). The anti-MMP activity of some of these compounds is likely due to the presence of a ring hydroxyl and/or carbonyl that chelates the active-site zinc(II) ion.

Synthetic MMP inhibitors (MMPi) have been designed based upon knowledge of the MMP structure (peptide sequence) and subsites, combined with the capability to chelate zinc at the active catalytic site (Skotnicki et al., 1999). As shown in table 2, the MMP affinity profiles vary among the different generations of MMP inhibitors. The first generation was broad-spectrum and could inhibit a large number of MMPs with the same effectiveness due to high homology among the MMP catalytic domains. These inhibitors were also nonspecific and inhibited a broad range of non-MMP proteases. This MMPi group includes collagen peptidomimetic and nonpeptidomimetic inhibitors, tetracycline derivatives, bisphoshonates (currently in use to treat bone re-absorption), thiols, and metal chelators. The most widely used zinc-chelating compounds contain a hydroxamic acid group (Skotnicki et al., 1999). Improving the effectiveness in chelating zinc, in combination with efforts to modulate the P1′ substituents to achieve more selectivity, has been the guiding direction for this first class of compounds. Batimastat (BB-94, British Biotech Pharmaceuticals, Oxford UK; see Table 2) was the first synthetic MMP inhibitor to be tested in clinical trials, based on early studies showing that batimastat covalently binds the zinc atom of the MMP active site. However, batimistat has poor solubility and its use was discontinued due to lack of oral bioavailability. A second generation hydroxymate MMP inhibitor, marimistat, has increased bioavailability (Table 2). Overall, these agents have shown no benefit in multiple cancer trials and have demonstrated several poorly tolerated side effects such as a dose-dependent joint and muscular pain (Ikejiri et al. 2005). Additional factors that have contributed to the failure of the first generation inhibitors include the ability of some of these agents to chelate other metals such as iron, calcium, magnesium, and copper, even more prominently than zinc itself (Farkas et al. 2004). Likely, this indiscriminate metal chelating power has greatly contributed to the toxic effects seen with these drugs.

The adjective "selective" implies that at lower concentrations, a given inhibitor will preferentially target one or only a few MMPs. As reported by Verma and Hansch, in order to confer such selectivity, two dominant molecular features should be considered and manipulated: (1) chelating moiety that interacts with the zinc ion and (2) hydrophobic extensions that protrude from the catalytic side into the hydrophobic S′ pocket (P1′ group) (Verma and Hansch 2007). Since the latter subsite is the one

that shows structural differences between MMP families, changes in P1′ should be considered in order to confer inhibitor selectivity (Engel et al. 2005; Verma and Hansch 2007).

Several strategies have been employed to generate new generation of peptidomimetic hydroxamate structures that are extremely effective. However, these inhibitors need to be further optimized to prevent metabolism and improve selectivity. Four major groups of nonhydroxamate inhibitors are under consideration: (a) zinc-binding inhibitors, (b) tetracycline derivatives, (c) bisphosphonate derivatives, and (d) endogenous MMP inhibitors. Developing MMP inhibitors that are selective for specific MMP will allow us to target specific processes and limit deleterious side effects observed with current inhibitors. Additionally, using MMP structure information will allow the design of more suitable inhibitors. Combined, these recent advances give us hope for the development of clinically viable MMP inhibitors.

As recently reported by J.T. Peterson (Peterson 2006), at least 56 MMP synthetic inhibitors have been studied as potential clinically relevant drugs, mostly to target cancer and arthritis. Ten of them also appear to have indications for cardiovascular diseases (systolic heart failure and post-ischemic cardiac remodeling). One of these compounds is PD166793, a MMP catalytic site inhibitor, the main topic of this review. However, only 7 of these 10 drugs remain on the stage at the moment, and only one (Periostat®) has been FDA-approved for periodontal diseases (Peterson 2004).

PD166793

PD166793 ($C_{17}H_{18}BrNO_4S$) is a cell-permeable biphenylsulfonylvaline compound [(S)-2-(4′-Bromo-diphenyl-4-sulfonylamino-3-methylbutyric acid)] with a molecular weight of 412.3 kDa (Fig. 2A). PD166793 is soluble in DMSO or ethanol. This MMP inhibitor was developed by Parke-Davis and its structure is tailored to bind to the active domain of all MMP types and endowed with a high (nanomolar range) inhibitory potency (Peterson et al. 2000). Thus, PD166793 is designated as a broad-spectrum and fairly potent MMP inhibitor. Moreover, in an extensive pharmacokinetic study conducted in the rat to identify the best possible candidates for chronic dosing of MMP inhibitors *in vivo*, O'Brien et al.(2000) have found that biphenyl derivatives substituted in the 4′-position with nonmetabolizable groups (such as PD166793) yield compounds that achieve high plasma concentrations with long elimination half-lives ($t_{1/2}$ = 34–117 h) when compared to a biphenylsulfonamide derivative ($t_{1/2}$ = 3.35 h). Conversely, when the substitution in the 4′-position is made with metabolizable groups the resulting MMP inhibition is potent but very short $t_{1/2}$ (<30 min).

As shown in table 1, (modified from Spinale et al. 1999), PD166793 acts as a potent inhibitor against MMP−2, −3, and −13 (IC_{50} = 47, 12, and 8 nM, respectively) and a weaker inhibitor of MMP−1, −7, −9, and −14 (IC_{50} = 6.1, 7.2, 7.9, and 0.24 μM, respectively)(Ye et al. 1992; Ye et al. 1994; Ye et al. 1995). Its mechanism of action is depicted in figure 2B. As previously reported (Spinale et al. 1999), at concentrations up to 100 μM/L PD166793 did not exhibit any inhibitory activity against angiotensin converting enzyme (ACE from rabbit lung)(Bunning et al. 1983), neutral endopeptidase from Burkitt lymphoma cells (Shipp et al. 1989), endothelin converting enzyme (Ahn et al. 1998), and tumor necrosis factor-α convertase (Welker et al. 1996). We have shown that in rat heart homogenates, PD166793 at 0.1 μM leads to a 20% inhibition in AMP deaminase (AMPD) activity (Paolocci et al. 2006). Conversely, additional data from our group indicate that PD166793 may have selective inhibitory action on AMPD in that other crucial enzymatic activities involved in the purine catabolism such as adenosine deaminase (ADA), purine nucleotide phosphorylase (PNP), 5′-nucleotidase (5′-NT), and xanthine oxidase are not inhibited by PD166793, at the same concentration (Tavazzi, Paolocci, and Lazzarino, unpublished data). Interestingly in rabbit skeletal muscle, the region 48–61 of AMPD contains a zinc-binding site (Mangani et al. 2007). It is, therefore, possible that, in addition to the well-known effects on MMPs, PD166793 may have inhibited AMPD, though only partially, by chelating the active zinc. Any zinc binding protein, therefore, is potentially inhibited by PD166793. Conversely, PD166793 does not appear to act as a "general" antioxidant in that, at least in a subacute regimen (5 mg/kg/day p.o., for 1 week) myocardial *in vivo* redox balance is not significantly affected (Paolocci et al. 2006)

Genetic Ablation or Inhibition of MPPs in Heart Failure

The involvement of MMP activity in contributing to normal and disease structure/function in the cardiovascular system is documented at many levels (Davis and Saunders 2006; Deschamps and Spinale 2006; Johnson 2007; Newby 2006; Rutschow et al. 2006; Spinale 2007; Ye 2006). MMPs have major implications in the normal growth of numerous tissues/organs, and vasculature and myocardium are no exceptions. For the MMP role in blood vessel remodeling and cardiac development, we refer you to other excellent reviews (Page-McCaw et al. 2007; Brauer 2006). Here, we limit our scope by

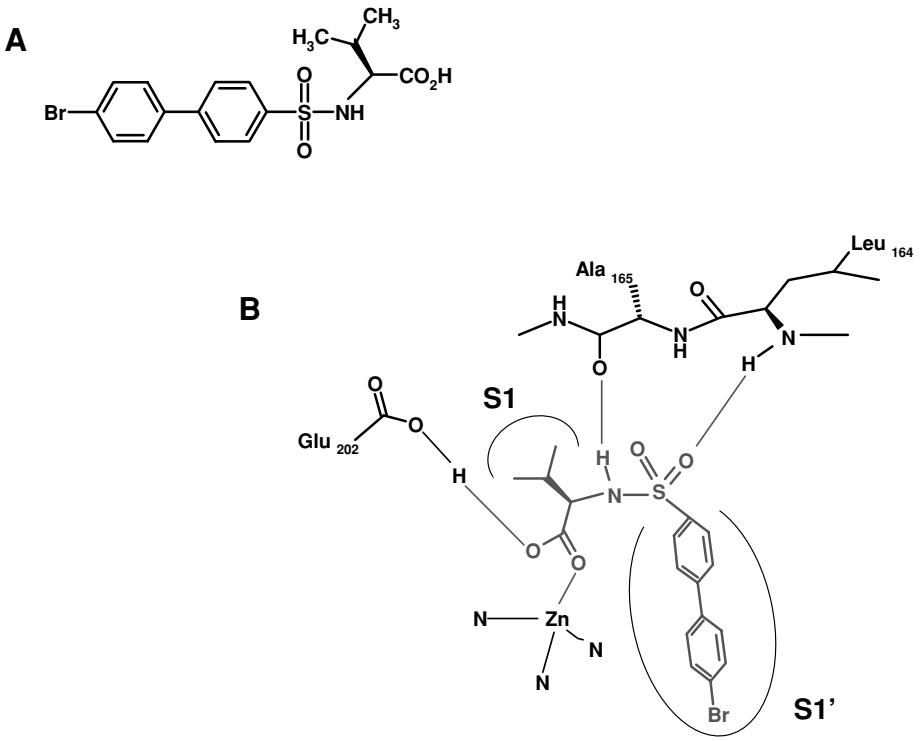

Figure 2 Structure of PD166793 (*panel A*). The tight binding of the inhibitor in the catalytic site of the enzyme is due to carboxylic acid—zinc ligation, the carboxylate hydrogen bonding with Glu202 and hydrogen bonding between the sulfonamide moiety and Leu164 and Ala165. In addition, S1' pocket present in MMP-3 is occupied by 4'-bromo- substituted biphenyl ring system resulting in a more potent inhibition (panel B). Modified from O'Brien et al. 2000.

Table 1 PD166793 inhibitory activity (modified from Spinale et al. 1999.)

Enzyme	Size (kD)	IC_{50}* or other inhibiting concentrations
MMPs		
MMP-1 (interstitial collagenase)	52/57	6.1 μmol/L*
MMP-3 (stromelysin 1)	52/58	0.012 μmol/L*
MMP-7 (matrilysin)	28	8.100 μmol/L*
MMP-2 (gelatinase A)	72	0.047 μmol/L*
MMP-9 (gelatinase B)	92	9.900 μmol/L*
Other Enzymes		
AMP deaminase	80–85	20% inhibition at 0.1 μmol/L

MMPs activity was determined using recombinant human MMP constructs of the catalytic domain and an artificial substrate assay system. For AMP deaminase activity, rat heart homogenates were incubated in presence of saturating AMP (3 mM) and increasing PD166793 concentrations. The amount of IMP produced in the reaction mixtures was assayed by HPLC and used to calculate the enzyme activity.

focusing on the appearance (activity/expression) of MMPs during the progression of congestive heart failure and LV remodeling, from the initial cardiac ischemic insult (acute myocardial infarction and LV remodeling) to the overt dilation and congestive heart failure. Then the impact of pharmacological MMP inhibition on CHF unfolding *in vivo* will be considered, emphasizing the structural and functional outcome observed with PD166793 treatment. Some of the most relevant *in vitro* studies employing PD166793 with respect to cardiac function are also discussed.

MMP Activation after Myocardial Infarction

After an ischemic insult, the myocardium undergoes a reparative process, involving inflammation, the

formation of new scar tissue, and LV remodeling. In this setting, MMP activation may intervene at different timepoints (Lindsey 2004; Vanhoutte et al. 2006).

Changes in MMP plasma levels in patients with acute MI have been carefully reviewed elsewhere (Lindsey 2004). Briefly, levels of MMP-9 and TIMP-1 are elevated in the plasma of patients with acute coronary syndrome (Inokubo et al. 2001). After 5 days from the ischemic insult, serum levels of MMP-1, a 54 kDa interstitial collagenase, and TIMP-1 inversely correlate with LV end-systolic volume index and directly with LV ejection fraction (Hirohata et al. 1997). The authors suggested that this likely reflects the healing phases of the infarct, although no further mechanistic explanations are provided for these correlations.

In humans, the temporal pattern of MMP and TIMP appearance in post-MI patients can be followed by evaluating the MMP/TIMP plasma profile. Recently, Webb et al. have found that MMP-8 and -9 acutely rise in the plasma of patients at day 3 post-MI. Others, such as MMP-2, were not changed in this study (Webb et al. 2006). TIMP-1 levels were constantly higher than the normal range (with a peak at 5 days), whereas TIMP-4 was markedly reduced, particularly at day 5 post-MI. In an earlier article, levels of inflammatory mediators such C-reactive protein (CRP), interleukin-6 (IL-6), and MMP-9 were positively correlated with the serum levels of troponin I, suggesting a contribution of MMP-9 to myocardial injury (Manginas et al. 2005). All in all, these and other clinical studies (Hirohata et al. 1997; Inokubo et al. 2001; Kaden et al. 2003; Li et al. 1999;) support and complement studies using animal models showing that MMPs and TIMPs are upregulated following MI (Webb et al. 2006). However, monitoring the patterns of MMP changes in the plasma of ischemic patients provides only a partial reflection of what is occurring within the myocardium. Certainly, plasma monitoring does not provide detailed information on regional distribution and actual proteolytic activity. Furthermore, different MMPs may sustain different aspects of the ischemic cardiomyopathy unfolding. For instance, serum levels of MMP-3 are significantly lower in the acute stage post-MI patients (48 h after MI) when compared to the recovery phase (3 months after the initial ischemic episode) (Samnegard et al. 2006).

Genetic Manipulation of MMPs and Ischemic Cardiomyopathy

Using a genetic approach, several studies employing MMP or TIMP gene knockout mice have proved a major role for MMPs in LV remodeling, particularly for gelatinases such MMP-2 and MMP-9. Hearts from MMP-9 $^{-/-}$ appear to be less prone to cardiac rupture after MI (Hey-

Table 2 Comparison of the affinity profiles of broad-spectrum MMP inhibitors.

MMP Inhibitor	Chemical name	Route of administration	MMP affinity profile
PD 166793	S)-2-(4′-Bromo-diphenyl-4-sulfonylamino-3-methylbutyric acid	p.o.	MMP-2, -3, -13, and -14
Batimastat (BB-94)	N^1-[(1S)-2-(methylamino)-2-oxo-1-(phenylmethyl)ethyl]-2-[2-methylpropyl)-3-[(2-thenylthio)methyl]buta-nediamide	i.p. (poor solubility and oral absorption)	MMP-1, -2, -3, -7, -9
CP-471,474	2-[4-(4-fluoro-phenyoxy)-benzenesulfonylamino]-N-hydroxy-isobutyramide	p.o.	MMP-2, -3, -9, -13
Ilomastat (GM 6001, galardin)	N′-hydroxy-N-((S)-2-indol-3-yl-1-(methylcarbamoyl)ethyl)-2-isobutylsuccinamide	p.o.	MMP-1, -2, -3, -7, -8, -9
Marimastat (BB-2516)	N-[2,2-dimethyl-1-(methylcarbamoyl)propyl]-2-[hydroxyl-(hydroxycarbamoyl)methyl]-4-methyl-pentanamide	p.o.	MMP-1, -2, 7, 9, 14
Ro 28–2653	5-biphenyl-4-yl-5-[4-(4-nitro-phenyl)-piperazin-1-yl]-pyrimidine-2,4,6-trione	p.o.	MMP-2, -9, -14
Ro 31–9790	*N*-2-((2S)-[(hydroxycarbamoyl) methyl)-4-methylvaleryl]-N-1,3-dimethyl-L-valinamide	p.o.	MMP-1, -2, -9

mans et al. 1999) while the deletion of the MMP-9 gene reduces LV dilation at 7 and 15 days (Ducharme et al. 2000). Romanic and coworkers have shown that knocking MMP-9 out (that is paralleled by TIMP-1 upregulation) reduces infarct size (Romanic et al. 2002). For a more extensive summary of the phenotypic effects observed after genetic ablation of MMPs, particularly of MMP-2 and -9, see elsewhere (Chow et al. 2007; Vanhoutte et al. 2006). However, it should be noticed that most of the MMP mutants created so far survive to birth and show only subtle phenotypic changes in the unchallenged setting (Page-McCaw et al. 2007). MMP-14 is an exception to this, as MMP-14 null mice are born with dwarfism and do not survive past 1 month of birth (Holmbeck et al. 1999). It is possible that during the embryonic development there is a high degree of enzymatic redundancy/compensation or an adaptive development. Alternatively, MMPs proteolytic activity may be relevant only after embryonic development.

MMP Inhibition during CHF Progression: The *In vivo* Impact of PD166793

A growing body of literature indicates that MMPi treatment may represent a useful therapeutic strategy to treat a variety of cardiovascular diseases, particularly to prevent cardiac remodeling in heart failure. In this setting, PD166793 has been widely used as a general inhibitor of MMP activity. Table 3 offers an exhaustive synopsis of almost all the *in vivo* studies conducted using the broad-spectrum MMP inhibitor PD166793 in animal models generating features of congestive heart failure and LV remodeling. A common denominator appears to be the ability of PD166793 to prevent or limit LV dilation, regardless of the specific model chosen, and of the presence/absence of genetic manipulation of key ECM enzymatic activities such as TIMPS (Fig. 3). While the mechanisms for these beneficial effects appear to vary from model to model, the theme is consistent. Using an MMP-2 antibody capture assay, Spinale et al. showed that PD166793 inhibits MMP-2 in a concentration-dependent manner in LV myocardial extracts (50% inhibition by 5 μmol/L PD166793), which is the same concentration as achieved in the heart after oral dosing (Spinale et al. 1999). With this PD166793 regimen, LV dilation was reduced in tachypacing-induced heart failure in pigs. The authors attributed this effect, at least in part, to increased myocardial collagen content from both untreated (tachypacing) heart failure hearts and controls. From an inotropic point of view, resting myocyte length was reduced in cardiac cells isolated from tachypacing-CHF pigs treated with PD166793 as compared to cardiac cells isolated from untreated pigs with CHF. Yet, steady-state myocyte contractile function remained the same in the two cells groups, and PD166793 did not improve β-adrenergic response in myocytes from failing hearts (Spinale et al. 1999). Thus, no inotropic benefit was noticed after PD166793, and studies in a subacute tachypacing (plus 2 days angiotensin II) confirmed the latter finding (Paolocci et al. 2006). Actually, in the Spinale's group study LV chamber stiffness increased with MMP inhibition, likely due to an absolute increase in LV myocardial stiffness. The latter effect is likely ascribable to the increased amount of collagen content and/or to changes in collagen patterns (i.e., subtype and/or crosslinking). At the same dose PD166793, given 5 days after infarction, reduced postinfarction dilation (end-diastolic cross-section area 8 weeks post-MI) and regional myocardial wall stress in pigs (Yarbrough et al. 2003). Moreover, MMP inhibition also lowered radial stress in the posterolateral MI wall. However, myocardial collagen content increased equally in the MI region of PD166793-treated or untreated pigs. Using a different model (hypertensive heart failure in rats), Peterson et al. found that MMP inhibition by PD166793 preserved LV geometry, attenuating LV remodeling and improving LV function during the transition to heart failure. Elevated peak wall stress persisted and, despite the beneficial effects on LV chamber dilation, inhibition of several MMPs (i.e., MMP-2 and -9 and -13) did not reduce the degree of LV hypertrophy. This study is relevant in that, differently from previous reports (Spinale et al. 1999), it demonstrates that MMP inhibition, even during CHF progression (and not at the onset of the CHF trigger), still exerts a favorable impact on LV remodeling, particularly when MMP inhibition is provided over a long period of time (4-month treatment) (Peterson et al., 2001). These studies do not provide unequivocal evidence for a role of total collagen in the beneficial action of MMP inhibition on LV remodeling. However, all concur in showing that wide-broad MMP inhibition prevents LV enlargement (Table 3 and Fig. 3). Perhaps, in addition to the collagen volume, changes in structure (collagen subtype ratios, extent of cross-linked collagen, etc.)(Holmes et al. 2005) may occur after chronic MMP inhibition with agents such as PD166793. For instance, in hearts subjected to rapid pacing the drug favors the appearance of increased fibrillar collagen weaves as opposed to the reduced and disrupted ones surrounding individual myocytes in failing, untreated hearts (Spinale et al. 1999). MMP inhibition via PD166793 seems also to affect the fate or to rescue the function of TIMPs. In mice deficient in the TIMP-1 gene and subjected to MI, the administration of PD166793 (30 mg/kg/day, p.o., started 3 days after MI) improved LV geometry and function, suggesting that the structural benefits lost after TIMP-1 gene deletion can be pharmacologically "rescued" by

Table 3 *In vivo* effects of PD166793 in animal models of congestive heart failure and left ventricle remodeling.

LV remodeling stimulus and reference	Species	Dose or plasma level of PD166793	Effects on examined MMPs and TIMPs	Cardiac effects
Tachypacing (chronic rapid pacing, systo-diastolic dysfunction) Spinale et al. (1999)	Pigs	20 mg/kg/day p.o. (3 days + 3 weeks of pacing) 93±10 μmol/L (plasma)	↓ MMP-2 (activity with capture assay) ↓ MMP activity (LV myocardial zymographic activity)	↓ *LV dilation* ↓ *LV peak wall stress* ↓ *LV load* ↑ *Fibrillar collagen thickness*
SHHF (spontaneously hypertensive heart failure) Peterson et al. (2001)	Rats	5 mg/kg/day p.o. 100μmol/L	↓ MMP-2 activity ↓MMP-9 (abundance) MMP-13 (no effect)	↓ *LV dilation* ↑ *Contractility* ↑*Thickness of fibrillar collagen*
Tachypacing McElmurray III et al. (1999)	Pigs	2 mg/kg/day p.o. + ACE inhibition	In normal porcine LV samples PD166793 (3μg/ml) led to >50% reduction in MMP zymographic activity	↓ *LV dilation* ↑ *LV pump function* ↓ *Chamber/myocardial stiffness*
SHHF Li et al. (2000)	Rats	5 mg/kg/day p.o. or ACE inhibition	↓ MMP-2 and -9 (abundance/zymographic activity) ↓ TIMP-1 and –2 ↑ TIMP-4	↓ *LV dilation* *Preserved LV systolic function* *Normalized MMP/TIMP express.* ↓ *collagen volume fraction*
Infrarenal aortocaval fistula (LV volume overload) Chancey et al. (2002)	Rats	1 mg/kg/day p.o.	-	↓ *LV dilation* ↓ *LV hypertrophy* *Preserved LV function*
Myocardial Infarct (circumflex art. ligation) Mukherjee et al. (2003)	Pigs	20 mg/kg/day p.o.	↓ MMP-13 abundance (infarct area) ↓ MMP-9 and MMP-2 levels (remote area) MT1-MMP normalized abundance (infarct area) ↑ TIMP-1 (infarct area)	↓ *Post-MI LV dilation* ↓ *Expansion of LV infarct*
Pressure overload in timp -/-3 mice Kassiri et al. (2005)	Mice	15 mg/kg/day p.o. + ablation of TNFα	↓ gelatinase activity ↓ collagenase activity	*Improved survival* *Prevention of LV dilation, cardiac dysfunction and hypertrophy*
Tachypacing + Angiotensin II (Subacute systodiastolic dysfunction) Paolocci et al. (2006)	Dogs	5 mg/kg/day p.o.	↓ MMP-2 and MMP-9 (abundance and activity)	↑ *diastolic compliance* ↓ *HEP depletion* ↓ *AMP catabolism*

p.o.- per oral; ↑- increase; ↓-decrease

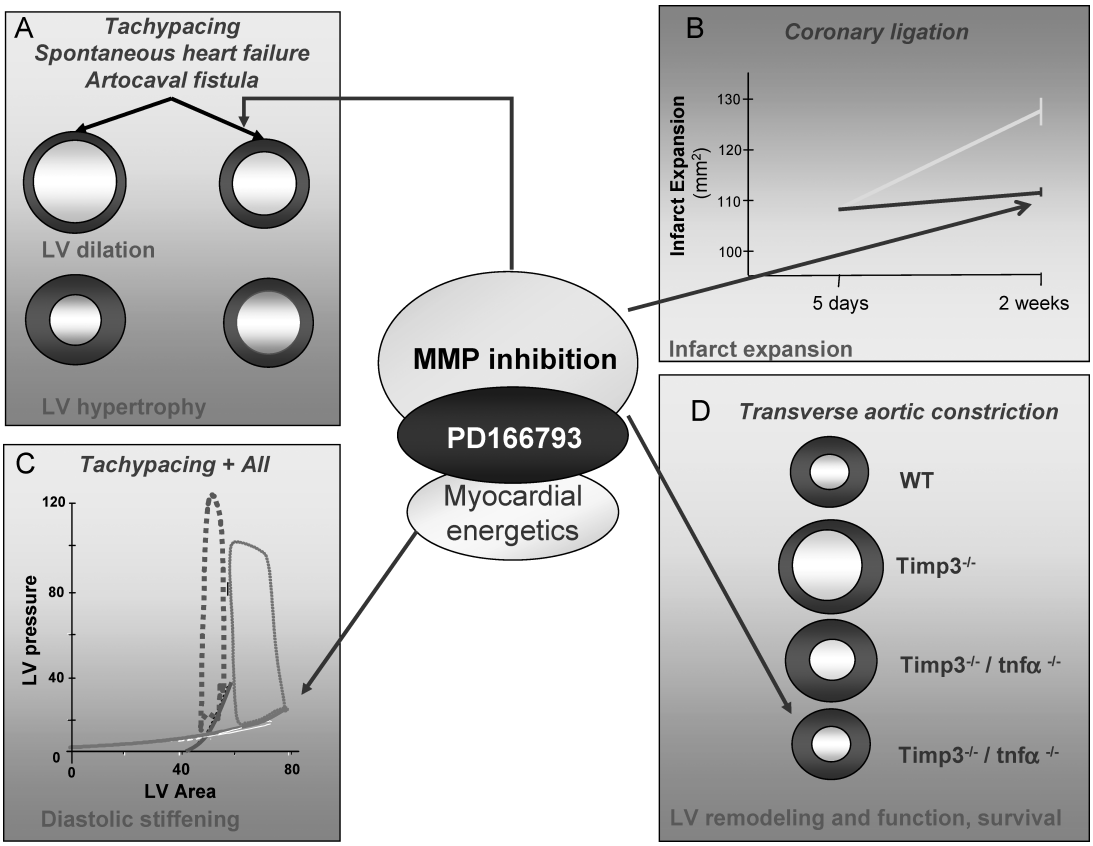

Figure 3 Effects on MMP inhibition by PD166793 in *in vivo* models of LV remodeling and pump failure. Panel B modified from Mukherjee et al. (2003); Panel C modified from Paolocci et al. (2006); Panel D modified from Kassiri et al. (2005).

MMP inhibition (Ikonomidis et al. 2005). These results define the importance of local endogenous control of MMP activity, that is, the effective balance between factors promoting and countering ECM remodeling, with respect to regulating LV structure and function after MI.

Another interesting aspect of MMP inhibition via PD166793 is the possibility of successfully combining it with angiotensin converting enzyme (ACE) inhibitors. In the tachypacing pig model, MMP and ACE inhibition (via fosinopril, 5 mg/kg/day) reduced LV wall stress and improved LV pump function (McElmurray, III. 1999). Furthermore, this combined intervention prevented the increased chamber and myocardial stiffness reported with MMP inhibition alone (Spinale et al. 1999). One initial explanation for this "interactive" (or apparent additive/synergistic) effects could be the profound impact exerted on arterial pressure and systemic vascular resistance. By positively modulating vascular smooth muscle tone and ameliorating loading conditions, these two drugs may contribute to reduce LV chamber and myocardial stiffness. The same combination (ACE and MMP inhibition by PD166793) was also proven to reduce fibrosis and normalize MMP/TIMP expression in SHHF rats (Li et al. 2000). Yet, in this setting only ACE inhibition was able to lower mRNA levels of collagen α1(I) and LV hypertrophy, suggesting that at least in this setting ACE and MMP inhibition affect LV geometry and function using distinctive pathways, which are yet to be fully determined. All in all, this suggests that inhibiting MMPs may not be sufficient to counter all the structural/functional abnormalities occurring during LV remodeling, particularly the decrease in diastolic ventricular compliance (Borlaug and Kass 2006). Very recently, Brower et al. found that ACE inhibitors directly prevent MMP-2 activity in LV tissue obtained from rats 24 h after creating an A-V fistula. ACE inhibition prevents the negative structural/functional changes occurring in this model (Brower et al. 2007), and it is likely that ACE inhibitors prevent MMP activity by virtue of their ability of binding to the catalytic zinc ion in MMPs, inactivating them. In the same model, the same group has shown

earlier that PD166793 is able to prevent adverse remodeling by attenuating LV dilation and improving contractility with respect to untreated A-V fistula (failing) group (Chancey et al. 2002). The combination of the two drugs may have amplified the effects of MMP inhibition alone, acting on several upstream pathways responsive to ACE inhibition and leading to MMP-2 activation. A cooperative effect of MMP inhibition by PD166793 has been also shown in mice lacking timp3 (timp3$^{-/-}$) (Kassiri et al. 2005) when these mice were subjected to TNF$_\alpha$ ablation. TIMP-3 lies upstream of both systems (MMPS and TNF$_\alpha$) and is reduced in CHF patients while its deficiency results in severe LV dilation and dysfunction in response to cardiac pressure overload (Fig. 3). Thus, this not only highlights again that matrix degradation and cytokines are both responsible for heart structural/functional changes, but also reiterates the idea that combined therapies may have more advantageous effects than MMP inhibition alone.

Lastly, it should be noted that other drugs, different from specifically designed MMP inhibitors, but still endowed with the ability of chelating zinc, may exert beneficial effects on LV remodeling and function similarly to those attributed to well-known MMP inhibitors. Along the same line, using an accelerated canine model of systo-diastolic dysfunction [angiotensin II infusion for 1 week with tachypacing superimposed in the last 2 days (AII+P)], our group has shown that AII+P activated MMP-2 and MMP-9 and specifically increased diastolic stiffening (+130% in chamber stiffness). Cotreatment with PD166793 prevented these changes, although myocardial collagen content, subtype, and crosslinking were unaltered in PD166793-treated versus the AII+P group. Conversely, myocardial energetics was profoundly affected by AII+P, that is, lowered ATP content, free energy of ATP hydrolysis [ΔG(ATP)], and phosphocreatine with increased free *ADP*, AMP catabolites (nucleoside-total purines), and lactate (Paolocci et al. 2006). PD166793 reversed most of these changes, in part due to its unexpected inhibition of AMP (AMPD) deaminase activity, likely due to PD166793 ability of chelating the active zinc that is crucial for AMPD activity (Ranieri-Raggi et al. 2003; Mangani et al. 2007). Admittedly, our study does not establish a direct, causal link between changes in myocardial energetics and diastolic stiffening with AII+P, nor does it prove that the beneficial effect on ventricular compliance was due to MMP inhibition or direct effects from the MMP inhibitor. In contrast to some reports (Spinale et al. 1999), in our laboratory MMP inhibition with PD166793 did not worsen diastolic stiffening, which is in agreement with SHHF rat studies showing improved diastolic compliance after MMP inhibition (Peterson et al. 2001). This could be due to the different doses and/or length of administration, and perhaps do not necessarily correlate with the total of collagen amount in all models.

In vitro Studies with PD166793 Relevant to Cardiac Failure

In addition to *in vivo* studies, PD166793 effects have been also tested in several different *in vitro* cell systems. These studies provide additional details about mechanisms that explain the beneficial actions of broad-spectrum MMP inhibitors such as PD166793 on LV remodeling while, at the same time, introduce new questions. For instance, in isolated rat working hearts, Qun Gao et al. have shown that PD166793 preserves cardiac work after 2 h infusion with a buffer containing interleukin-1β, interferon-γ and tumor necrosis factor-α, an effect mimicked by another broad-spectrum MMP-2 inhibitor (Ro31–9790) (Table 2) or by a MMP-2 neutralizing antibody (Gao et al. 2003). Collagen content was unchanged by the cytokine treatment whereas this study points to inflammation and proteolytic cleavage of troponin I induced by MMP-2 as one of the possible culprits of cytokine-induced myocardial dysfunction. Interestingly, Chapman et al. have shown that, in normal human cardiac fibroblasts, PD166793 (100 μM, for up to 36 h) significantly reduces MMP-9 activity. However, it appears that cells responded to MMP inhibition with an increased release of MMP-9 in the conditioned media, leading an overall increase of the MMP-9/TIMP-1 ratio of approximately 20% from time-matched values (Chapman et al. 2003). Similar results were obtained with batimastat, suggesting a common feature among broad-spectrum MMP inhibitors, whereby inhibiting MMP activity induces a feedback loop that stimulates MMP synthesis. These data suggest that inhibiting MMPs at the transcription level may be more effective than inhibiting at the activation level. This also ties back in with the clinical trials that documented efficacy of Periostat® in blocking MMPs through effects at the level of transcription, not activation.

Limitations of Current MMP Inhibitors

Multiple reasons potentially explain why more MMP inhibitors are not under development, the primary of which is the detrimental side effect observed in early clinical trials. As J. Thomas Peterson explains in his recent reviews, achieving an optimal therapeutic index remains a challenge (Peterson 2004; Peterson 2006). The development of the musculoskeletal syndrome (MSS) was the most severe side effect observed with first generation inhibitors. Characterized by joint pain, stiffness, edema, skin discoloration, and reduced mobility, MSS is a tendonitis-like

fibromyalgia that was so severe in MMP inhibitor-treated patients that they requested to be removed from the study (Peterson 2004; Peterson 2006). We first thought that MSS was caused by MMP-1 inhibition. Later studies with MMP-1 sparing inhibitors, however, have documented that MMP-1 inhibition is not essential for MSS. We now believe that the sheddase activity of non-MMP metalloproteinases, such as adamalysins or tumor necrosis factor alpha converting enzyme are being inhibited by the first generation inhibitors. While we still need to more clearly define sheddase-sparing activity, maintaining sheddase activity continues to be a design objective with MMP inhibitors (Fingleton 2007). While PD166793 was never used in clinical trials due to its poor water solubility, we predict that this inhibitor would yield similar MSS symptoms.

Another reason attributed to the lack of success with MMP inhibitors is that most MMP inhibitors are also nonspecific for individual MMPs, which is due to the particular loop residues in the S1′ subsite pocket. Differences in the subsite pocket differentiate between shallow pocket (MMP-1 and -7) and deep pocket (MMP−2, −3, −8, −9, −12, −13) MMPs (Overall and Kleifeld 2006). MMP inhibitors are nonspecific for individuals within a classification. Some MMP−12 and −13 inhibitors are exceptions, because these MMPs have an additional side pocket that can be targeted to achieve specificity (Chen et al. 2000).

MMP Inhibition in Cardiovascular Diseases: From PD166793 to Future Drugs

Some of the novel, and somewhat serendipitous, aspects of PD166793 pharmacology are important not only because they go beyond LV remodeling but also for the fact that much of our current knowledge of the effects produced by MMPs and their inhibition comes from studies employing this agent. Equally important is to confirm, using different CHF models, whether other signals, different from those that regulate ECM turnover, can be influenced by MMP inhibition, or whether changes in energetics of the myocardium may contribute to alter MMP and TIMP expression. The use of new, more selective MMP inhibitors (less toxic than current inhibitors, including PD166793) may help to address these points.

The good news is that we now have much more information on which relevant clinical markers and diagnostic tools are most appropriate for judging efficacy. Early inhibition versus late inhibition, will likely yield a more beneficial response for some cardiovascular disease processes but not others. Using this knowledge, we can structurally adjust existing inhibitors to increase selectivity, remove toxicity and improve bioavailability, which would greatly improve clinical relevance (Fingleton 2007). A review by B. Govinda Rao summarizes recent developments using structural and computational studies that are currently being employed to design highly specific MMP inhibitors (Rao 2005). MMP inhibitors in the future will take advantage of structural similarities and differences between MMP family members to improve outcomes.

Conclusions

Experimental evidence supports a major role of MMP inhibition (by PD166793) to counter the maladaptive LV remodeling process taking place in multiple animal models of congestive heart failure. However, we are currently limited by the lack of MMP inhibitors approved for clinical trials to treat decompensated hearts, in part due to unwanted side effects of drugs such as PD166793. Moreover, while MMP inhibition can prevent LV structural and functional changes, it is likely not sufficient to offset the multitude of changes that occur in the myocardium during the transition from compensated hypertrophy to maladaptive remodeling and pump dysfunction.

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Conflict of Interest

The authors have no conflict of interest.

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