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Selective Targeting and Timing of Matrix Metalloproteinase Inhibition in Post–Myocardial Infarction Remodeling

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Background—A cause-and-effect relationship exists between matrix metalloproteinase (MMP) induction and left ventricular (LV) remodeling after myocardial infarction (MI). Whether broad-spectrum MMP inhibition is necessary and the timing at which MMP inhibition should be instituted after MI remain unclear. This study examined the effects of MMP-1 and MMP-7–sparing inhibition (sMMPi) on regional and global LV remodeling when instituted before or after MI.

Methods and Results—Pigs instrumented with coronary snares and radiopaque markers within the area at risk were randomized to MI only (n=11) or sMMPi (PGE-530742, 10 mg/kg PO TID) begun 3 days before MI (n=11) or 3 days after MI (n=10). Eleven weight-matched noninstrumented pigs served as reference controls. At 10 days after MI, infarct size was similar between groups (47±3% of the area at risk). Marker area increased from baseline in the MI-only group (10±3%, P<0.05) but was unchanged with sMMPi. LV end-diastolic volume increased in the MI-only group (82±3 mL) compared with controls (56±3 mL, P<0.05) but was attenuated with pre-MI and post-MI sMMPi (69±3 and 69±4 mL, respectively, P<0.05). Collagen content increased in the infarct zone of the MI-only group (34±5%) compared with control (2±1%, P<0.05) but was reduced with pre-MI and post-MI sMMPi (24±1% and 23±2%, P<0.05). Collagen content increased in the border zone (12±2%) and decreased in the remote zone (3±1%) of the pre-MI sMMPi group compared with post-MI sMMPi values (7±1% and 5±1%, P<0.05).

Conclusions—Inhibition of MMP-1 and -7 is not required to favorably influence LV remodeling after MI. Moreover, a temporal difference exists with respect to the timing of sMMPi and regional and global myocardial remodeling patterns after MI. (Circulation. 2003;108:1753-1759.)

Key Words: myocardial infarction ■ metalloproteinases ■ inhibitors

Myocardial infarction (MI) evokes changes within the architecture of the left ventricular (LV) wall leading to chamber dilation. This process, called LV remodeling, is an independent determinant of morbidity and mortality after MI.1 The matrix metalloproteinases (MMPs) are an endogenous family of endopeptidases that degrade all components of the myocardial extracellular matrix.2 Experimental studies using pharmacological compounds that inhibit all MMPs (broad-spectrum inhibitors) have been demonstrated to directly affect LV remodeling after MI.3–5 Thus, a cause-and-effect relationship between induction of MMP activity and LV remodeling after MI is emerging to emerge. However, whether broad-spectrum MMP inhibition is necessary to favorably modulate LV remodeling after MI remains unclear. MMP-1 is reduced in the myocardium of patients with LV failure, and experimental studies suggest that this MMP may not be causative to pathological LV remodeling.6 Moreover, the potential role of MMP-7 in adverse LV remodeling after MI remains to be defined. Accordingly, the first objective of the present study was to test the hypothesis that selective MMP inhibition (sMMPi) that spared MMP-1 and -7 would favorably alter LV remodeling after MI. Past studies of MI suggest that early MMP inhibition may adversely affect normal wound-healing responses.5,7,8 Whether and to what degree differences exist between the institution of MMP inhibition at the time of MI or after initial wound-healing responses remains unknown. Therefore, a second objective was to examine whether differential effects on LV remodeling were achieved with sMMPi instituted before MI as opposed to delayed administration.

Methods

Pharmacokinetics/MMP Inhibitory Profile

Yorkshire pigs (n=3, 30 to 32 kg, Hambone Farms, Orangeburg, SC) were instrumented with arterial lines as previously described4,9 to
Figure 1. Top, Oral administration of 10 mg/kg PGE-530742 achieved plasma concentrations below that necessary to inhibit MMP-1 and -7 (IC₅₀=1000 ng/mL) and above that required for inhibition of other MMPs. Computed steady-state plasma level for PGE-530742 was 113 ng/mL, with a minimum level of 15 ng/mL. PGE-530742 (10 mg/kg) was administered 3 times daily to yield this targeted plasma level. Bottom, Ability of PGE-530742 to inhibit human recombinant MMP-1,-2,-3,-7,-8,-9, and -13 in vitro was evaluated kinetically with a quenched fluorescence substrate assay. Concentrations producing 50% inhibition of enzyme activity (IC₅₀) were calculated from mean±SD of percentage inhibition for PGE-530742 that yielded between 10% and 90% inhibition of enzyme activity. PGE-530742 potently inhibited MMP-2, -3, -9, and -13 but spared MMP-1 and -7.

Experimental Design

Instrumentation

Pigs (n=41, 30 to 32 kg) were instrumented with a disengaged snare that encircled the circumflex coronary artery immediately distal to the first branch. A quadrilateral array of radiopaque markers was sutured onto the myocardial area at risk as described previously. 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, DC, 1996), and the Institutional Animal Care and Use Committee approved the protocol.

Randomization

After recovery from instrumentation, the pigs were randomized to MI only (n=11), sMMPi initiated 3 days before MI (n=11, PGE-530742 10 mg/kg PO TID; pre-MI sMMPi), or sMMPi initiated 3 days after MI (n=10, PGE-530742 10 mg/kg PO TID; post-MI sMMPi). PGE-530742 was dispensed with normal food by an automated delivery system to ensure regular dosing. The pigs were monitored to confirm compound consumption. Eleven weight-matched noninstrumented pigs were used as reference non-MI controls (Figure 2).

Baseline Measurements

Long-axis echocardiographic (3-MHz transducer; Sonos5500, Agilent Technologies) and fluoroscopic (Philips Cardio-Diagnost) imaging was used to establish a pharmacokinetic profile (Figure 1) for a single 10-mg/kg oral dose of PGE-530742 (Procter and Gamble Pharmaceuticals). This compound potently inhibited MMP-2, -3, -9, and -13 but spared MMP-1 and -7 (Figure 1). A selective MMP inhibitor with a similar structure was previously demonstrated to achieve myocardial MMP inhibition in a porcine model.

Terminal Measurements

Echocardiographic and fluoroscopic measurements were repeated at 10 days after MI. Systemic hemodynamics, LV volume, and LV function were assessed under general anesthesia as described previously. Infarct size was computed as a percentage of the myocardial area at risk by tetrazolium chloride staining. The LV was sectioned into remote, border, and infarct zones to perform histomorphometric measurements that included myocyte cross-sectional area, relative collagen content, and immunolocalization for myofibroblasts (anti-α-smooth muscle actin, Sigma), macrophages (anti-Mac-3, Genetex), and lymphocytes (anti-CD-3, Genetex). Myocyte length was determined from cells isolated from formalin-fixed sections by potassium hydroxide digestion (50 myocytes per zone per pig) as described previously. MMP-2 activity was determined within remote and border zones by use of an MMP-2 Biotrak Activity Assay System (Amersham Biosciences UK).

Data Analysis

Changes in systemic hemodynamics, regional marker area, and global LV dimensions were compared between the MI groups by 2-way ANOVA. The main treatment effects were the presence or absence of sMMPi and the time of sMMPi institution. Specific pair-wise comparisons were performed with a Bonferroni adjusted t test. Statistical analyses were performed with statistical software programs (BMDP Statistical Software Inc, University of California Press, Los Angeles). Results are presented as mean±SEM, and values of P<0.05 were considered statistically significant.

Results

Myocardial Injury and Systemic Hemodynamics

At 24 hours and 10 days after MI, there was no difference in troponin-I values or infarct size between the MI groups (Table). Mean aortic pressure, LV peak pressure, and LV peak positive developed pressure decreased in each MI group compared with control values (Table).
Regional LV Geometry and Function

LV end-diastolic marker area increased from baseline values in the MI-only group but was unchanged from baseline in both sMMPi groups (Figure 3). Marker area in the post-MI sMMPi group appeared to be reduced from MI-only values, but statistical significance was not achieved (P=0.14). Fractional shortening of the marker area decreased from baseline in the MI-only group (Figure 3) but remained unchanged from baseline in both sMMPi groups.

Global LV Geometry and Function

LV end-diastolic volume was similarly reduced in both sMMPi groups compared with MI-only values but exceeded reference control values (Table). LV end-diastolic area increased by 32±3% in the MI-only group but by only 24±2% in pigs randomized to sMMPi (P<0.05). LV stroke volume decreased in the post-MI sMMPi group compared with MI-only and pre-MI sMMPi values but was similar to reference control values (Table). LV ejection fraction decreased in the MI-only and post-MI sMMPi groups compared with control values but remained unchanged from controls in the pre-MI sMMPi group.

Myocardial Morphometrics

Myocyte cross-sectional area in the remote and border zones of the MI-only group and the border zone of the pre-MI sMMPi group compared with control values (Figure 4). However, myocyte cross-sectional area was reduced in the post-MI sMMPi group compared with MI-only values. Isolated myocyte length was 136±4 μm in the control group and was consistent with lengths previously reported by this laboratory.17 Myocyte length in the remote and border zones of the MI-only group was increased compared with control (181±6 and 173±9 μm, respectively, P<0.05) and was similar to values obtained from both sMMPi groups. Relative collagen content increased in the remote, border, and infarct zones of the MI-only group compared with control values (Figure 5). In the pre-MI sMMPi group, collagen content was similar to MI-only values in the remote and border zones but was reduced in the infarct region. Region-specific changes in relative collagen content were observed in the post-MI sMMPi group. Specifically, collagen content was increased in the remote zone, decreased in the border zone, and similar in the infarct zone compared with the pre-MI sMMPi group. Linear regression analysis was performed to determine whether a relationship existed between relative collagen content in the remote and border zones and the change in LV end-diastolic area at 10 days after MI. A positive relation was observed between collagen accumulation in the border zone and change in LV end-diastolic area (r=0.38, P<0.05). In contrast, a tendency toward a negative relation was evident between collagen content in the remote zone and the change in LV end-diastolic area (r=−0.32, P=0.08).

MMP-2 Activity and Immunohistochemistry

Compared with MI-only values, myocardial MMP-2 activity was decreased in the remote and border zones of the pre-MI and post-MI sMMPi groups (pre-MI sMMPi, 54±29% and 39±19%, respectively, P<0.05; post-MI sMMPi, 37±20% and 9±7%, respectively, P<0.05). Qualitative analysis of the

### Systemic Hemodynamics and Ventricular Volumes After MI: Effects of Timing With sMMPi

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MI Only</th>
<th>Before MI</th>
<th>After MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>101±6</td>
<td>111±7</td>
<td>94±5</td>
<td>97±6</td>
</tr>
<tr>
<td>Pressures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean aortic, mm Hg</td>
<td>91±2</td>
<td>79±2*</td>
<td>78±4*</td>
<td>80±4*</td>
</tr>
<tr>
<td>Mean pulmonary artery, mm Hg</td>
<td>15±1</td>
<td>14±2</td>
<td>14±1</td>
<td>14±2</td>
</tr>
<tr>
<td>LV peak, mm Hg</td>
<td>114±2</td>
<td>102±3*</td>
<td>101±4*</td>
<td>103±4*</td>
</tr>
<tr>
<td>LV end-diastolic, mm Hg</td>
<td>9±1</td>
<td>10±1</td>
<td>11±1</td>
<td>12±2*</td>
</tr>
<tr>
<td>Peak positive dP/dt, mm Hg/s</td>
<td>2035±81</td>
<td>1542±139*</td>
<td>1248±75*</td>
<td>1223±161*</td>
</tr>
</tbody>
</table>

LV volumes
- End-diastolic volume, mL: 56±3 vs 82±3* vs 69±3*† vs 69±4*†
- End-diastolic volume indexed to tibial length, mL/cm: 4.3±0.3 vs 6.4±0.3* vs 5.4±0.3*† vs 5.3±0.4*†
- Stroke volume, mL: 40±2 vs 46±3 vs 46±3 vs 38±2†‡
- Ejection fraction, %: 71±2 vs 62±2* vs 68±2 vs 62±2*
- Infarct size, % of area at risk: ... vs 43±4 vs 44±6 vs 49±6
- 24-Hour troponin-I, ng/mL: ... vs 155±11 vs 144±13 vs 189±18
- Regional wall stress, g/cm²: 291±5 vs 479±32† vs 395±24† vs 403±34†
- LV weight, g: 110±4 vs 127±4* vs 137±7* vs 128±6*
- Sample size, n: 11 vs 11 vs 11 vs 10

sMMPi, PGE-530742, administered at 10 mg·kg⁻¹·d⁻¹ PO TID. Values are presented as mean±SEM.

*P<0.05 vs Control, †P<0.05 vs MI Only, ‡P<0.05 vs pre-MI sMMPi.
Infarct zone of the MI-only group revealed a well-organized pattern of positive staining for \( \alpha \)-smooth muscle actin consistent with myofibroblast replacement (Figure 6). Although similar degrees of staining for \( \alpha \)-smooth muscle actin were observed in the infarct zones of both sMMPi groups, the matrix morphology appeared to be disorganized compared with the MI-only group. Moreover, prominent perivascular staining for \( \alpha \)-smooth muscle actin was evident in both sMMPi groups, suggesting increased neovascularization.

There were no qualitative differences in Mac-3 or CD-3 staining within the infarct zones of the MI groups, suggesting that similar degrees of macrophage and lymphocyte infiltration occurred after MI.

**Discussion**

MMP inhibition has been shown to attenuate LV remodeling in animal models of MI.\(^9\)\(^,\)\(^4\)\(^,\)\(^7\)\(^,\)\(^18\) Although these past studies provide proof of concept that MMP activation directly influences post-MI LV remodeling, a number of issues regarding MMP inhibition after MI remain to be resolved. These issues include (1) a need for evaluation of MMP inhibition in a large animal model of MI that closely recapitulates human cardiac structure and function, (2) establishment of the structural basis for these effects, (3) development of targeted MMP inhibitory profiles, and (4) determination of whether a temporal influence for MMP inhibition after MI exists. Accordingly, this study was performed in an effort to resolve these issues and to build on concepts previously established. Specifically, MMP-1 and -7-sparing sMMPi was applied to a porcine model of MI to quantify the effects of sMMPi on regional and global LV remodeling processes. The unique findings of this investigation were 2-fold. First, inhibition of MMP-1 and -7 is unnecessary to influence LV remodeling after MI. Second, temporal differences exist with respect to the timing of sMMPi institution and patterns of regional and global myocardial remodeling after MI.

**Timing of sMMPi after MI**

LV remodeling after MI encompasses progressive phases of myocyte death, tissue inflammation and granulation, and extracellular matrix remodeling.\(^9\) MMPs are most likely involved in each phase of the wound-healing response and have been shown to contribute directly to LV remodeling after MI.\(^3\)\(^,\)\(^4\)\(^,\)\(^7\)\(^,\)\(^18\) For instance, a past study demonstrated MMP activation within the myocardial interstitium within 1 hour of ischemia.\(^20\) However, early interruption of MMP activity\(^5\)\(^,\)\(^7\)\(^,\)\(^8\) in rodent models of MI has been associated with reductions in wound neutrophil\(^7\) and macrophage\(^8\) infiltration, larger areas of necrosis,\(^5\) and decreased infarct collagen deposition.\(^5\)\(^,\)\(^8\) Therefore, it is unclear when MMP inhibition should be initiated after MI to favorably modulate LV remodeling without impairing necessary wound-healing processes. The present study instituted sMMPi 3 days before or 3 days after MI to compare the effects of sMMPi operative at the time of MI induction, with sMMPi introduced during resolution of the acute MI period. Importantly, there was no difference in plasma troponin-I values at 24 hours or infarct size at 10 days after MI between the MI groups. Thus, equivalent degrees of myocardial injury were incurred in each MI group.

**Regional and Global LV Geometry**

Determinants of MI expansion include patency of the infarct-related artery, LV chamber pressure, tethering of the border-zone, and LV remodeling. Several studies have reported that LV remodeling after MI is influenced by the site of MI infarction.\(^19\) The present study demonstrates that sMMPi, when given 3 days before or 3 days after MI, is effective in modifying LV remodeling processes. The unique findings of this investigation were 2-fold. First, inhibition of MMP-1 and -7 is unnecessary to influence LV remodeling after MI. Second, temporal differences exist with respect to the timing of sMMPi institution and patterns of regional and global myocardial remodeling after MI.

**Figure 3.** Top, End-diastolic marker area increased from baseline in MI-only group. Marker areas appeared decreased from MI-only values in both sMMPi groups and were not significantly different from baseline. Bottom, Fractional shortening of marker area decreased from baseline in MI-only group but remained unchanged from baseline and increased vs MI-only values in both sMMPi groups. \(*P<0.05\) vs baseline, \(+P<0.05\) vs MI-only.

**Figure 4.** Myocyte cross-sectional area increased in remote and border zones of MI-only group vs controls. Cross-sectional area decreased in remote and border zones of post-MI sMMPi group vs MI-only values and were similar to controls. A regional disparity was observed in pre-MI sMMPi group; however, there was no difference in cross-sectional area between sMMPi groups. \(*P<0.05\) vs control, \(+P<0.05\) vs MI-only, \(#P<0.05\) vs remote region.
zone myocardium to the infarct, and the intrinsic material properties of the infarct. Of these potential determinants, alterations in the extracellular matrix of the border zone and the material properties of the infarct most likely accounted for the observed differences in regional LV remodeling and function. In the MI-only group, end-diastolic marker area was increased compared with both sMMPi groups. It is likely that sMMPi instituted before and after MI improved the continuity of the tissue interface between the border zone and infarct, thus accounting for the smaller areas. The pre-MI sMMPi group appeared to be associated with a greater change in marker area compared with the post-MI sMMPi group. If incorporation of the border-zone myocardium into the infarct begins at \( \approx 3 \) days after MI, sMMPi operative at the time of MI induction may have impaired initial wound-healing processes, thereby providing a basis for the increase in marker area in this group. Compared with both sMMPi groups, the MI-only group was associated with reduced fractional shortening of the marker area, a measure of temporal changes in regional LV geometry. Thus, the smaller end-diastolic marker areas observed with sMMPi may have been a result of maintained structural characteristics within the infarct that translated into reduced marker area during systole. Although a greater degree of variation in the parameters of regional function and geometry were observed with sMMPi, LV chamber volume was reduced to similar degrees in both pre-MI and post-MI sMMPi groups, suggesting that heterogeneous changes in regional geometry may be translated into significant attenuations in LV dilation after MI. However, the observed effects on indices of global LV function, namely, stroke volume and ejection fraction, were not uniform. Taken together, these results suggest that a temporal window for the institution of MMP inhibition after MI probably exists and may be associated with functional consequences.
Myocyte and Matrix Remodeling

Increases in myocyte length and cross-sectional area observed in each MI group suggest that LV remodeling ensued after MI and that residual myocytes responded appropriately to the presentation of an increased load. This hypertrophic effect may have occurred in response to elevated wall stresses encountered with establishment of a larger chamber volume. Although myocyte length increased to similar degrees in each MI group, cross-sectional area was decreased with pre-MI and post-MI sMMPi groups. G, Examination of sMMPi sections revealed robust staining for $\alpha$-smooth muscle cell actin (C), CD-3 antibody (D), and Mac-3 antibody (E), indicating presence of myofibroblasts, lymphocytes, and macrophages, respectively. F, Low-power view of a myocardial section obtained from pre-MI sMMPi group. Significant vascularity was observed within infarct zone, particularly at border-zone region (arrows). This prominent vascular effect was observed in both pre-MI and post-MI sMMPi groups. Magnification: A and F, $\times$100; C, D, and E, $\times$200; B and G, $\times$400.

Study Limitations and Clinical Implications

This study used permanent coronary artery occlusion in a large animal model. Arguably, a more relevant methodology might have used a finite period of occlusion followed by reperfusion to evoke apoptotic cell death as a result of ischemia-reperfusion injury, a common clinical scenario. In the present study, the beneficial effects of sMMPi on regional LV remodeling were not translated into improvements in LV pump function. On the basis of the results of past studies, it is likely that a significantly longer follow-up period would be necessary to determine whether and to what extent sMMPi influences global LV function after MI. Considered in conjunction with past reports that demonstrated that early interference in MMP activity interrupted wound-healing responses, the results of the present study suggest that introduction of sMMPi may be most appropriate after resolution of the acute MI period. Past studies provide proof of concept that broad-spectrum MMP inhibition attenuates LV dilation after MI. However, broad-spectrum MMP inhibition has been associated with undesirable musculoskeletal syndrome, thus limiting clinical utility. Although the cause of this syndrome is unclear, inhibition of particular MMPs, namely, that of the interstitial collagenase (MMP-1), as well as other nonmatrix metalloproteinases may be responsible. Importantly, initial clinical studies using more selective MMP inhibitory profiles have shown that MMP inhibition may be achieved in the absence of adverse musculoskeletal effects. The present study builds on the possibility that selective targeting of MMPs causative to pathological post-MI LV remodeling can be achieved. Because more than 20 distinct MMP species have been identified and cloned, continued efforts to narrow the portfolio of MMPs targeted for post-MI inhibition may hold significant therapeutic potential.

Acknowledgments

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