Targeted deletion of MMP-2 attenuates early LV rupture and late remodeling after experimental myocardial infarction

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Myocardial Infarction (MI) leads to complex structural alterations (remodeling) involving both the infarcted and noninfarcted left ventricular (LV) myocardium (21). Early remodeling as LV cavity dilatation occurs during the early phase of MI, which is likely due to wall thinning in the infarct region. This might lead to a cardiac rupture, thereby accounting for the 5–30% of in-hospital mortality after acute MI (1). During the first several days, LV enlargement follows, and, thereafter, a progressive dilatation of the noninfarcted LV occurs over weeks (21). These progressive changes in LV geometry contribute to the development of depressed cardiac function, clinical heart failure, and increased mortality. Accordingly, it is of critical importance to explore the mechanisms of LV remodeling and develop therapeutic strategies that will effectively inhibit this deleterious process.

The dynamic synthesis and breakdown of extracellular matrix (ECM) proteins play an important role in post-MI LV remodeling. In particular, the increased expression and activation of matrix metalloproteinases (MMPs) have been implicated in this process (4, 5). Several studies have demonstrated that MMPs are involved not only in LV remodeling and failure (7, 15, 24) but also in cardiac rupture (9). Among the various known MMPs, MMP-9 has been shown to play an important role in post-MI remodeling (7), and it is mainly expressed in such infiltrating inflammatory cells as neutrophils and macrophages (14, 25). Conversely, MMP-2 is ubiquitously distributed in cardiac myocytes and fibroblasts (3), and it has been shown to be persistently upregulated after MI (2, 6, 23). Therefore, MMP-2 may play an important role in early myocardial healing and the late postinfarct remodeling process. However, no previous studies have yet determined the pathophysiological significance of MMP-2 in post-MI hearts. In the present study, we evaluated the effects of a targeted deletion of the MMP-2 gene on both LV structural and functional alterations after induction of experimental MI in mice.

Materials and Methods

Animals

The study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. We used the
progeny of heterozygous breeding pairs of C57BL/6J mice with targeted disruption of MMP-2 ranging in age from 8 to 10 wk old (10, 11). The original breeding pairs used to develop the mice for this study were obtained from Dr. Shigeyoshi Itohara (Laboratory for Behavioral Genetics, Riken).

Creation of MI

We created a MI in male MMP-2 knockout (KO) mice (KO + MI group) and sibling wild-type (WT) mice (WT + MI group) by ligating the left coronary artery (8). A sham operation without coronary artery ligation was also performed in both WT (WT + sham) and MMP-2 KO (KO + sham) mice. Tail clips and a PCR protocol to confirm the genotype were performed by a group of investigators (S. Hayashidani and T. Kubota). Next, MI was induced in these mice by another subset of investigators (H. Matsusaka and H. Tsutsui), who were not informed of the genotyping results. This assignment procedure was performed using numeric codes to identify the animals.

Experimental Protocol 1: 28-Day Post-MI Study

Survival. A survival analysis was performed for the WT + sham (n = 13), KO + sham (n = 17), WT + MI (n = 36), and KO + MI (n = 20) mice. During the 4-wk study period, the cages were inspected daily to identify any deceased animals. All deceased mice were examined for the presence of MI as well as pleural effusion (serous fluid within the chest wall cavity) and cardiac rupture, based on a diagnosis of the presence of a blood clot within the pericardial sac in the postmortem examination.

Echocardiographic and hemodynamic measurements. Echocardiographic studies were performed under light anesthesia with tribromoethanol-amylene hydrate [Avertin; 2.5% (wt/vol), 8 μg/g ip] and spontaneous respiration (8). A two-dimensional parasternal short-axis view of the LV was obtained at the level of the papillary muscles. In general, the best views were obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was maintained at the level of the papillary muscles. In general, the best views were obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was then gently moved cephalad or caudal and angulated until desirable images were obtained. After it was confirmed that the imaging was on axis (based on roundness of the LV cavity), two-dimensional targeted M-mode tracings were recorded at a paper speed of 50 mm/s. Next, a 1.4-Fr micromanometer-tipped catheter (Millar) was inserted into the right carotid artery and then advanced into the LV to measure the LV pressures. One subset of two investigators (M. Ikuchi and T. Shioi), who were not informed of the experimental groups, performed in vivo LV function studies. Our recent validation study (27) has shown that the intraobserver and interobserver variabilities of our echocardiographic measurements for LV cavity dimensions and fractional shortening were small and measurements made in the same animals on separate days were highly reproducible.

Infarct size. The heart was excised, and the right ventricle and LV including the septum were dissected. Infarct size was determined by the methods described by Pfeffer et al. (22) in rats and also in mice (19, 26). The LVs were cut from apex to base into three transverse sections. Five-micrometer sections were cut and stained with Masson’s trichrome. Infarct length was measured along the endo- and epicardial surfaces from each of the LV sections, and the values from all specimens were summed. Total LV circumference was calculated as the sum of endo- and epicardial segment lengths from all LV sections. Infarct size (in %) was calculated as total infarct circumference divided by total LV circumference. In our preliminary study, we confirmed excellent reliability of infarct size measurements, in which a morphometric methodology similar to that used in this study was employed. The intraobserver and interobserver variabilities between two measurements divided by the mean of the two measurements, expressed as a percentage, were <5%. Therefore, our technique could be considered to allow reliable assessment of infarct size in mice.

Experimental Protocol 2: 3-Day Post-MI Study

Because a substantial portion of the MI animals died within 7 days after MI, in vivo LV function and myocardial histopathology were also evaluated in a separate group of additional mice after 3 days of surgery treated identically to experimental protocol 1. The LV function was determined by using the same echocardiographic and hemodynamic measurements as described in Experimental Protocol 1: 28-Day Post-MI Study.

Myocardial histopathology. From the mid-LV transverse sections, 5-μm sections were cut and stained with hematoxylin and eosin and Masson’s trichrome. The wall thickness was determined by measuring the width of the thinnest part of the infarct and the thickest part of the septum (15). The collagen volume fraction was determined by the quantitative morphometry of Masson’s trichrome-stained mid-LV sections (8). Interstitial collagens were also stained with Sirius red to determine the interstitial yellow-red (type I collagen) and green (type III collagen) fibers (18).

Experimental Protocol 3: LV MMPs

In a separate group of additional mice treated identically to experimental protocol 1, the time-dependent changes of zymographic MMP levels including MMP-2 and MMP-9 were determined in the noninfarcted LV using gelatin zymography (12). The LV myocardial samples were homogenized (~30-s bursts) in 1 ml of an ice-cold extraction buffer containing cacodylic acid (10 mmol/l), NaCl (0.15 mol/l), ZnCl2 (20 mmol/l), NaNO3 (1.5 mmol/l), and 0.01% Triton X-100 (pH 5.0). The homogenate was then centrifuged (4°C, 10 min, 10,000 g), and the supernatant was decanted and saved on ice. The pH levels of the samples were adjusted to 7.5 using Tris (1 mol/l). The final protein concentration of the myocardial extracts was determined using a standardized colorimetric assay. The extracted samples were then aliquoted and stored at ~80°C until the time of assay. The myocardial extracts were then directly loaded onto electrophoretic gels (SDS-PAGE) containing 1 mg/ml gelatin under nonreducing conditions. The myocardial extracts at a final protein content of 5 μg were loaded onto the gels using a 3:1 sample buffer (10% SDS, 4% sucrose, 0.25 mol/l Tris·Cl, and 0.1% bromphenol blue; pH 6.8). The gels were run at 15 mA/gel through the stacking phase (4%) and at 20 mA/gel for the separating phase (10%) while the running buffer temperature was maintained at 4°C. After SDS-PAGE, the gels were washed twice in 2.5% Triton X-100 for 30 min each, rinsed in water, and incubated for 24 h in a substrate buffer at 37°C (50 mmol/l Tris·HCl, 5 mmol/l CaCl2, and 0.02% NaNO3; pH 7.5). After incubation, the gels were stained with Coomassie brilliant blue R-250. The zymograms were digitized, and the size-fractionated bands, which indicated the MMP proteolytic levels, were measured by the integrated optical density in a rectangular region of interest.

MMP-13, the predominant murine form of interstitial collagenases, protein levels were determined by immunohotting analysis (29).
of 36 mice) and MMP-2 KO mice. Animals were followed up to 28 days after surgery. There was no significant difference in survival after the sham operation between MMP-2 KO and WT mice (data not shown). *P < 0.05 vs. WT + MI. B: numbers of cardiac ruptures in the WT + MI (n = 14 of 36 mice) and MMP-2 KO + MI mice (n = 2 of 20 mice).

Statistical Analysis

All data are expressed as means ± SE. A survival analysis was performed by the Kaplan-Meier method, and the between-group difference in survival was tested by the logrank test. Between-group comparisons of the means were performed by one-way ANOVA, followed by t-tests. The Bonferroni’s correction was done for multiple comparisons of the means.

RESULTS

Experimental Protocol 1: 28-Day Post-MI Study

Mortality and cardiac rupture. Early operative mortality (within 24 h after ligation) was comparable between the groups [16% for WT + MI vs. 17% for KO + MI, P = not significant (NS)]. There were no deaths in the sham-operated groups.

MMP-2 KO mice had significantly better survival after MI (KO + MI) compared with WT + MI mice (Fig. 1A). The number of the mice that died of LV rupture, which occurred within 7 days, was significantly greater in WT + MI mice than in KO + MI mice (39% vs. 10%, P < 0.05; Fig. 1B).

Infarct size. The infarct size determined by the morphometric analysis 28 days after ligation was comparable (50 ± 3% vs. 51 ± 3%, P = NS) between the WT + MI (n = 7) and KO + MI (n = 6) mice. In a separate group of additional animals 3 days after ligation, it was also comparable (54 ± 4% vs. 53 ± 3%, P = NS) between the WT + MI (n = 4) and KO + MI (n = 4) mice.

Echocardiography and hemodynamics. The echocardiographic and hemodynamic data of the surviving mice at 28 days are shown in Table 1. LV diameters increased significantly more in the WT + MI mice than in the WT or KO mice with sham operations. KO + MI mice exerted less LV cavity dilatation and improved fractional shortening compared with WT + MI mice.

There was no significant difference in the heart rate and mean aortic blood pressure among the four groups of mice. The LV end-diastolic pressure (LVEDP) increased in the WT + MI group, which was significantly attenuated in the KO + MI group.

Experimental Protocol 2: 3-Day Post-MI Study

Data for the echocardiographic, hemodynamic, and histomorphometric studies at 3 days of MI are summarized in Table 2.

Echocardiography and hemodynamics. LV diameters and fractional shortening were similar between WT + MI and KO + MI mice after 3 days of MI. The heart rate and mean aortic pressure were also comparable between the groups (Table 2).

Myocardial histopathology. Low-power photomicrographs of Masson’s trichrome-stained LV cross sections obtained from 3 days post-MI mice demonstrated that the wall thickness at the infarcted LV was similar between WT + MI and KO + MI hearts (Fig. 2, A and B). In addition, the average thinning ratio, in which the thickness of the infarct LV wall normalized to that of the septum, was also comparable between the groups (Table 2).

High-power photomicrographs of LV cross sections demonstrated that collagen deposition at the border zone between the infarcted and noninfarcted LV was similar between WT + MI and KO + MI hearts (Fig. 2, C–F). Furthermore, interstitial fibrosis, measured as the collagen volume fraction, in the noninfarcted LV was also comparable between the groups (2.9 ± 0.3% vs. 2.7 ± 0.4%, P = NS). Moreover, in Sirius red-

Table 1. Echocardiographic and hemodynamic data at 28 days after surgery

<table>
<thead>
<tr>
<th></th>
<th>WT + Sham</th>
<th>KO + Sham</th>
<th>WT + MI</th>
<th>KO + MI</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Echocardiographic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>404 ± 8</td>
<td>416 ± 10</td>
<td>410 ± 6</td>
<td>422 ± 7</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>4.5 ± 0.1‡</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>3.8 ± 0.1‡</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>35.6 ± 0.5</td>
<td>37.5 ± 1.4</td>
<td>12.1 ± 0.8</td>
<td>14.7 ± 0.7‡</td>
</tr>
<tr>
<td>Hemodynamic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>424 ± 14</td>
<td>434 ± 20</td>
<td>416 ± 9</td>
<td>422 ± 14</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>76 ± 2</td>
<td>74 ± 4</td>
<td>70 ± 3</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>2.8 ± 0.4</td>
<td>2.2 ± 0.7</td>
<td>14.7 ± 2.3</td>
<td>8.9 ± 1.6†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. WT, wild-type mice; KO, MMP-2 knockout mice; MI, myocardial infarction; LVEDD, left ventricular (LV) end-diastolic diameter; LVESD, LV end-systolic diameter; LVEDP, LV end-diastolic pressure. *P < 0.05 vs. WT + sham; †P < 0.05 and ‡P < 0.01 vs. WT + MI.
stained sections, the structure of interstitial yellow-red (type I collagen) and green (type III collagen) fibers was similar between the WT/H11001MI and KO/H11001MI mice.

Experimental Protocol 3: LV MMPs

First, the time-dependent changes of LV zymographic MMP-2 levels were determined after the coronary artery ligation. The zymographic MMP-2 levels in the noninfarcted LV increased and peaked at day 7 after MI and remained elevated above the sham level for up to 28 days (Fig. 3). The same level also increased in the infarcted LV after MI (2.2-fold increase compared with WT/H11021sham, \(P < 0.01\)). Second, the baseline differences in MMPs between WT and KO mice were determined (Fig. 4). In MMP-2 KO mice, no zymographic MMP-2 levels were detected in the LV. It is important to note that the zymographic MMP-9 levels, even though they were very faint at baseline, did not increase in MMP-2 KO mice. Moreover, the MMP-13 protein levels were similar between WT and KO mice. Third, the changes of MMPs after MI were compared between WT and KO mice. Again, the zymographic MMP-2 levels in the noninfarcted LV significantly increased in the WT mice after 3 and 28 days of MI. This increase was completely prevented in KO mice (Fig. 5A). Similarly, the zymographic MMP-2 levels in the infarcted LV were not detected in KO/H11001MI mice. The MMP-9 levels in the noninfarcted LV also increased after MI; however, no difference in this regard was seen between WT and KO mice (Fig. 5B).

DISCUSSION

The major new finding of the present study is the significant improvement in the survival after MI in MMP-2 KO mice, which was mainly attributable to the inhibition of early cardiac rupture and the development of subsequent LV dysfunction. After MI, the zymographic MMP-2 level significantly and persistently increased in the LV, which might contribute to cardiac rupture as well as LV remodeling progression. Our observations thus suggest that an anti-MMP-2 strategy may be of therapeutic benefit against the evolution of cardiac rupture after MI.

Consistent with previous studies (2, 6, 23), the LV MMP-2 level was persistently activated after MI (Fig. 3). Although the mechanisms responsible for this acti-

Table 2. Echocardiographic, hemodynamic, and histomorphometric data after 3 days of MI

<table>
<thead>
<tr>
<th></th>
<th>WT + MI</th>
<th>KO + MI</th>
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<tr>
<td>n</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Echocardiographic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>420 ± 8</td>
<td>424 ± 8</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.0 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>3.5 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>16.1 ± 1.3</td>
<td>18.0 ± 0.8</td>
</tr>
<tr>
<td>Hemodynamic data</td>
<td></td>
<td></td>
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<tr>
<td>Heart rate, beats/min</td>
<td>443 ± 15</td>
<td>443 ± 15</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>74 ± 3</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>Histomorphometric data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>54 ± 4</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Infarct wall thickness, mm</td>
<td>0.53 ± 0.02</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>Infarct thinning ratio</td>
<td>0.61 ± 0.01</td>
<td>0.65 ± 0.03</td>
</tr>
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</table>

Values are means ± SE; n, no. of mice.
vation remain to be determined, cellular constituents of cardiac muscle, including fibroblasts, inflammatory cells, and myocytes, are known to be capable of expressing MMP-2 in response to specific stimuli (28).

To investigate the specific role of MMP-2 activation in post-MI hearts, we used MMP-2 KO mice. As expected, no MMP-2 expression was observed in the myocardium at baseline from these KO mice (Fig. 4), and their levels were not elevated in KO mice even after MI (Fig. 5A). The most striking finding was the inhibition of an LV rupture in MMP-2 KO mice during the first week after infarction (Fig. 1B). A previous study (9) demonstrated that the MMP-9 deficiency in mice almost completely protected them against cardiac rupture after MI. In addition, adenoviral human tissue inhibitor of metalloproteinase (TIMP)-1 overexpression in the mouse after MI resulted in a diminished leukocyte influx, less neovascularization, larger residual necrotic areas, and decreased collagen content in the infarct itself (9). In that study (9), the complete prevention of cardiac rupture was achieved by TIMP-1 overexpression, thus indicating that TIMP-1 is an important endogenous inhibitor of myocardial MMP activity. The present study provided strong evidence that cardiac rupture could be attenuated in MMP-2 KO mice as well. Collectively, these findings suggest that increases in MMPs in MI may contribute to cardiac rupture. The prevalence of a cardiac rupture in our WT mice with MI was as high as 39%, which might be, at least in part, due to the large infarct size (>50%). However, this prevalence value appears to be similar to that (33%) reported previously (9). The beneficial effects of MMP-2 deletion shown in the present study were not due to its MI size-sparing effects because the infarct size was comparable between WT and KO mice after 28 days as well as after 3 days. Furthermore, its effects might not be attributable to those on hemodynamics because the blood pressure and heart rate showed no alterations (Table 1 and 2).

Cardiac rupture is an acute fatal complication that occurs during the early phase after MI. It is difficult to predict its occurrence by previously reported clinical risk factors such as aging, hypertension, and delayed thrombolysis (16). ECM proteins play an important role in the healing process after MI. An important aspect of early infarct healing is the deposition of collagen, which stabilizes the damaged myocardium. Furthermore, agents that inhibit collagen synthesis have been shown to be associated with an increased risk of cardiac rupture (20). Therefore, defective infarct healing and the resultant inadequate or disrupted ECM at the site of infarction may lead to myocardial rupture. In addition, inadequate fibrosis may result in myocyte separation in the infarct area, which can enhance inflammatory cell infiltration and thus lead to eventual cardiac rupture. Defective infarct healing may be associated with expansion of the infarcted tissue, and, in extreme cases, it may result in the rupture of the infarcted ventricular wall. However, in the present study, there were no significant changes
observed in collagen deposition between WT and MMP-2 KO mice after MI (Fig. 2). Furthermore, the thickness of the infarcted LV wall was comparable. Therefore, the inhibition of cardiac rupture in MMP-2 KO mice was not due to the impairment of the reparative process of interstitial collagens. However, given a previous study by Heymans et al. (9), we could not rule out the possibility that activated MMP-2 may enhance the infiltration of inflammatory cells and the degradation of ECM components other than collagens, which might thus lead to LV rupture.

Furthermore, the present study demonstrated the long-term beneficial effects of MMP-2 deletion on the decline in LV systolic function at 28 days after MI (Table 1). These findings are consistent with a study by Rohde et al. (24) in which MMP inhibition was shown to decrease early LV dilatation after MI, but this was observed only 4 days after MI. Mukherjee et al. (17) recently demonstrated that MMP inhibition reduced the regional infarct size and expansion rate, and these effects persisted when treatment was continued for up to 2 wk. The present study demonstrated that the target deletion of MMP-2 attenuated the degree of post-MI LV dilatation during the late phase, but it did not alter the expansion of the infarct. The beneficial effects of MMP-2 deletion on LV function were very modest, although they were statistically significant, which might be, in part, due to the fact that the measurements were performed in the 28-day MI survivors. Whatever the reason, the major effects of MMP-2 deletion are the prevention of cardiac rupture.

There are several limitations to be acknowledged in this study. First, even though in vivo assessment of LV function with echocardiography is feasible and reproducible in the mouse, it might still be difficult to interpret the indexes in dilated post-MI LV. Our validation study has shown that the intraobserver and interobserver variabilities of our echocardiographic measurements for LV cavity dimensions and fractional shortening were small and the measurements made in the same animals on separate days were highly reproducible (8). Therefore, our technique could be considered to allow for a noninvasive assessment of the LV structure even in mice with a large MI. Importantly, even though the differences between WT + MI and MMP-2 KO + MI mice after 28 days were small, they are still considered to be meaningful. Second, the heart rate values in the present study (420–450 beats/min) were lower than those (600 beats/min) measured in conscious mice. Therefore, the LV size and function results might be greatly influenced by differences in anesthetic regimens and experimental conditions such as heart rate. Third, in addition to the findings of gelatin zymographic studies, either an ELISA or a Western blot analysis is needed to quantify the MMP protein levels in MI and the effects of MMP-2 deletion on MMP induction. Finally, TIMPs may also have played an important role in the pathogenesis of myocardial remodeling as an endogenous inhibitor of the myocardial MMP activity. Therefore, further studies are needed to clarify the modulation of TIMP species in our MMP-2 KO model.

A cardiac rupture usually occurs unexpectedly, and it is often fatal, thereby resulting in one of the major causes of in-hospital death in patients with acute MI. Our results in mice with deletion of MMP-2 may provide some insight regarding the pathophysiological role of MMP-2 activation in cardiac rupture and thus help to establish an effective therapeutic strategy. Furthermore, myocardial MMP-2 activation after MI is also involved in the process of LV structural remodeling, which is a central feature of heart failure progression (13).

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DISCLOSURES

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