Differential effects of pressure or volume overload on myocardial MMP levels and inhibitory control

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The induction of a hemodynamic load to the left ventricle through either increased pressure (pressure overload, PO) or increased volume (volume overload, VO) results in significant myocardial remodeling (2, 6, 14, 17, 20, 24, 27, 29, 33, 41, 45). However, the pattern of myocardial remodeling is very different between PO and VO and has been hypothesized to be primarily due to differences in the left ventricular (LV) wall stress patterns, which result from these overload states (17). Specifically, PO due to aortic stenosis has been demonstrated to acutely increase LV systolic wall stress, which is normalized over time by increased LV wall thickness or concentric hypertrophy (2, 17, 24, 27). Whereas in VO such as that of mitral regurgitation, increased diastolic wall stress occurs and results in LV chamber enlargement or eccentric hypertrophy (24, 29). A fundamental event in the LV myocardial remodeling process with both forms of overload hypertrophy are changes in myocyte size and alignment within the LV free wall (6, 14, 20, 29, 41). The maintenance of myocyte shape, alignment, and overall architecture are critically dependent on the structural support provided by the extracellular collagen matrix (3, 10, 37, 45, 46).

Past studies have demonstrated that alterations in the LV myocardial collagen interface, both in structure and composition, occur with both PO and VO states (29, 41, 45). The matrix metalloproteinases (MMPs) are an endogenous family of enzymes, which have been identified to be responsible for extracellular tissue remodeling in a number of physiological processes (8, 9, 19, 35, 43, 47). Increased activity of different species of MMPs can result in the degradation of a number of extracellular components, which include the collagens and basement membrane components such as laminin and fibronectin (8, 9, 19, 35, 43, 47). Thus changes in myocardial MMP activity may influence the stability of the LV myocyte-extracellular interface, which in turn would facilitate LV myocardial remodeling. Changes in LV myocardial MMP activity and expression have been identified to occur in end-stage human cardiomyopathic disease, as well as in several animal models of heart failure (18, 23, 40, 44). These past studies would suggest that myocardial MMP activation may contribute to the LV remodeling process. However, whether and to what degree changes in MMP expression and activity occur following either a PO or VO stimulus remained unexplored. Accordingly, the overall goal of the present study was to examine myocardial MMP activity and species expression following the induction of acute PO and VO, as well as following a more prolonged period of both LV overload conditions.
The classification of MMPs was originally developed to reflect substrate specificity, but several MMPs exhibit activity against a wide range of matrix proteins. Whereas a large number of MMPs have been identified in various tissues and cell systems, there are specific classes of MMPs that likely have particular relevance to myocardial remodeling (8, 18, 23, 40, 44). These include the gelatinases such as MMP-9 and MMP-2, interstitial collagenases such as MMP-1, and the stromelysins such as MMP-3. Accordingly, the present study examined the relative myocardial abundance of these MMP species following PO or VO. An important control point of MMP activity is inhibition of the active MMP enzyme. Inhibition of active MMP enzyme can occur through an endogenous class of proteins called the tissue inhibitors of the MMPs, or TIMPs (5, 15, 31). Thus increased myocardial MMP activity may be due to increased abundance of MMPs or alterations in inhibitory control by TIMPs. To address this issue, the present study quantified the myocardial expression of TIMP-1, a TIMP that has been identified to play an important regulatory role in MMP activity (5, 15, 31). The process by which TIMPs modulate MMP activity is through direct binding to the catalytic site of the activated MMP by forming a tight covalent complex (5, 31). Thus control of MMP activity in vivo is not only dependent on the total amount of TIMP available but the degree of actual MMP-TIMP complexes that are formed. Accordingly, the present study directly measured myocardial MMP/TIMP complex formation following the induction of PO or VO. The guiding hypothesis for the experiments performed was that increased MMP activity and expression occur early following the induction of an LV overload stimulus, which is coupled by a loss of MMP inhibitory control.

METHODS

Induction of acute and prolonged PO and VO. This study measured MMP activity, expression, and TIMP profiles after the acute and prolonged PO induced by aortic stenosis in dogs as described previously (24). For the VO studies, measurements were performed after the creation of VO due to mitral regurgitation in dogs (6, 29, 41). The effects on systemic hemodynamics and indexes of LV ejection performance in these overload states have been well characterized previously (2, 6, 24, 29, 41). For the present series of studies, adult mongrel dogs (21–23 kg) were anesthetized with 0.15 ml/kg of fentanyl-droperidol (Innovar Vet, Pitmen-Moore, Washington, DC, 1996). All animals were treated and cared for in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, Washington, DC, 1996.

LV zymographic MMP activity. The LV myocardial samples were prepared for zymographic studies as described previously (40, 44). Briefly, the samples were homogenized in an ice-cold extraction buffer solution maintaining a low pH to prevent proteolytic activation during the extraction process. The homogenate was then centrifuged (4°C, 10 min, 1,200 g), the supernatant concentrated (Centriplus; Millipore, Bedford, MA), and the final protein concentration of the myocardial homogenate was determined (Bio-Rad Protein Assay; Bio-Rad, Richmond, CA). The LV zymographic MMP activity was examined by substrate-specific zymographic analysis as described previously (18, 23, 40, 44). The LV myocardial extracts (6 µg total protein) were loaded onto electrophoretic gels (SDS-PAGE) containing 1 mg/ml of gelatin. After SDS-PAGE, the gels were washed and incubated for 12 h in an MMP substrate buffer at 37°C. After incubation, the gels were stained using 0.1% amido black and destained in water. To provide an internal control with respect to the zymographic activity, samples were collected from the conditioned cell culture media of a human fibrosarcoma HT-1080 cell line (40, 44). 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

(22). A constant heart rate was maintained during balloon inflation by atrial pacing at 100 beats/min. Prolonged PO was produced through direct aortic banding using an external balloon dilation catheter, which was placed by a left thoracotomy (22, 24). After recovery from the procedure, the balloon was inflated to produce a 75% increase in LV systolic pressure, which was maintained for 10 days (n = 5). Acute VO was produced by chordal rupture with urolithic stone grasping forceps as described previously (6, 29, 41). Adequate VO due to mitral regurgitation was defined as a pulmonary capillary wedge pressure of 20 mmHg and over a 50% reduction in thermodilution stroke volume (6, 29, 41). Severe mitral regurgitation was also visually confirmed angiographically in each case. Acute VO was defined as 6 h following the induction of mitral regurgitation (n = 6), and prolonged VO was defined as 14 days following the induction of the mitral regurgitation (n = 7). A separate group of sham-operated controls were used for comparison purposes (n = 11). At the end of the PO or VO protocols, LV pressures and volumes were obtained and wall-stress computations were performed as well described previously (6, 24, 29, 41). Briefly, through the carotid artery, a 5-Fr pigtail catheter was advanced into the LV for contrast dye injection and a micromanometer catheter (7.5 Fr; Millar Instruments, Houston, TX) was advanced through the same artery into the LV. Nonionic contrast material was injected into the LV, and the opacified image was filmed at 60 frames/s in the 30° right anterior oblique position (Cardio Diagnostics; Philips Medical Systems, Sherton, CT) while simultaneously recording LV pressure. LV end-diastolic and end-systolic volumes and wall thickness were computed from the ventriculograms, and with the temporally aligned LV pressures, peak-systolic and end-diastolic circumferential wall stress was computed (2, 6, 17, 24, 29, 41). After this procedure the animals were euthanized by anesthetic overdose, and the LV was quickly harvested, weighed, and placed in a phosphate-buffered ice slush. Full thickness sections of the LV myocardium were then placed in liquid nitrogen and stored at −80°C until the time of assay. 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.

LV zymographic MMP activity. The LV myocardial extracts were prepared for zymographic studies as described previously (40, 44). Briefly, the samples were homogenized in an ice-cold extraction buffer solution maintaining a low pH to prevent proteolytic activation during the extraction process. The homogenate was then centrifuged (4°C, 10 min, 1,200 g), the supernatant concentrated (Centriplus; Millipore, Bedford, MA), and the final protein concentration of the myocardial extracts was determined (Bio-Rad Protein Assay; Bio-Rad, Richmond, CA). Relative LV MMP gelatinase activity was examined by substrate-specific zymographic analysis as described previously (18, 23, 40, 44). The LV myocardial extracts (6 µg total protein) were loaded onto electrophoretic gels (SDS-PAGE) containing 1 mg/ml of gelatin. After SDS-PAGE, the gels were washed and incubated for 12 h in an MMP substrate buffer at 37°C. After incubation, the gels were stained using 0.1% amido black and destained in water. To provide an internal control with respect to the zymographic activity, samples were collected from the conditioned cell culture media of a human fibrosarcoma HT-1080 cell line (40, 44). 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
LV myocardial MMP, TIMP, and MMP/TIMP complex measurements. An ELISA was used for quantitation of specific species of MMPs in the LV myocardial samples. With a two-site ELISA sandwich format and internal standards, quantitative analysis for MMP-1, MMP-2, MMP-9, TIMP-1, as well as the MMP-1/TIMP-1 complex, was performed (RPN 2610–2614, BIOTRAK; Amersham Life Sciences, Buckinghamshire, UK) using previously described techniques (32, 49). For this procedure, the MMP, TIMP-1, or MMP-1/TIMP-1 antibody was coated to the bottom of microtiter wells (mouse anti-human). MMP or TIMP standards and LV myocardial samples (100 µg total protein) were then incubated at 25°C for 2 h. The standards and samples were then aspirated, the plates were washed with 0.1 M phosphate buffer (pH 7.5), and the wells were filled with a polyclonal rabbit antibody and incubated for 2 h. The wells were then washed, and a conjugated donkey anti-rabbit antibody was placed in each well and incubated for 1 h. After another series of washes, the reaction was initiated by the addition of 3,3′,5,5′-tetramethylbenzidine-hydrogen peroxide in dimethylformamide (30% vol/vol). The reaction was allowed to proceed for 30 min and then terminated by the addition of 100 µl of 1 M sulfuric acid, and the plate was read at 450 nm (Vmax Kinetic Microplate Reader; Molecular Devices). This ELISA method was highly linear with increasing concentrations of MMP, TIMP, and MMP/TIMP complex standards. With these standards a regression curve was generated, and results were expressed as nanograms per gram of LV myocardium. All assays were performed in triplicate, and the intra-assay variability was less than 15%.

Immunoblotting for MMP-3. An internally validated ELISA method was not available for MMP-3, and therefore immunoblotting was performed using previously described methods and a well-characterized antibody (40, 44). LV extracts (10 µg total protein) were loaded on an 8% SDS-polyacrylamide gel and separated by SDS-PAGE. The separated proteins were transferred to a polyvinylidene fluoride membrane (0.45 µm; Millipore, Bedford, MA) in 0.025 M Tris base, 0.2 M glycine, pH 6.8, containing 0.1% SDS. The separated proteins were transferred to a polyvinylidene fluoride membrane (0.45 µm; Millipore, Bedford, MA) in 0.025 M Tris base, 0.2 M glycine, pH 6.8, containing 0.1% SDS. The transferred proteins were blocked with 0.2 M Tris base, 1.4 M NaCl, pH 7.6, containing 10% nonfat dry milk, 0.1% Tween 20, and 0.02% NaN3. After a wash with 0.2 M Tris base, 1.4 M NaCl, pH 7.6, containing 0.1% Tween 20, membranes were incubated overnight at 4°C in a monoclonal antibody solution (1.0 µg/ml, IM36L Oncogene Research Products, Cambridge, MA). After stringent washing, the membranes were incubated for 1 h in a horseradish peroxidase-conjugated goat anti-mouse antibody (1:5,000 dilution, Bio-Rad Laboratories, Hercules, CA). The membranes were washed again, and the horseradish peroxidase-conjugated secondary antibody was activated with peracid and luminol (ECL-Plus; Amersham Life Science, Arlington Heights, IL). The luminescent signal was detected by exposure to X-ray film (Eastman Kodak, Rochester, NY). A positive control was included in all immunoblots (AG770; Chemicon International, Temecula, CA). Prestained molecular weight markers (Bio-Rad Laboratories) were used to ensure adequate protein separation and transfer. The intensity of the signal was quantitated using densitometric methods and normalized to control values.

Data analysis. For comparisons of hemodynamics with each treatment, an ANOVA was first performed and if the ANOVA revealed significant differences, pair-wise tests of individual group means were compared using Bonferroni probabilities. The comparisons of MMP zymographic activity and abundance by ELISA were performed in a similar fashion. For the relative comparisons between groups for the MMP-3 immunoblotting results, the Kruskal-Wallis ANOVA was employed. A relationship was determined between the quantitative ELISA and zymographic data and the end-systolic and end-diastolic wall stress values using regression analysis. Linear regression was first performed in which the independent variable was LV wall stress. Multiple linear regression modeling was then performed in which the independent variables were LV wall stress and the treatment intervention (none, PO, or VO). All statistical procedures were performed using the BMDP statistical software package (BMDP Statistical Software, Los Angeles, CA). Results are presented as means ± SE.

RESULTS

The LV-aorta pressure gradient during the 6 h of acute PO due to aortic stenosis was 126 ± 4 mmHg, and after 10 days of prolonged PO it was 84 ± 18 mmHg. After 6 h of acute VO due to mitral regurgitation the regurgitant fraction was 70 ± 3%, and after 14 days of VO it was 63 ± 5%. LV mass increased in prolonged PO compared with control values (6.3 ± 0.2 vs. 4.3 ± 0.3 g/kg, respectively, P < 0.05). LV mass was increased from control values after prolonged VO (5.1 ± 0.4 g/kg), but this did not reach statistical significance (P = 0.08). The LV mass-to-end-diastolic volume ratio decreased with acute PO compared with control values (0.76 ± 0.02 vs. 1.26 ± 0.12 g/ml, respectively, P < 0.05). With prolonged PO, LV mass-end-diastolic volume increased from control values (2.06 ± 0.17 g/ml, P < 0.05). With acute VO, there was a small but significant fall in this value from control (1.19 ± 0.02 g/ml, P = 0.047). The LV mass-to-end-diastolic volume ratio returned to control values with prolonged mitral regurgitation (1.21 ± 0.11 g/ml). Thus, as reported previously, these two forms of LV overload resulted in a different hypertrophic response and subsequently different patterns of LV wall stress (6, 24, 29, 41).

LV myocardial MMP zymographic activity. Total MMP zymographic activity was computed by densitometric analysis of all gelatinolytic zones from 60 to 100 kDa, which likely reflect several species of MMPs (9, 19, 43, 47). Total MMP zymographic activity did not increase from control values with acute PO (1,365 ± 142 vs. 1,237 ± 118 pixels, respectively) or with prolonged PO (1,027 ± 96 pixels). However, as shown in Fig. 1, an emergence of a strong 92-kDa lytic zone was observed with acute PO, which corresponds to the substrate specificity and molecular weight of MMP-9 (43, 47). With acute and prolonged VO, total zymographic activity was unchanged from controls (1,190 ± 142 vs. 1,247 ± 316 pixels, respectively). However, as with acute PO, acute VO resulted in a significant increase in 92-kDa gelatinolytic activity (Fig. 2). Thus, after the acute induction of either PO or VO, increased MMP-9 zymographic activity occurred.

LV myocardial MMP abundance. LV myocardial content for different species of MMPs, TIMP-1, and the MMP/TIMP-1 complex following acute and prolonged PO and VO is summarized in Table 1. With prolonged PO, MMP-1 abundance was reduced from control val-
After both acute and prolonged VO, MMP-1 levels were reduced from controls. MMP-2 levels were unchanged with acute PO and were reduced with prolonged PO. MMP-2 levels remained unchanged with either acute or prolonged PO. MMP-9 levels remained unchanged with either acute or prolonged PO and tended to decrease with acute and prolonged VO, but this did not reach statistical significance (P = 0.09 and 0.10, respectively). Overall, TIMP-1 levels were unchanged with either acute or prolonged PO or VO. The abundance of MMP-1/TIMP-1 myocardial complexes was unchanged with acute PO or VO. MMP-1/TIMP-1 levels were significantly reduced with prolonged PO and tended to decrease with prolonged VO (P = 0.16). Thus differential responses were elicited with respect to MMP-1, MMP-2, and MMP-1/TIMP-1 abundance between PO and VO states.

Immunoblotting was performed on LV myocardial extracts for MMP-3, and the results from these studies of acute and prolonged PO are shown in Fig. 3, and the results for acute and prolonged VO are shown in Fig. 4. A strong immunoreactive signal was observed in all LV myocardial extracts, which localized to approximately 58 kDa, consistent for MMP-3 (21, 30, 31, 42, 43, 47). The signal from the LV myocardial extracts aligned with the positive control preparation, which was obtained from a human fibroblast cell line as described previously (Figs. 3 and 4) (40, 44). With acute PO, the relative abundance of MMP-3 was not increased significantly from control values but was increased with prolonged PO. In acute VO, the relative abundance of MMP-3 significantly increased and fell to within normal values with prolonged VO. Thus, with PO and VO, temporal differences were observed with respect to MMP-3 expression.

The relationship between MMP zymographic activity and MMP abundance was examined by computing the ratio of MMP-9 (92 kDa) activity to MMP-9 abundance for all of the treatment groups (Fig. 5). In both acute PO and VO, the MMP-9 activity-to-abundance ratio increased from control values. This ratio was over twofold greater than normal values with prolonged PO and VO.
higher in the acute VO group compared with acute PO. The MMP-9 activity-to-abundance ratio fell to within normal limits with both prolonged PO and VO. The ratio of MMP-9 activity to TIMP-1 abundance was also computed for all treatment groups and is shown in Fig. 5. The MMP-9 activity-to-TIMP-1 ratio increased in both acute PO and VO, with the highest values observed in the acute VO group. This ratio returned to control values in both prolonged PO and VO. Thus the increased MMP-9 zymographic activity with acute PO and VO was not paralleled by a proportionate increase in MMP-9 or TIMP-1 content and would suggest a heightened activational state of MMP-9.

LV wall stress patterns and MMP expression. The potential relationship between changes in LV wall stress patterns, which were induced with either PO or VO, were examined with respect to quantifiable indexes of MMP activity and inhibitory control. With MMP-9 zymographic activity as the dependent variable, there was a positive relationship with LV peak-systolic wall stress after the induction of acute PO (Fig. 6). With VO, LV end-diastolic wall stress was significantly associated with MMP-9 zymographic activity (Fig. 6). LV peak-systolic wall stress was strongly associated with a change in TIMP-1 levels in PO, whereas increased diastolic stress was associated with decreased TIMP-1 values in VO (Fig. 7). There was an apparent association between LV myocardial levels of the MMP-1/TIMP-1 complex and changes in LV wall stress patterns with PO and VO; however, there was a significant degree of variability associated with this parameter (Fig. 8). Overall, LV myocardial MMP-1/TIMP-1 complex levels were positively associated with

### Table 1. MMP and TIMP abundance: effects of acute and prolonged pressure or volume overload

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>TIMP-1</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 11)</td>
<td>808 ± 144</td>
<td>1,483 ± 510</td>
<td>75 ± 15</td>
<td>779 ± 129</td>
<td>904 ± 134</td>
</tr>
<tr>
<td>Pressure overload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute (n = 9)</td>
<td>1,055 ± 317</td>
<td>1,600 ± 393</td>
<td>69 ± 14</td>
<td>651 ± 147</td>
<td>1,337 ± 251</td>
</tr>
<tr>
<td>Prolonged (n = 5)</td>
<td>432 ± 69*</td>
<td>256 ± 39†</td>
<td>56 ± 8</td>
<td>527 ± 39</td>
<td>505 ± 114†</td>
</tr>
<tr>
<td>Volume overload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute (n = 6)</td>
<td>309 ± 52*</td>
<td>1,730 ± 493</td>
<td>45 ± 7</td>
<td>417 ± 40</td>
<td>682 ± 212</td>
</tr>
<tr>
<td>Prolonged (n = 7)</td>
<td>440 ± 54*</td>
<td>931 ± 249</td>
<td>46 ± 3</td>
<td>455 ± 63</td>
<td>576 ± 176</td>
</tr>
</tbody>
</table>

Values are means ± SE in nanograms per gram LV. MMP, matrix metalloproteinases; TIMP, tissue inhibitor of MMP; Complex, MMP-1/TIMP-1 complex; LV, left ventricle. *P < 0.05 vs. control. †P < 0.05 vs. acute.
LV peak-systolic stress in PO and negatively correlated in VO. Multiple linear regression was performed in which both LV wall stress and the overload state (PO, VO) were considered as independent variables. With respect to MMP-9 zymographic activity, the type of overload was not considered an independent predictive variable when considered with either LV peak-systolic ($r = -0.30, P = 0.18$) or end-diastolic ($r = -0.18, P = 0.42$) wall stress. For TIMP-1 levels, linear regression modeling revealed that the type of overload was a significant independent variable when considered with peak-systolic stress ($r = -0.63, P < 0.01$). The type of overload stimulus was not identified as an independent variable when considered with LV end-diastolic wall stress in a multiple linear regression model with TIMP-1 as the dependent variable ($r = -0.03, P = 0.17$). The type of overload state was not an independent variable for MMP-1/TIMP-1 levels for either peak systolic ($r = 0.01, P = 0.96$) or end-diastolic ($r = -0.26, P = 0.31$) wall stress.

DISCUSSION

The myocardial collagen scaffold has been postulated to play an important role in maintaining the alignment of myofibrils within the myocyte through a collagen-cytoskeletal-myofibril interface, which may also be important for the transduction of extracellular mechanical signals (3, 10, 37, 45, 46). Thus alterations in the normal three-dimensional extracellular network due to enzymatic cleavage by the MMPs may facilitate changes in LV function and geometry. Several recent clinical and basic studies have demonstrated increased LV myocardial MMP activity with end-stage LV failure (18, 23, 26, 31, 40, 44). However, there have been no studies to date that have systematically examined LV myocardial MMP activity and species expression following the imposition of either a PO or VO. The present study measured MMP activity, species abundance, and indexes of MMP activational control in dogs following the induction of either PO or VO. Acute PO and VO resulted in increased MMP activity in control myocardial preparations ($n = 11$), following A-PO ($n = 9$), with P-PO ($n = 5$), with A-VO ($n = 6$), and with P-VO ($n = 7$). With A-PO and A-VO, MMP-9 activity-to-abundance ratio increased, and returned to within normal values with P-PO or P-VO. A-VO resulted in significantly higher MMP-9 activity compared with A-PO values. B: 92-kDa MMP activity-to-tissue inhibitor of MMP (TIMP)-1 ratio was computed for all treatment groups. With A-PO and A-VO, MMP-9 activity-to-TIMP-1 ratio increased from controls, and fell to within normal values with P-PO or P-VO. This ratio was significantly higher with A-VO compared with A-PO. *$P < 0.05$ vs. control. **$P < 0.05$ vs. respective acute values. *$P < 0.05$ vs. A-PO values.

LV peak-systolic stress in PO and negatively correlated in VO. Multiple linear regression was performed in which both LV wall stress and the overload state (PO, VO) were considered as independent variables. With respect to MMP-9 zymographic activity, the type of overload was not considered an independent predictive variable when considered with either LV peak-systolic ($r = -0.30, P = 0.18$) or end-diastolic ($r = -0.18, P = 0.42$) wall stress. For TIMP-1 levels, linear regression modeling revealed that the type of overload was a significant independent variable when considered with peak-systolic stress ($r = -0.63, P < 0.01$). The type of overload stimulus was not identified as an independent variable when considered with LV end-diastolic wall stress in a multiple linear regression model with TIMP-1 as the dependent variable ($r = -0.03, P = 0.17$). The type of overload state was not an independent variable for MMP-1/TIMP-1 levels for either peak systolic ($r = 0.01, P = 0.96$) or end-diastolic ($r = -0.26, P = 0.31$) wall stress.

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Myocardial MMP abundance with PO or VO. In the present study, acute VO but not acute PO, was associ-
Fig. 6. Relationship between LV wall stress and myocardial MMP-9 zymographic activity was examined for control values (●), with acute induction of LV overload (■), and with prolonged LV overload (▲). A: results for PO. B: results for VO. With PO increased LV peak-systolic stress was associated with increased MMP-9 zymographic activity, but there was no relationship observed for MMP-9 activity and end-diastolic stress. With VO LV end-diastolic wall stress and MMP-9 zymographic activity were positively related.

Fig. 7. Relationship between LV wall stress patterns and myocardial TIMP-1 content was examined for control values (●), with acute induction of LV overload (■), and with prolonged LV overload (▲). A: results for PO. B: results for VO. LV peak-systolic wall stress was strongly and positively associated with TIMP-1 levels in A-PO state. There was no relationship between LV end-diastolic stress and TIMP-1 levels with PO, but significant negative relation was observed for VO.
ated with a significant reduction in the myocardial content of MMP-1 and increased MMP-3. With prolonged PO, a reduction in MMP-1 and MMP-2 and increased MMP-3 was observed. Thus some difference in the temporal profile of MMP species abundance was observed between these two LV overload conditions. These results suggest that different chemical and mechanical signals are produced with PO and VO, which in turn would influence MMP expression. Although a diverse repertoire of signals can influence changes in MMP expression, one important intracellular event appears to be linked to the AP-1 binding site which also forms the phorbol-ester-responsive element on certain MMP genes (12, 35, 38, 39). It is likely that protein kinase C (PKC) is involved in the intracellular induction of MMP transcription because the exposure of several different cell systems to phorbol esters, which increase PKC levels and activation, causes increased MMP mRNA expression (12, 35, 38, 39). Bioactive molecules such as catecholamines, angiotensin II, and endothelin, through specific receptor activation, can modulate intracellular PKC levels. There are several past reports that have demonstrated that the local production of angiotensin II and endothelin occur with PO- or VO-induced hypertrophy (6, 7, 11, 13). Local activation of these receptor transduction pathways following the induction of PO or VO would likely influence MMP expression via a PKC-dependent mechanism. The MMP-1 and MMP-3 genes contain elements in the promoter region, which bind protooncogene products of the fos and jun family (12, 39). However, the present study did not measure circulating or tissue levels of bioactive peptides or cytokines, which may have directly contributed to the changes in MMP levels that were observed. Bioactive peptides and cytokines, such as tumor necrosis factor-α and interleukin-1, which stimulate the production of protooncogenes, have been demonstrated to increase MMP transcription in several cell systems (4, 28, 36). However, it is also possible that the imposition of an LV overload caused alterations in MMP mRNA stability or protein synthesis rates. Based on the results obtained in the present study, future studies that more carefully determine the basis for the changes in MMP myocardial levels in LV overload states would be warranted.

In addition to receptor-mediated effects on MMP expression, it has been demonstrated previously that MMP activity can be modulated by physical stimuli (1, 48). For example, Werb et al. (48) demonstrated that cytoskeletal disruption in isolated cell preparations increased MMP expression. In addition, past studies have demonstrated that changes in cell shape can cause alterations in MMP synthesis and activity (1, 19). In both PO and VO, the cells within the myocardium are exposed to increased stress and deformation, which may have caused alterations in MMP expression and activation. Changes in LV myocardial MMP-9 zymographic activity and TIMP-1 levels appeared to be associated with changes in LV wall stress patterns. Specifically, with increased LV wall stress, MMP-9 zymographic activity increased. TIMP-1 levels changed in relation to both types of overload stimuli, as well as
in association with changes in LV wall stress patterns. In PO, MMP/TIMP complex formation was higher, suggesting increased MMP inhibitory control was operative in this overload state. This distinct profile in MMP activity and TIMP levels with LV wall stress patterns may have important implications in the LV remodeling process. For example, these results would suggest that with increased LV systolic wall stress, such as that with a pure PO, an overall decrease in MMP activity may occur within the LV myocardium, which would potentially favor extracellular matrix accumulation. A common histological observation in the LV remodeling that occurs with severe PO-induced hypertrophy is increased extracellular matrix accumulation (45, 46). Conversely, with increased LV diastolic wall stress, which occurs in VO, a net increase in myocardial MMP activity may occur. In VO states, such as mitral regurgitation, changes in extracellular matrix composition and structure have been reported (6, 41). Although remaining speculative, the net changes in MMP zymographic activity and TIMP-1 levels that occurred in the present study following the induction of mitral regurgitation would favor extracellular remodeling and thereby facilitate the LV dilation that invariably occurs with this form of VO (6, 29, 41).

The activation of MMP-1 involves an enzymatic cascade that ultimately results in degradation of the nascent enzyme (21, 31, 42). Thus, with acute VO, enhanced proteolytic MMP-1 activation and subsequent degradation was a probable contributory factor for the significant reduction in MMP-1. The promoter region of the MMP-1 and the MMP-3 genes have some common regulatory DNA sequences, but there are several additional upstream response elements in the MMP-3 gene (9, 28, 36, 47). Thus increased extracellular stimuli such as neurohormones and cytokines may induce differential levels of MMP-1 and MMP-3 expression. The gelatinases MMP-9 and MMP-2 also contain dissimilar promoter sequences and regulatory elements (9, 28, 34, 47). This laboratory has reported previously that MMP-3 and MMP-9 levels increased and MMP-1 levels decreased in human dilated cardiomyopathy, providing evidence for differential regulation of myocardial MMP expression (44). The differences in MMP myocardial abundance that occurred with PO or VO provide additional support for the concept that MMP species expression is differentially regulated and is dependent on the local stimulus produced.

Myocardial MMP activity with PO or VO. Because MMPs are primarily secreted in an inactive or pro-MMP form, MMP activation occurs following secretion into the extracellular space (8, 9, 19, 21, 31, 42, 43, 47). Thus an important control point for MMP activity is proteolytic processing of the pro-MMPs. One of the most frequently studied MMPs with respect to zymogen activation is the interstitial collagenase MMP-1 (19, 21, 30, 42, 43, 47). The first step in MMP-1 activation is proteolytic cleavage ahead of the cysteine residue, which results in a partially active intermediate form that is then quickly converted to the active form by autolytic means or through cleavage by MMP-3 (21, 30, 42). Thus an important regulatory step in overall MMP activation involves the expression and activational state of MMP-3. Increased myocardial levels and activity of MMP-3 have been reported to occur in both humans and animals with LV dilation and failure (40, 44). In the present study, MMP-3 was increased with acute VO and therefore provided an increased capacity for MMP-1 proteolytic activation. The reduced MMP-1 levels which occurred concomitantly with increased MMP-3 levels following the induction of acute VO would support this hypothesis.

In the present study increased 92-kDa zymographic activity occurred with both acute PO and VO, which is consistent with the activity and substrate specificity of the 92-kDa gelatinase MMP-9 (8, 9, 19). The increased MMP-9 zymographic activity with PO and VO was not associated with changes in absolute enzyme abundance. These findings would suggest that the increased MMP-9 activity observed with acute PO and VO was due to an increased activational state. As discussed in the following section, this is probably due to a loss in MMP inhibitory control. However, it must be recognized that the in vitro zymographic technique requires electrophoretic separation of the MMP proteins and therefore only provides an index of potential MMP activational states which may exist in vivo.

TIMPs with PO or VO. The TIMPs are low-molecular-weight proteins that can complex with high efficiency to activated MMPs. Therefore, TIMPs form an important endogenous system for regulating actual MMP activity in vivo. The first well-characterized TIMP was TIMP-1, which binds to a number of activated MMPs (5, 15). In the present study, TIMP-1 levels were unchanged with either PO or VO. However, the ratio of MMP-9 activity to MMP-9 abundance and the ratio of MMP-9 activity to TIMP-1 levels were both significantly increased, particularly with acute VO. These results would suggest a loss of inhibitory control of MMP activity. Furthermore, the abundance of MMP-1/TIMP-1 complexes within the myocardium were reduced in prolonged PO, which would also suggest that alterations in MMP-TIMP stoichiometry occurred in this overload state. Several past studies have provided evidence to suggest that changes in the stoichiometric ratio of MMPs to TIMPs have occurred with end-stage cardiomyopathic disease (26, 44). At present, there are four TIMPs that have been characterized with respect to being separate gene products which influence the activity of MMPs (5, 8, 9, 15, 16, 19, 25). More recently a high expression pattern for TIMP-4 was reported in the myocardium (16). However, whether TIMP-4 possesses different MMP inhibitory activity within the myocardium remains to be established. In light of the findings from the present study, future investigations focused on the function and regulation of these various TIMP species in LV overload states would be warranted.

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REFERENCES


