Myocardial Infarct Expansion and Matrix Metalloproteinase Inhibition

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Background—A potential mechanism for left ventricular (LV) remodeling after myocardial infarction (MI) is activation of the matrix metalloproteinases (MMPs). This study examined the effects of MMP inhibition (MMPi) on regional LV geometry and MMP levels after MI.

Methods and Results—In pigs instrumented with radiopaque markers to measure regional myocardial geometry, MI was created by ligating the obtuse marginals of the circumflex artery. In the first study, pigs were randomized to MMPi (n=7; PD166793, 20 mg·kg⁻¹·d⁻¹) or MI only (n=7) at 5 days after MI, and measurements were performed at 2 weeks. Regional MI areas were equivalent at randomization and were increased in the MI-only group at 2 weeks after MI compared with the MMPi group. In the second study, pigs randomized to MMPi (n=9) or MI only (n=8) were serially followed up for 8 weeks. At 8 weeks after MI, LV end-diastolic dimension was lower with MMPi than in the MI-only group (4.7±0.1 vs 5.1±0.1 cm, P<0.05). Regional MI area was reduced with MMPi at 8 weeks after MI (1.3±0.1 vs 1.7±0.1 cm², P<0.05). MMPi reduced ex vivo MMP proteolytic activity. In the MI region, membrane-type MMP levels were normalized and levels of the endogenous tissue inhibitor of MMPs (TIMP-1) were increased compared with normal levels with MMPi. These effects were not observed in the MI-only group.

Conclusions—MMPi attenuated the degree of post-MI LV dilation and expansion of the infarct during the late phase of MI healing. In addition, exogenous MMPi caused region-specific modulation of certain MMP and TIMP species.

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Key Words: myocardial infarction ■ metalloproteinases ■ inhibitors

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t ventricular (LV) regional myocardial dysfunction and remodeling that occur immediately after myocardial infarction (MI) can persist long after the acute insult.1-6 The summation of cellular and extracellular events that occur in the post-MI period results in changes in LV geometry and has been called “infarct expansion.”3-6 Past studies have demonstrated that a structural determinant of infarct expansion is extracellular remodeling.1,5,6 A family of proteolytic enzymes that have been implicated in tissue remodeling are the matrix metalloproteinases (MMPs).7 Increased MMP expression has been reported in patients with end-stage heart failure and in several animal models of developing LV dysfunction.8-16 Increased interstitial MMP activity has been demonstrated to occur directly within the ischemic myocardium.17 Past studies have demonstrated that exogenous MMP inhibition (MMPi) can influence the myocardial remodeling process.13-16 However, the effects of MMPi on infarct expansion during the later phases of post-MI healing remain unknown. Moreover, the effect of post-MI MMPi on regional MMP species expression remains unclear. The present study tested the hypothesis that increased myocardial MMP activation is a contributory mechanism for continued expansion of the healing infarct.

Methods

Mature Yorkshire pigs (23 to 25 kg, Hambone Farms, Orangeburg, SC) were instrumented to measure the effects of MMP inhibition on global and regional LV geometry after MI. To examine the effects of MMPi in the early and late post-MI periods, this study was performed as 2 substudies. In the first substudy, changes in global LV and regional MI geometry that occurred within 2 weeks after MI were examined. In the second substudy, the effects of sustained MMPi on global and regional MI geometry were measured serially for 8 weeks after MI. Ten weight-matched noninstrumented pigs were used as reference, non-MI controls. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, 1996).

Substudy 1: Early Post-MI Remodeling

The animals were anesthetized with 2% isoflurane, and an arterial line was placed as described previously.13,17 Through a left thoracotomy, snare occluders were placed loosely around the first 2 obtuse marginals of the circumflex coronary artery (OM1, OM2), and the distal ends of the snare were secured in a subcutaneous
Cinefluoroscopic images of the myocardial markers were recorded with a high-speed cinefluoroscope (Philips Cardio-Diagnost) and were digitized (ATI ELISA). The distal ends of the snare were exteriorized, and MI was induced by tightening of the snare. Occlusion of the coronary arteries resulted in discrete, transmural MI of 23±1%. At 5 days after MI, echocardiographic and fluoroscopic measurements were repeated, blood was collected, and mean arterial pressure was monitored. Plasma MMP-2 levels were measured by ELISA. The distal ends of the snare were exteriorized, and MI was induced by tightening of the snare. Occlusion of the coronary arteries resulted in discrete, transmural MI of 23±1%.

At 5 days after MI, echocardiographic and fluoroscopic measurements were repeated, blood was collected, and the animals were randomized to remain untreated (n=8) or receive MMPi (n=9) at 5 days after MI. Echocardiographic and fluoroscopic measurements were made at 2-week intervals up to 8 weeks after MI. At the conclusion of the study period, the LV was quickly extirpated. Full-thickness sections from the MI, border (0.5-cm perimeter of the MI), and remote regions were obtained.

LV Myocardial MMP Levels

At 8 weeks after MI, the abundance of MMP-13, MT1-MMP (membrane-type MMP), and the endogenous tissue inhibitors of MMP (TIMP-1 and TIMP-4) were examined by immuno blotting. Relative LV MMP-2 and MMP-9 levels were examined by use of substrate-specific zymography. Specific ex vivo MMP-2 activity was measured in crude tissue homogenates with an antibody capture assay.

Data Analysis

Temporal changes in global LV dimensions and regional marker area were compared between the MI groups by 2-way ANOVA. Hemiodynamic changes at terminal study were compared by 1-way ANOVA. Regional LV myocardial MMP levels were compared by 2-way ANOVA. Temporal changes in global LV dimensions and regional marker area were compared by 2-way ANOVA. Time-Dependent LV Remodeling in the Late Post-MI Period: Effects of MMPi

At 8 weeks after MI, LV end-diastolic dimensions were lower in the MI+MMPi group than the MI-only group (Figure 2C). In the MI-only group, the wall thinning index was 4.6% lower (28 ± 2%; P<0.05) than pre-MI values, with no difference between the 2 groups. At 2 weeks after MI, LV end-diastolic dimension was increased over pre-MI values by 14 ± 2% (P<0.05) in the MI-only group and by 12 ± 3% (P<0.05) in the MMPi (MI+MMPi) group. The increase in LV end-diastolic dimension from pre-MI values, however, was not different between the 2 groups. The baseline marker area was 1.00±0.01 cm² and was not different between the 2 groups. At 5 days after MI and before randomization, marker areas were increased from pre-MI values (Figure 1A), and the rate of increase in marker area was similar between the 2 groups (Figure 1B). At 2 weeks after MI, the extent and rate of change in marker area were lower in the MI+MMPi group than the MI-only group (Figure 1A and B). Plasma MMP-2 levels (Figure 1C) were similar between the 2 MI groups before randomization. At 2 weeks after MI, plasma MMP-2 levels were increased in the MI-only group.

Time-Dependent LV Remodeling in the Late Post-MI Period: Effects of MMPi

At 8 weeks after MI, LV end-diastolic dimensions were lower in the MI+MMPi group than the MI-only group (Figure 2C). In the MI-only group, the wall thinning index was 4.6% lower (28 ± 2%; P<0.05) than pre-MI values, with no difference between the 2 groups. At 2 weeks after MI, LV end-diastolic dimension was increased over pre-MI values by 14 ± 2% (P<0.05) in the MI-only group and by 12 ± 3% (P<0.05) in the MMPi (MI+MMPi) group. The increase in LV end-diastolic dimension from pre-MI values, however, was not different between the 2 groups. The baseline marker area was 1.00±0.01 cm² and was not different between the 2 groups. At 5 days after MI and before randomization, marker areas were increased from pre-MI values (Figure 1A), and the rate of increase in marker area was similar between the 2 groups (Figure 1B). At 2 weeks after MI, the extent and rate of change in marker area were lower in the MI+MMPi group than the MI-only group (Figure 1A and B). Plasma MMP-2 levels (Figure 1C) were similar between the 2 MI groups before randomization. At 2 weeks after MI, plasma MMP-2 levels were increased in the MI-only group.

MMP Abundance and Zymographic Levels With Chronic MI: Effects of MMPi

In the remote and border regions, MMP-13 abundance was reduced from controls in both MI groups (MI-only group: remote, 52 ± 6%; border, 62 ± 7%; MI+MMPi group: remote, 64 ± 11%; border, 55 ± 10%; P<0.05 versus control level of 100%). MMP-13 abundance in the MI region was lower than in controls in the MI+MMPi group (72 ± 11%, P<0.05).

By substrate-specific zymography, proteolytic bands consistent with that for MMP-9 could be identified in 20 of 42 samples for the MI-only group and in 18 of 54 samples for the MI+MMPi group (χ² = 0.16). In the remote region, detectable MMP-9 levels were lower in the MI+MMPi group than the MI-only group (25 742 ± 9211 versus 82 223 ± 26 418 pixels, P<0.05, Mann-Whitney test). Gelatinolytic bands consistent with that for MMP-2 were detected in all myocardial samples (Figure 3). Compared with the MI-only group, MMP-2 levels in the remote region were reduced with MMPi. In the MI-only group, tissue MMP-2 activity measured as a function of proteolytic product formation (Figure 4) revealed...
that MMP activity in the MI region was higher than control values. With MMPi, MMP-2 activity at the MI region was lower than that of the MI-only group and was normalized to control values.

MT1-MMP abundance was significantly higher in the MI region in the MI-only group than reference control values (Figure 5). With MMPi, MT1-MMP abundance was reduced from reference control values in the remote and border regions and was normalized in the MI region. In the MI and border regions, MT1-MMP abundance was lower with MMPi.

Prominent bands for TIMP-1 were detected at 28 and 56 kDa. The 56 kDa band was a dimer for TIMP-1 and was analyzed for each region (Figure 6A). TIMP-1 levels were increased over control values in the MI region of the MI+MMPi group. In the remote and MI regions of the MI+MMPi group, TIMP-1 levels were higher than in the MI-only group. In the remote region, TIMP-4 levels were higher in the MI+MMPi group than in the MI-only group (Figure 6B).

**Discussion**

LV remodeling that occurs after MI has been demonstrated to result in progressive LV dilation. At the infarcted region, alterations in myocardial composition and structure occur after MI and can lead to infarct thinning and expansion. Because the MMPs are enzymes that influence myocardial remodeling, the goal of the present study was to examine the relation between MMP activation and infarct expansion. The important and unique findings of this study were that MMPi in the post-MI period attenuated the degree of global LV dilation, which was a direct result of a reduction in the extent and rate of infarct expansion during the later phase of post-MI healing. Contributory mechanisms for these LV remodeling effects with MMPi after MI were 2-fold. First, there was a direct pharmacological inhibitory effect on MMP

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Figure 1. A, Time-dependent changes in regional MI size, defined as end-diastolic area encompassed within myocardial markers for pigs without MMPi (MI Only) or with MMPi (MI+MMPi). Before randomization at 5 days after MI, marker area was similarly increased from baseline values in both MI groups. At 2 weeks after MI, marker area was higher in MI-only group than MI+MMPi group. B, Rate of change in marker area was similar between MI groups before initiation of MMPi. At 2 weeks after MI, rate of increase in marker area was lower in MI+MMPi group. C, Plasma MMP-2 levels were similar in MI groups before randomization and increased in MI-only group at 2 weeks after MI. +P<0.05 vs MI only, #P<0.05 vs baseline.
activity in the MI region. Second, post-MI MMPi caused region-specific modulation of certain species of MMPs and TIMPs. Thus, these results demonstrate that localized alterations in MMP/TIMP levels are a contributory mechanism for late expansion of the healing infarct.

Infarct sizes obtained in the present study are consistent with those reported previously. Moreover, similar MI size in patients was associated with post-MI LV dilation.

Therefore, in the present study, MI induction in pigs most likely recapitulated a commonly encountered clinical phenotype of post-MI remodeling. However, it must be recognized that the location and size of the MI may play an important role in the determination of post-MI LV remodeling and LV function and/or dysfunction in the post-MI

Figure 2. A, Time-dependent changes in regional MI size, defined as end-diastolic area encompassed within myocardial markers. Regional MI size remained smaller in MI + MMPi group than MI-only group throughout post-MI period. B, Rate of change in regional MI size, computed relative to week 2 values, was significantly lower in MI + MMPi group than MI-only group. C, Time-dependent changes in LV dimensions in post-MI period for pigs in MI-only or MI + MMPi groups were determined as change from baseline values. Change in end-diastolic dimensions from baseline values was lower in MI + MMPi group than MI-only group at 8 weeks after MI. $P < 0.05$ vs MI only, $# P < 0.05$ vs baseline or week 2.

Figure 3. MMP zymographic bands were detected in all myocardial samples at 68 to 72 kDa, consistent with that for MMP-2. In remote region, zymographic MMP-2 levels were lower in MI + MMPi group than MI-only group. In MI region, zymographic MMP-2 levels were higher than remote and border regions for both MI groups. *$P < 0.05$ vs controls, +$P < 0.05$ vs MI only, $\# P < 0.05$ vs remote region, $\& P < 0.05$ vs border region.
period. For example, in a past report, Jugdutt reported that infarcts created in the anterior LV wall were associated with a greater degree of LV dilation and infarct expansion than infarcts in the posterior LV created by occlusion of the circumflex artery. In the present study, the size and location of the MI were chosen to prevent acute changes in ejection performance and systemic hemodynamics. Nevertheless, future studies are warranted to examine the effects of MMPi on post-MI LV remodeling that is superimposed with reduced ejection performance and the effect of infarct location.

The rationale for selecting the duration and the initiation of MMPi in the post-MI period was based on past observations. In a mouse model of MI, Rohde et al demonstrated that MMPi instituted immediately after MI attenuated the degree of LV dilation that occurred over a period. For MI + MMPi group, MMP-2 activity was lower than control values in remote and border regions and was normalized in MI region. MMP-2 activity in MI region was lower in MI + MMPi group than MI-only group. *P < 0.05 vs controls, +P < 0.05 vs MI only, §P < 0.05 vs remote region, ¶P < 0.05 vs border region.

Figure 4. Ex vivo MMP-2 activity in tissue homogenates. Inset, Time-dependent proteolytic product formation with a recombinant MMP-2 standard. Double-reciprocal plots constructed to compute maximal velocity of substrate formation and Michaelis-Menten constant were linear, and these values were used to determine MMP-2 proteolytic product formation. In MI-only group, MMP-2 proteolytic activity was higher than control values at MI region. For MI + MMPi group, MMP-2 activity was lower than control values in remote and border regions and was normalized in MI region. MMP-2 activity in MI region was lower in MI + MMPi group than MI-only group. *P < 0.05 vs controls, +P < 0.05 vs MI only, §P < 0.05 vs remote region, ¶P < 0.05 vs border region.

Figure 5. Robust signals for MT1-MMP were detected in all myocardial samples. In remote region, MT1-MMP abundance was lower in MI + MMPi group than control values. At border and MI regions, MT1-MMP abundance in MI + MMPi group was lower than MI-only values. *P < 0.05 vs controls, +P < 0.05 vs MI only, §P < 0.05 vs remote region, ¶P < 0.05 vs border region.
period of 4 days after MI. However, a previous study demonstrated that the levels of various MMP species can remain elevated for several weeks in the post-MI period. Therefore, the present study was performed to define the spatiotemporal relationship between regional MI and chamber remodeling with prolonged MMPi. MMPi instituted at 5 days after MI reduced regional MI size and expansion rate by 2 weeks, and these effects persisted when treatment was continued for up to 2 months. Furthermore, the early attenuation of regional MI expansion was translated to a reduction in LV chamber dilation at 2 months after MI.

The gelatinases, such as MMP-2 and MMP-9, proteolytically process fibrillar collagen fragments and other critical basement membrane proteins of the extracellular matrix. In the remote myocardium, zymographic MMP-2 levels were reduced with MMPi. Therefore, this observation suggests that MMPi modulated MMP-2 levels in a region-specific manner after MI, which in turn would probably influence interstitial proteins such as basement membrane components. MMP-9 was variably expressed within the myocardium at 8 weeks after MI. Levels of the collagenase MMP-13 were reduced from control values in the viable myocardium at 8 weeks after MI. Because MMP-9 and MMP-13 abundance in the post-MI myocardium has been demonstrated to increase early after MI and then decrease later in the healing process, the reduction in the levels of these MMP species at 8 weeks after MI may
be a result of a time-dependent response in the post-MI period. The MT-MMPs have been demonstrated to degrade components of the extracellular matrix and activate other MMPs.\textsuperscript{10} In the present study, the relative reduction in MT1-MMP levels with MMPi may have reduced the degree of pericellular matrix degradation and reduced the activation state of other MMP species.

The TIMPs provide endogenous control of MMP activity.\textsuperscript{9,10,12,14} TIMP-1 and TIMP-4 have been demonstrated to be present in the myocardium.\textsuperscript{9,10} In the present study, exogenous MMPi was associated with increased TIMP-1 abundance after MI, suggesting that pharmacological MMPi increased TIMP-1–mediated MMP regulation. However, TIMP-4 abundance appeared to be reduced in the post-MI myocardium. Interestingly, chronic MMPi was associated with a region-specific increase in TIMP-4 levels in the region remote to the MI. The region-specific changes in MMP/TIMP levels with pharmacological MMPi puts forth the intriguing possibility that exogenous MMPi may actually influence endogenous synthesis and release of MMP/TIMP species in the post-MI period. Nevertheless, it must be recognized that TIMP-2 levels were not measured in the present study. Because past studies have demonstrated that time-dependent changes in TIMP-2 levels can occur after MI,\textsuperscript{12,22} the effects of MMPi on TIMP-2 expression in the post-MI setting warrant further investigation.

In the present study, regional ex vivo MMP-2 proteolytic activity was higher in the MI region than in controls. In contrast, chronic MMPi reduced ex vivo MMP activity within the MI region, thereby confirming that local pharmacological MMPi was achieved. Because broad-spectrum MMPi was used in the present study, the question of which MMP species were causative in the post-MI LV remodeling process remains unclear. Therefore, identification of those MMP species that emerge in LV remodeling after MI would be necessary to develop more targeted MMPi.

Study Limitations and Summary

In the present study, transthoracic echocardiograms were used to serially determine global LV geometry in the post-MI period. Although these measurements provided information on LV dimensions and fractional shortening, computations of LV volumes based on multiple short-axis sections or apical views could not be performed. Consequently, changes in LV geometry in the peri-MI region, ie, bulging or expansion indices,\textsuperscript{5,6} were not determined.

A well-defined temporal sequence of cellular and extracellular events occurs after acute MI and determines the initial wound-healing response.\textsuperscript{5–7} The early, or acute, phase of post-MI wound healing is associated with structural remodeling and neutrophil infiltration within the infarcted myocardium.\textsuperscript{5,6} Early abrogation of MMP activity, either through genetic modification or pharmacologically, has been associated with abnormalities in the post-MI wound-healing response.\textsuperscript{19–21} Accordingly, the present study deployed MMPi 5 days after MI to avoid the confounding influences surrounding the acute phase of an MI and the potential deleterious effects of early modulation of MMP activity. Because MMPi was instituted at 5 days after MI, it is unlikely that this pharmacological approach resulted in significant myocardial salvage within the MI region. Rather, it is likely that MMPi reduced MMP activation within the border zone of the MI and may have contributed to the attenuation in infarct expansion. However, because myocardial MMP/TIMP levels were not measured in the early post-MI period, this issue remains speculative and warrants further investigation. Finally, it must be recognized that these studies were performed in an animal model of MI, and future studies must be performed to further elucidate the mechanistic aspects of MMPi in the post-MI setting. Nevertheless, the unique results of the present study provide proof of concept that post-MI MMPi can attenuate the extent and rate of infarct expansion and modify local MMP/TIMP species expression during the late phase of post-MI healing.

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