Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs

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Etoh, Takuma, Cassandra Joffs, Anne M. Deschamps, Jennifer Davis, Kathryn Dowdy, Jennifer Hendrick, Simona Baicu, Rupak Mukherjee, Marlina Manhaini, and Francis G. Spinale. Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. Am J Physiol Heart Circ Physiol 281: H987–H994, 2001.—A structural event during the evolution of a myocardial infarction (MI) is left ventricular (LV) remodeling. The mechanisms that contribute to early changes in LV myocardial remodeling in the post-MI period remain poorly understood. Matrix metalloproteinases (MMPs) contribute to tissue remodeling in several disease states. Whether and to what degree MMP activation occurs within the myocardial interstitium after acute MI remains to be determined. Adult pigs (n = 15) were instrumented to measure regional myocardial function and interstitial MMP levels within regions served by the circumflex and left anterior descending arteries. Regional function was measured by sonomicrometry, and interstitial MMP levels were determined by selective microdialysis and zymography as well as by MMP interstitial fluorogenic activity. Measurements were performed at baseline and sequentially for up to 3 h after ligation of the obtuse marginals of the circumflex artery. Regional fractional shortening fell by over 50% in the MI region but remained unchanged in the remote region after coronary occlusion. Release of soluble MMPs, as revealed by zymographic activity of myocardial interstitial samples, increased by 2 h post-MI. The increased zymographic activity after MI was consistent with MMP-9. Myocardial interstitial MMP fluorogenic activity became detectable within the ischemic region as early as 10 min after coronary occlusion and significantly increased after 1 h post-MI. MMP fluorogenic activity remained unchanged from baseline values in the remote region. The present study demonstrated that myocardial MMP activation can occur within the MI region in the absence of reperfusion. These unique results suggest that MMP release and activation occurs within the ischemic myocardial interstitium in the early post-MI period.

zymography; microdialysis; myocardial ischemia

IT IS WELL RECOGNIZED that structural changes occur within the left ventricular (LV) myocardium after myocardial infarction (MI). The LV myocardial remodeling that occurs after MI can lead to progressive dilation and eventually pump dysfunction (8, 24, 35). After acute MI, regional myocardial dysfunction occurs and is accompanied by local neurohormonal/cytokine activation (14, 24, 32). However, the downstream cellular and molecular mechanisms that directly contribute to the changes in myocardial structure and function after MI remain poorly understood. Matrix metalloproteinases (MMPs) are an endogenous family of enzymes that have been identified to be responsible for collagen matrix remodeling in a number of physiological processes (10, 19, 20, 29). Increased LV myocardial MMP activity and expression have been identified to occur in end-stage human cardiomyopathic disease as well as in animal models of heart failure (3, 15, 28, 30). While dependent on the severity and duration of the ischemic insult, alterations in the LV myocardial collagen structure can occur after MI, which would implicate changes in MMP activity within the myocardial interstitium (33, 35, 36). Moreover, recent studies (5, 11, 25) using rodent models have suggested a functional role of myocardial MMPs in post-MI remodeling. The primary site for the proteolytic activity of MMPs is the interstitial compartment (10, 19, 20, 29). However, previous studies (3, 4, 15, 16, 28, 30) have employed tissue extracts or ex vivo assays to determine relative MMP activity. Thus whether changes in myocardial MMP activity occur within the interstitial compartment in vivo after acute MI remain to be established. Accordingly, the objective of the present study was to measure time-dependent changes in myocardial interstitial MMP activity in both the MI and remote regions after acute MI.

METHODS

Instrumentation and MI induction. Mature pigs (n = 20, Yorkshire, 35–40 kg, Hambone Farms) were instrumented to obtain indexes of LV global and regional function as well as for the measurement of myocardial interstitial MMPs. Anesthesia was initially induced with intravenous administration of 50 mg etomidate (Amidate, Elkins-Sinn), and an endotracheal tube was placed. A loading dose of 50 μg sufentanil (Elkins-Sinn) was then administered, and a maintenance infusion of lactated Ringer solution (400 ml/h) and morphine

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(60 mg/h, Elkins-Sinn) was initiated. A continuous infusion of tubocurare (3 mg/h, Bristol-Myers-Squibb) and lidocaine (120 mg/h, Elkins-Sinn) was established. In preliminary studies, this anesthetic protocol provided a deep anesthetic plane and stable hemodynamic profiles for at least 8 h. 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 Institutes of Health, 1996).

After a surgical plane of anesthesia was established, an arterial line (7-Fr) was placed in the right carotid artery, and a multilumen thermodilution catheter (7.5-Fr, Baxter Healthcare; Irvine, CA) was positioned in the pulmonary artery from the right external jugular vein. The fluid-filled aortic and pulmonary artery catheters were connected to externally calibrated transducers (Statham P23ID, Gould; Oxnard, CA). A sternotomy was performed, and a vascular ligature was placed around the inferior vena cava to perform transient caval occlusion. A precalibrated microtipped transducer (7.5-Fr, Millar Instruments; Houston, TX) was placed in the LV through the apex and sutured in place. Two pairs of piezoelectric crystals (2 mm, Sonometrics; Ontario, Canada) were positioned within the midmyocardium to measure segmental wall motion (17, 28). The first crystal pair was placed between the first and second diagonal branches of the left anterior descending artery. The second crystal pair was placed between the first (OM1) and second obtuse marginal (OM2) branches of the circumflex coronary artery. The pressure waveforms and crystal signals were digitized on a computer for subsequent analysis at a sampling frequency of 250 Hz (Pentium Processor, Dell; Round Rock, TX). A microdialysis probe was inserted in the midmyocardial region between each pair of crystals, sutured in place, and prepared as described in Myocardial interstitial MMP measurements by microdialysis. Snare occluders were then loosely placed around the OM1 and OM2 branches. A schematic that outlines the placement of the instrumentation is shown in Fig. 1. After instrumentation and a 60-min equilibration period, baseline measurements were recorded. These measurements included heart rate, cardiac output, stroke volume, aortic pressure, LV end-systolic and -diastolic pressure, peak rate of pressure development (+dP/dt), and LV regional segmental shortening. To more carefully determine regional myocardial performance, LV end-diastolic pressure and segmental shortening values obtained during caval occlusion and release were subjected to regression analysis (17). From the regression analysis, the slope of the regional preload recruitable stroke-work (PRSW) relationship was computed (17).

After the microdialysis systems were equilibrated, baseline interstitial fluid was collected, and baseline hemodynamics were obtained, regional ischemia was induced through closure of the OM1 and OM2 ligatures. This region of the LV was chosen for ischemic injury because it does not provide blood supply to a major myocardial conduction pathway and, therefore, did not result in atrioventricular block or refractory arrhythmogenesis. LV function and microdialysis samples were collected for up to 3 h after coronary ligation. A 3-h total ischemic period was chosen because it has been previously demonstrated to result in permanent myocyte injury and infarction (12, 23, 26). In the present study, blood samples were collected at baseline and at each hour after occlusion to measure troponin-I levels, a specific marker of myocyte injury and infarction (6). In preliminary studies (n = 5) (6), selective coronary ligation of the OM1 and OM2 branches for 3 h resulted in an infarct size of 25 ± 3% based on the triphenyltetrazolium chloride staining technique. Moreover, core temperature was rigidly maintained at 37°C during the entire 3-h occlusion period because small fluctuations in temperature have been previously demonstrated to influence infarct size in swine (6). None of the animals used in this study required electrical cardioversion after coronary occlusion. At the conclusion of the 3-h study period, the LV was quickly harvested and placed in ice-cold Krebs solution. The LV free wall was dissected into the ischemic region, the border region, and the remote region. The border region was defined as a 0.5-cm perimeter surrounding the infarcted region. The myocardial samples were then flash-frozen in liquid nitrogen for subsequent zymographic analysis.

Myocardial interstitial MMP measurements by microdialysis. The microdialysis system used in the present study was adapted from previously described techniques to measure bioactive peptides (7, 31, 34). In the first set of experiments (n = 9), the microdialysis probe contained a 4-mm-long membrane with a 100-kDa molecular mass cutoff (CMA12, CMA/Microdialysis; North Chelmsford, MA). The pore size of the dialysis membrane allowed for traversal of MMPs, which are predominantly below this molecular weight cutoff (10, 19, 20, 29, 30). A Krebs buffer solution containing 0.5% BSA was perfused through the probe using a computer-controlled syringe pump at 2.5 μl/min (Baby Bee and Beehive Controller, CMA/Microdialysis). After a 60-min infusion period in which the interstitial fluid equilibrated with the dialysate (7), the dialysate was collected into chilled microtubes and flash-frozen. The long-term placement of the microdialysis probes within the midmyocardium produced no effect on LV regional...
function, as assessed by sonomicrometry. A previously performed postmortem study (7) of myocardial sections containing the probe showed no extravasations of blood cells or inflammatory response after long-term placement (>3 h). A flow rate of 2.5 μl/min was chosen for this system based on initial measurements using an in vitro calibration system (7). For this in vitro approach, the microdialysis probe was immersed in a Krebs solution containing purified gelatinase (MMP-2 and MMP-9, 1.25 μg/ml, Chemicon International; Temecula, CA), and dialysate was collected every 60 min using a flow rate of 1–5 μl/min. The immersion solution and the dialysate were subjected to zymographic analysis as described in MMP zymography and presented in detail previously (3, 28, 30). For the microdialysates collected in vivo, these samples were stored at −70°C until the time of zymographic analysis.

In a second set of studies (n = 6), interstitial myocardial MMP activity was directly quantified using a MMP fluorescent substrate (18, 22). For these studies, microdialysis probes containing a 20-kDa molecular mass cutoff (CMA/20, CMA/Microdialysis) membrane were placed within the mid-myocardium. This molecular mass cutoff effectively excluded MMP species from directly traversing from the interstitial space into the microdialysis membrane. Instead, the microdialysis infusate contained a quenched fluorescent substrate (50 μM DNP-Pro-Leu-Gly-Leu-Gly-Met-Trp-Ser-Arg-OH; Calbiochem) specific for the gelatinases MMP-2 and MMP-9 (22). Initial in vitro studies were performed in which the concentration of the MMP substrate contained within the microdialysate was varied (10–200 μM) and infused at 2.5 μl/min in an immersion solution containing purified MMP-2/9 (50 μM, 37°C). The entire microdialysis system was protected from ambient light, and the dialysate was collected at 10-min intervals into chilled amber tubes and immediately processed for fluorimetry measurements. The samples were read using excitation and emission wavelengths of 280 and 360 nm, respectively (Gilford Fluoro IV; Oberlin, OH). A time-dependent increase in relative fluorescence was observed, which plateaued by 60 min of infusion. A representative in vitro MMP fluorescence measurement using 50 μM of the MMP substrate is shown in Fig. 5 (inset). This MMP substrate did not autofluoresce, and no fluorescence could be detected in the absence of MMP in the immersion solution [Fig. 5 (inset)]. For the in vivo studies, the MMP substrate was infused for 60 min to reach equilibrium, and baseline interstitial MMP fluorescent proteolytic activity was determined. The interstitial fluorescent measurements were converted to nanograms per microliter of MMP-2/9 activity based on the in vitro calibration curves. In preliminary studies (n = 3), coinfusion of the MMP substrate and a broad-spectrum MMP inhibitor (5 mM galardin, British Biotech) (9, 28) abolished all fluorescent activity in the interstitial microdialysate samples. To compare the relative changes in MMP interstitial activity after coronary occlusion, the time-dependent change in fluorescent activity from baseline values was computed for the ischemic and remote regions.

**MMP zymography.** LV MMP gelatinase activity and abundance were examined by substrate-specific zymography (3, 28, 30). The LV myocardial extracts (5 μg total protein) were subjected to electrophoresis followed by zymography (28, 30). The microdialysate (5 μl) was suspended in 5 μl of electrophoresis buffer (10% SDS, 4% sucrose, 0.25 M Tris-HCl, and 0.1% bromophenol blue; pH 6.8) and subjected to zymographic analysis. To provide an internal control with respect to the zymographic activity, cell culture media samples from a cultured human fibrosarcoma HT 1080 cell line were included (3, 30). The zymograms were digitized, and the size-fractionated banding pattern, which indicated MMP proteolytic activity, was determined by quantitated image analysis (Gel Pro Analyzer, Media Cybernetics; Silver Spring, MD). The lysis areas were measured by two-dimensional integrated optical density computations and expressed in pixels.

**Data analysis.** Changes in LV regional function, hemodynamics, and MMP levels after the induction of an MI were initially compared with baseline values by ANOVA. This approach was based on using a two-way ANOVA for repeated measures. The main treatment effects were myocardial region (MI and remote regions) and time post-MI. Specific comparisons between baseline and post-MI values were performed by a Bonferroni adjusted t-test. Cumulative changes in fluorescent activity obtained in the MMP substrate studies were subjected to a two-way ANOVA for repeated measures. Statistical analysis was performed using statistical software programs (BMDP Statistical Software, University of California Press; Los Angeles, CA). Results are presented as means ± SE. Values of P < 0.05 were considered to be statistically significant.

### RESULTS

Steady-state hemodynamics and global LV function at baseline and after coronary occlusion are summarized in Table 1. Mean aortic blood pressure and LV peak systolic pressure significantly fell by 3 h after coronary occlusion, but all other parameters were unchanged from baseline values. Despite a significant elevation in the S-T segment of the electrocardiogram, all of the animals remained in sinus rhythm throughout the 3-h postocclusion period. Plasma levels of tro-

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**Table 1. Steady-state hemodynamics and LV function after acute myocardial infarction**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60</th>
<th>120</th>
<th>180</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>93 ± 5</td>
<td>94 ± 5</td>
<td>95 ± 6</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>3.22 ± 0.27</td>
<td>2.94 ± 0.30</td>
<td>2.88 ± 0.22</td>
<td>3.19 ± 0.43</td>
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<tr>
<td>Stroke volume, ml</td>
<td>36.0 ± 3.4</td>
<td>31.9 ± 3.2</td>
<td>31.4 ± 2.6</td>
<td>33.8 ± 4.2</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>92 ± 4</td>
<td>88 ± 3</td>
<td>88 ± 4</td>
<td>79 ± 5*</td>
</tr>
<tr>
<td>LV pressures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak systolic, mmHg</td>
<td>121 ± 3</td>
<td>115 ± 3</td>
<td>118 ± 4</td>
<td>109 ± 6*</td>
</tr>
<tr>
<td>End diastolic, mmHg</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Peak +dP/dt, mmHg/s</td>
<td>2,363 ± 148</td>
<td>2,225 ± 155</td>
<td>2,194 ± 169</td>
<td>2,235 ± 276</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>9</td>
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Values are presented as means ± SE; n = no. of animals (sample size). LV, left ventricular; +dP/dt, peak rate of pressure development.

*P < 0.05 vs. baseline.
Tropinin-I increased by over twofold from baseline values after 3 h of coronary occlusion (10.5 ± 1.8 vs. 24.6 ± 4.7 ng/ml, \( P < 0.05 \), respectively). The increase in tropinin-I levels was consistent between preparations with a coefficient of variation of <18%. Thus this method of coronary occlusion caused a reproducible pattern of myocardial injury without a significant compromise on systemic hemodynamics.

Regional LV myocardial function was examined in both the MI and remote region, and the results are summarized in Fig. 2. Regional segmental shortening decreased in the MI region by 60 min of coronary occlusion and remained reduced throughout the ischemic period. Segmental shortening increased slightly in the remote region from baseline values, but this did not reach statistical significance. Regional PRSW decreased at 60 min of coronary occlusion and continued to fall with longer periods of myocardial ischemia. Regional PRSW remained unchanged in the remote region.

Representative MMP zymography of myocardial interstitial dialysate samples is shown in Fig. 3. A robust increase in zymographic activity was observed after 3 h of coronary occlusion, particularly within the range of 80–100 kDa. Densitometric analysis of the lytic regions from the zymograms was quantitated, and the results are summarized in Fig. 4. A significant increase in myocardial interstitial zymographic activity was observed after 3 h of coronary occlusion in the ischemic region. Myocardial interstitial zymographic activity tended to increase in the remote region after 3 h of coronary occlusion (\( P = 0.068 \)). A MMP-2/9 fluorogenic substrate was infused through the myocardial microdialysis system to measure in vivo MMP interstitial activity (Fig. 5). A time-dependent increase in the cleavage of this MMP substrate occurred after coronary occlusion within the ischemic region. In the remote region, MMP fluorogenic activity appeared unchanged from baseline values at any time point after coronary occlusion. At the conclusion of the 3-h coronary occlusion period, myocardial samples were collected and subjected to zymographic analysis (Fig. 6). Total zymographic activity was increased in the MI region compared with the remote region or the region surrounding the MI.

**DISCUSSION**

A number of cellular and extracellular events occur after coronary occlusion and the onset of an acute MI. It has been demonstrated previously that indexes of extracellular matrix degradation can occur during and after the onset of an acute MI (4, 16, 17, 33, 37). Recent experimental studies (4, 5, 11, 25) have reported that a family of zinc-dependent proteases, the MMPs, may potentially contribute to myocardial remodeling after MI. However, the temporal onset and the regional distribution of MMP activation within the LV after acute coronary occlusion remained to be defined. Accordingly, the present study utilized a porcine model of acute coronary occlusion to produce a discrete and localized MI and measured indexes of MMP activation in vivo and in vitro within the MI region as well as in the remote region. With the use of microdialysis techniques, the present study demonstrated that an early onset of MMP activation occurred within the interstitium of the MI region and that, with longer periods post-MI, release of soluble MMPs occurred within both the MI and remote regions. The present study demonstrated that myocardial MMP activation can occur within the MI region in the absence of reperfusion.

To our knowledge, this is the first study that has directly examined time-dependent indexes of MMP activation after an acute MI. However, there have been a number of previous studies (2, 5, 11, 25) that have identified a mechanistic role of MMP activation in the myocardial remodeling process after MI. For example, Rohde et al. (25) demonstrated that broad-spectrum MMP inhibition attenuated the degree of post-MI myocardial remodeling in mice. In a porcine model of myocardial stunning, Lu et al. (16) reported a threefold...

![Graph A](image1.png)  
**Fig. 2.** Regional myocardial function in the remote region served by the LAD coronary arteries and the myocardial infarcted (MI) region after coronary occlusion of the OM1 and OM2 branches of the circumflex artery. Segmental shortening (A) and the slope (B) of the regional preload recruitable stroke-work (PRSW) relationship significantly fell by 60 min of coronary occlusion. #: \( P < 0.05 \) vs. baseline values; *: \( P < 0.05 \) vs. remote region values.
increase in MMP-9 after a 90-min reperfusion period. The present study builds on these past reports by directly demonstrating that increased myocardial MMP activation occurs after an acute MI in vivo. Specifically, through the infusion of an MMP fluorogenic substrate into the myocardial interstitial space, this...
study provided direct evidence that MMP activation occurs as early as 10 min after an acute coronary occlusion.

A common event after myocardial ischemia and reperfusion is the influx of inflammatory cells into the ischemic region, which results in the release and activation of a number of proteolytic enzymes and systems. Neutrophils have been demonstrated to release several species of MMPs, including MMP-8 and MMP-9 (10, 19, 20, 29). Danielsen et al. (4) demonstrated that with MI and reperfusion in pigs, a strong immunoreactive signal for MMP-9 was detected and localized primarily to neutrophils. In the present study, myocardial interstitial fluid was collected and subjected to zymographic analysis after coronary occlusion without reperfusion. A relative increase in zymographic activity was observed in the 90-kDa region after 2 h in the ischemic region and after 3 h in both the ischemic and remote regions. This lytic activity, which was observed in the myocardial interstitial fluid samples, likely reflects soluble MMP-9 (19, 20). However, MMP-9 contains collagen/fibronectin-binding domains (10, 19, 20, 29), and, therefore, the solubilized MMP-9 that was captured within the microdialysis system may not necessarily reflect the total amount of MMP-9 contained within the interstitial compartment. Moreover, lytic activity was observed near the 100-kDa region of the microdialysate samples, which may be indicative of complexes of MMP-9 and the tissue inhibitors of MMPs (TIMPs) (10, 19, 20, 29). Thus, whereas the present study demonstrated an increased release of soluble MMP-9 into the interstitial compartment after MI, it is unlikely that this provides an accurate assessment of total MMP proteolytic activity. Nevertheless, the present study clearly demonstrates the emergence of MMP-9 into the interstitial space after the induction of an MI in the absence of reperfusion.

Fibroblasts and smooth muscle cells synthesize a complement of MMPs, including MMP-9. Previous studies from this laboratory (3, 27) have demonstrated that MMPs, particularly MMP-2 and MMP-9, can be synthesized by porcine myocytes. Thus the release of soluble MMP-9 into the interstitial space after MI was likely the result of liberated MMP-9 from these myocardial cell types. However, it remains to be defined whether this increased release of MMP-9 is a regulated response to the ischemic insult or a nonspecific release secondary to cell injury and lysis. Several recent studies (5, 11) have implicated a functional role for MMP-9 in the reparative response after MI using transgenic mice. For example, Heymans et al. (11) reported a reduced incidence of cardiac rupture in MMP-9 knockout mice after MI. Ducharme et al. (5) reported decreased collagen accumulation and a diminished macrophage infiltrate in MMP-9 knockout mice after coronary ligation and MI. The present study demon-

![Fig. 6.](image-url)
strates that MMP-9 released into the myocardial interstitium is an early event after MI. However, it must be recognized that a number of MMPs are expressed within the myocardium and potentially contribute to the remodeling process post-MI (2, 5, 11, 15, 27, 30). The present study employed gelatin zymography, which predominantly reveals the relative abundance of MMP-9 and MMP-2. Thus whether and to what degree other MMPs are released into the myocardial interstitial space after MI remains to be determined.

The primary approach for measuring myocardial MMP levels and indexes of activation has been through the collection of myocardial samples and the use of in vitro assays (2, 3, 15, 27, 28, 30). For example, with the use of MMP zymography, an induction of MMPs has been observed to occur after hours to days after MI (11, 16, 27). As a consequence, previous studies have been limited to measuring indexes of relative MMP abundance and activation at specific end points. The present study used a microdialysis approach to flood the interstitial compartment with a caged fluorogenic MMP-2/9 substrate and then measured the relative fluorescence within the MI and remote region after coronary occlusion. This approach provided a relative index of MMP activity within the interstitial space in vivo. The results from this set of experiments demonstrate that MMP gelatinolytic activity was increased early after coronary occlusion in the ischemic region but remained relatively unchanged in the remote region. These observations would appear at first to be in contradiction with the interstitial fluid measurements performed by zymographic analysis. MMP fluorogenic activity was detected by 10 min within the ischemic region, but soluble MMP zymographic activity within the interstitial fluid samples was not detected until 2 h after occlusion. These observations likely reflect the fact that MMPs bound to extracellular proteins were activated early after ischemia, and not until later periods of ischemia were detectable amounts of MMP released into the interstitial space and collected by the microdialysis system. There is a family of membrane-type MMPs (MT-MMPs) that are not released into the interstitial space as active enzymes and possess proteolytic activity against similar substrates to those of MMP-2 and MMP-9 (13, 20). These MT-MMPs have been localized to the myocardium and are upregulated in heart failure (27). Thus MMPs bound to extracellular proteins as well as MT-MMPs may have contributed to the fluorogenic activity observed early after acute coronary occlusion. Within the remote region, zymographic activity was increased in myocardial interstitial fluid samples taken from the remote region at 3 h post-MI. However, in vivo measurements of relative MMP fluorogenic activity within this remote region did not increase from baseline values. Total MMP activity is determined by the amount of activated enzyme and the abundance of the endogenous MMP inhibitors (TIMPs) (10, 19, 20, 29). TIMPs bind to activated MMPs in a stoichiometric ratio and inhibit overall proteolytic activity. In the present study, regional myocardial dysfunction occurred within the ischemic region and likely resulted in alterations in local myocardial stress patterns. We (21) have previously demonstrated that the induction of acute LV myocardial wall stress causes an induction of both MMPs and TIMPs. Thus a parallel induction of MMPs and TIMPs may have occurred in the remote region after coronary occlusion, which would have resulted in a neutral effect on MMP fluorogenic activity. The preparation of myocardial homogenates precludes the assessment of the relative effect of TIMPs on MMPs within the interstitial space as well as the in vivo proteolytic activity of these MMP species. This is due in part to the problematic issues that surround in vitro zymographic measurements. First, detergent extraction and electrophoretic separation displace MMP/TIMP complexes. Second, the zymographic assays were performed under optimal enzymatic conditions and substrate availability and in the absence of the influence of TIMPs. The quantity of interstitial fluid obtained from these studies prevented additional measurements of relative TIMP abundance. Thus future studies that utilize fluorogenic substrates of different specificities and measurements of relative TIMP abundance after MI are warranted. Nevertheless, this study clearly demonstrates that dynamic and differential effects on myocardial interstitial MMP activation occurred within the ischemic and remote regions after coronary occlusion.

In the present study, complete coronary occlusion without reperfusion was performed in the porcine LV to obtain a discrete and reproducible region of LV myocardial ischemia/MI. The size and location of the MI was chosen to avoid acute global LV pump failure. In this manner, confounding influences such as systemic hemodynamic compromise or neurohormonal activation on myocardial MMP activation could be minimized. However, it must be recognized that the size and location of the MI may likely influence the degree of myocardial MMP activation and that future studies will be required to define regional changes in MMP expression with different patterns of MI. The present study examined a limited number of MMP species over a very short time period after coronary occlusion. There are a number of MMP species that are endogenously expressed within the myocardium, and several have been identified to be upregulated in cardiac disease states (15, 27, 30). In addition, neutrophils release a specific portfolio of MMPs, which would be operative in the context of ischemia and reperfusion (5, 11, 16, 17, 24). Future studies directed at defining the specific portfolio of MMPs activated within the myocardium after MI are warranted. Nevertheless, the findings of the present study demonstrate an early and prolonged MMP activation occurred within the interstitial space after acute myocardial ischemia.

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