Selective Matrix Metalloproteinase Inhibition With Developing Heart Failure
Effects on Left Ventricular Function and Structure


Abstract—The matrix metalloproteinases (MMPs) are an endogenous family of proteolytic enzymes implicated to contribute to LV remodeling. However, broad-spectrum MMP inhibition (MMPi), particularly inhibition of interstitial collagenase (MMP-1), may not be clinically applicable. This study examined the effects of selective MMPi (sparing MMP-1) in a model of developing congestive heart failure. Pigs were randomly assigned to 3 groups: (1) rapid pacing for 3 weeks (240 bpm, n=10); (2) selective MMPi (20 mg/kg per day-PO;PGE7113313) and rapid pacing (n=12); and (3) controls (n=10). LV peak wall stress increased from controls with rapid pacing (140±6 versus 319±18 g/cm²; P<0.05) and was reduced with selective MMPi (208±9 g/cm²; P<0.05). Preload recruitable stroke work was reduced with rapid pacing (4.3±0.4 versus 1.2±0.2 dyne · cm/mm Hg; P<0.05) and was increased with selective MMPi (2.6±0.3 dyne · cm/mm Hg; P<0.05). Plasma norepinephrine increased by 6-fold in the rapid pacing group (P<0.05) and was reduced from untreated values with selective MMPi (P<0.05). At the myocardial level, myocyte cross-sectional area was increased with selective MMPi but fibrillar collagen volume fraction remained unchanged relative to control values. These results suggest that targeting a selective portfolio of myocardial MMP species for inhibition may provide a more rational therapeutic strategy in the setting of congestive heart failure. (Circ Res. 2003;92:177-185.)

Key Words: left ventricular systolic function □ myocardial stiffness □ myocardial structure □ matrix metalloproteinases □ heart failure

A structural milestone in the progression of congestive heart failure (CHF) is alterations in left ventricular (LV) geometry, commonly referred to as myocardial remodeling. The myocardial extracellular matrix contributes to the maintenance of LV geometry, structural alignment of adjoining myocytes, as well as modulating transmembrane signaling pathways. A family of enzymes that contributes to extracellular collagen degradation and tissue remodeling is the matrix metalloproteinases (MMPs). Increased expression and activity of MMPs within the LV myocardium occurs in both patients and animals with CHF. Oral active nonspecific MMP inhibitors, termed as broad spectrum MMP inhibitors, have been used in several animal models of CHF and demonstrated to attenuate the LV remodeling process. Therefore, modulating MMP activity represents a potential therapeutic target in the context of LV remodeling and CHF. However, long-term inhibition of all MMP species will likely interfere with normal tissue remodeling processes and can give rise to undesirable systemic effects. Thus, selective targeting of MMP species that contribute to pathological myocardial remodeling in developing CHF will likely hold greater therapeutic potential. Whereas a number of MMP species are expressed within the human myocardium, not all MMPs are upregulated in end-stage CHF. Specifically, the abundance of interstitial collagenase-1, or MMP-1, is significantly reduced in patients with cardiomyopathic disease. However, it remains unknown whether inhibition of MMP-1 is a fundamental requirement in order to alter the myocardial remodeling process during the initiation and development of CHF. Accordingly, the overall goal of this study was to institute selective MMP inhibition that would effectively spare MMP-1 inhibition in an animal model of developing CHF.

Materials and Methods
Selective MMP Inhibition
Past studies have demonstrated that several classes of MMPs are increased in CHF including the interstitial collagenase MMP-13, stromelysins such as MMP-3, and the gelatinases such as MMP-2 and MMP-9. In order to identify an MMP inhibition dosage regimen that would provide acceptable plasma profiles with respect to inhibition of these species but effectively spare MMP-1 inhibition, pharmacokinetic studies were performed on 6 chronically instru-
mented pigs using methods described previously. The MMP inhibitor chosen for this study was PGE7113313 (Procter and Gamble), which based on initial in vitro assay systems, exhibited a 50% inhibitory concentration (IC50) for MMP-2 of 1.5 nmol/L, MMP-3 of 13 nmol/L, MMP-8 of 1.9 nmol/L, MMP-9 of 1 nmol/L, and MMP-13 of 1.1 nmol/L. However, the IC50 of this compound for MMP-1 was greater than 4000 nmol/L. This MMP inhibitor did not influence TNF-α release in a THP-1 cell culture assay, nor affected angiotensin-converting enzyme activity based on an enzymatic assay. Thus, this MMP inhibitor did not influence other metalloprotease systems. Plasma MMP inhibitor levels were determined by high-performance chromatography and mass spectrometry. From initial dose determination studies and pharmacokinetic profiles, it was determined that a 20 mg/kg dose administered 3 times per day (TID) would result in the maintenance of plasma levels at approximately 10-fold higher than the IC50 for the MMP species of interest, but would not approach MMP-1 IC50 levels.

Experimental Design and CHF Model Preparation
For these studies, the rapid pacing pig model was used because this predictably causes progressive LV dilation, myocardial remodeling, and after 3 weeks of pacing, recapitulates the clinical features of the CHF phenotype. Weight-match pigs (22 to 23 kg, Hamborne Farms, Orangeburg, SC) were instrumented with an aortic access catheter and a modified atrial pacemaker (8329, Medtronic, Inc.). The pigs were randomly assigned to 3 groups: (1) rapid pacing (240 bpm for 3 weeks (n=10); (2) concomitant MMP inhibition (PGE7113313 20 mg/kg-TID PO) and rapid pacing (n=12); and (3) sham controls, instrumentation with no rapid pacing (n=10). Drug treatment was started 3 days before the initiation of pacing and continued for the entire 21-day pacing protocol. All animals described in these studies were treated and cared for in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (National Research Council, Washington, 1996).

LV Systolic and Diastolic Function
LV size and function were measured at weekly intervals in all of the pigs entered in the protocol using simultaneous pressure-echocardiography as described previously. After the final set of LV echocardiographic studies on day 21, plasma samples were collected for norepinephrine and endothelin levels, peak/trough drug levels, and MMP-2 quantitation. The pigs were then anesthetized (sufentanil-1 μg/kg bolus and 0.5 μg/kg per hour infusion) and intubated. A multilumen thermolodization catheter (7.5F, Baxter Healthcare Corp) was positioned in the pulmonary artery. A sternotomy was performed, and a vascular ligature was placed around the inferior vena cava. A previously calibrated microtip transducer (7.5 F, Millar Instruments Inc) was placed in the LV. Four piezoelectric crystals (2 mm, Sonometrics) were positioned in the LV endocardium in order to provide an orthogonal myocardial dimension across the short axis. LV preload was altered by sequential occlusion and release of the caval tape and the isochronal LV end-diastolic pressure-stroke work points subjected to linear regression in order to compute the preload recruitable stroke work relation (PRSWR). LV myocardial velocity of circumferential fiber shortening, corrected for heart rate (Vcf), was also computed from the digitized LV crystal and pressure data. Indices of LV diastolic function were determined by computations of the regional LV chamber stiffness constant (Kc) and myocardial stiffness constant (Km). As an index of LV active myocardial relaxation, the time constant of LV isovolumic relaxation, or Tau, was computed. The animals were then deeply anesthetized (5% isoflurane), a sternotomy performed, and the LV removed. The circumflex coronary artery was cannulated and the LV region served by this coronary perfusion fixed for morphometric assessment. Sections of the LV free wall served by the left anterior descending coronary artery were snap frozen for biochemical. The region that was perfusion-fixed or frozen was alternated with each preparation.

LV Myocardial Structure and MMP Levels/Zymography
Sections of perfusion-fixed LV myocardium were embedded, cut in the circumferential orientation, and examined by light microscopy in order to measure myocyte cross-sectional area. Perfusion-fixed LV sections were also subjected to immunohistochemical staining for the basement membrane protein laminin. Briefly, LV sections were incubated overnight at 4°C with an anti-laminin antibody (1:500 dilution, rabbit anti-laminin AB19012, Chemicon), followed by incubation with an anti-rabbit anti-sera conjugated to horse-radish peroxidase (1:1000, Vector Laboratories). Positive staining was visualized by incubation in a 3',3'-diaminobenzidine-hydrogen peroxide substrate. Negative controls included substitution with nonimmune primary anti-sera. LV myocardial sections were prepared for scanning electron microscopy and light microscopic examination of the collagen matrix. In addition to routine scanning electron microscopy, perfusion-fixed LV myocardial samples were subjected to potassium-hydroxide/tannic acid maceration treatment in order to remove cellular constituents and provide a greater relief of the fibrillar collagen matrix. LV full thickness myocardial sections (0.5 g) were subjected to biochemical assessment for hydroxyproline as well as collagen solubility. The sections underwent acid hydrolysis and hydroxyproline measured spectrophotometrically (550 nm) after reaction with Ehrlich’s reagent. Collagen solubility was determined as the quotient of hydroxyproline obtained from salt extraction and the total hydroxyproline from hydrolysis.

Relative LV MMP activity was examined by substrate-specific zymographic analysis as described previously. Before zymography, the myocardial extracts were incubated with trypsin (0.5 μg/mL, type I; EC 3.4.21.4, 5 minutes at 37°C) in order to unfold the MMP enzyme and cleave the propeptide sequence in order to reveal total MMP zymographic activity. The zymograms were quantitated by image analysis (Gel Pro Analyzer, Media Cybernetics). Using a 2-site ELISA sandwich format and internal standards, plasma and myocardial MMP-2 levels were measured (RFN 2617; 2611 BIO-TRAK, Amersham Life Sciences).

Data Analysis
Indices of LV function and systemic hemodynamics were initially examined using multiway analysis of variance (ANOVA) in which the main effects were treatment and time. Pair-wise tests of group means were compared using Bonferroni probabilities. For the biochemical and morphometrics data, the 3 treatment groups were compared by ANOVA and Tukey’s procedure. All statistical procedures were performed using the BMDP statistical software package (BMDP Statistical Software Inc). Results are presented as mean±SEM. Values of P<0.05 were considered to be statistically significant.

Results
LV Size and Function
Weekly changes in LV size and function obtained in the conscious, awake state for the rapid pacing groups are summarized in Figure 1. In both rapid pacing groups, LV end-diastolic dimension and peak wall stress increased, and fractional shortening decreased in a time dependent manner. However, at week 3 of the pacing protocol, LV fractional shortening was higher in with selective MMP inhibition when compared with untreated pacing values. LV peak wall stress was significantly lower in the selective MMP inhibition group at 2 and 3 weeks of pacing when compared with the untreated pacing group. A small but significant increase in LV mass occurred in the untreated rapid pacing group at 3 weeks of pacing when compared with baseline values (Figure 2). A significant and time-dependent increase in LV mass occurred in the selective MMP inhibition group.
LV Systolic and Diastolic Function
Systemic hemodynamics and pressures are summarized in the Table for the 3 groups. The changes in hemodynamics observed after 3 weeks of rapid pacing are consistent with past reports and the CHF phenotype. In the selective MMP inhibition group, mean arterial pressure, cardiac output, and systemic and pulmonary vascular resistance were similar to control values. In the selective MMP inhibition group, LV systolic pressure and dP/dt were increased from pacing-only values but remained reduced from control values.

The slope of the LV preload recruitable stroke work relation (PRSW) was reduced in both rapid pacing groups when compared with control values (Figure 3). In the selective MMP inhibition group, the slope of the PRSWR was increased from rapid pacing--only values. The slope of the Vcfc-stress relation was reduced from control values in the both rapid pacing groups when compared with control values (Figure 3) and was increased from rapid pacing--only values in the selective MMP inhibition group.

LV end-diastolic pressure was increased and the time constant of isovolumic relaxation (τ) was prolonged in the rapid pacing group (Table). In the selective MMP inhibition group, LV end-diastolic pressure was reduced from untreated pacing values, and τ was similar to control values. The LV chamber and myocardial stiffness constants were unchanged from control values with chronic rapid pacing (Figure 4). In the selective MMP inhibition group, LV myocardial stiffness was increased from both control and untreated pacing values. However, LV chamber stiffness was not significantly increased with selective MMP inhibition (P=0.19).

Plasma Drug Concentration, Neurohormones, and MMP-2 Levels
After the 21-day pacing protocol, peak plasma concentrations of the MMP inhibitor (drawn 2 hours after dose) were 455±120 nmol/L, and the trough values (drawn 8 hours after...
dose) were 97±25 nmol/L. These plasma concentrations were 10-fold lower than those necessary to inhibit MMP-1 and were within the target range for selective MMP inhibition. Plasma norepinephrine was increased from baseline control values (1231±271 pg/mL; P<0.05, respectively) and was reduced from untreated values in the selective MMP inhibition group (568±74 pg/mL; P<0.05), but remained increased from basal control values (P<0.05). Plasma endothelin increased from untreated pacing values (4.89±0.93 fmol/mL; P<0.05), but remained increased from normal baseline values (P<0.05). Baseline MMP-2 plasma levels were 90±33 ng/mL (range 40 to 160 ng/mL). The change in plasma MMP-2 levels were determined at the completion of the study protocol and are summarized in Figure 5. Plasma MMP-2 levels increased in the untreated pacing CHF group and decreased in the selective MMP inhibition group.

Myocardial Structure and Composition
The measurements for LV myocyte cross-sectional area approximated a Gaussian distribution in all treatment groups. LV myocyte cross-sectional area decreased from control values in the rapid pacing group (190±3 versus 210±4 μm²; P<0.05). In the MMP inhibition group, myocyte cross-sectional area increased from both control and the rapid pacing–only values (230±3 μm²; P<0.05). Representative picro-Sirius–stained LV myocardial sections are shown in Figure 6. In the pacing-only group, morphometric analysis revealed a reduction in the relative content of fibrillar collagen between myocytes (4.3±0.3 versus 6.2±0.2%; P<0.05). In control LV myocardial sections, immunohistochemical staining for laminin revealed a uniform linear staining pattern along myocyte fascicles (Figure 7). In the pacing CHF sections, disruptions in the continuity of the laminin staining pattern was observed. At higher power, a more fragmented staining pattern for laminin was observed in the pacing CHF sections. In the selective MMP inhibition group, laminin staining appeared similar to controls. Scanning electron microscopy revealed a disruption of the fibrillar collagen weave in the pacing CHF group (Figure 6). In the selective MMP inhibition group, the relative content the fibrillar collagen weave was similar to control values (5.8±0.05%). Maceration digestion revealed a highly structured fibrillar weave with clear profiles for individual myocytes (Figure 6). In marked contrast, cellular digestion of pacing CHF samples revealed a disorganized, collapsed, and emphysematous fibrillar matrix. In the selective MMP inhibition group, the organization of the collagen matrix appeared similar to normal control myocardium. Myocardial hydroxyproline content was 0.28±0.05 mg/g wet weight in the control group and was unchanged either pacing group. However, the salt extractable fraction of hydroxyproline was 0.3 versus 6.2±0.2%; P<0.05). In control LV myocardial sections, immunohistochemical staining for laminin revealed a uniform linear staining pattern along myocyte fascicles (Figure 7). In the pacing CHF sections, disruptions in the continuity of the laminin staining pattern was observed. At higher power, a more fragmented staining pattern for laminin was observed in the pacing CHF sections. In the selective MMP inhibition group, laminin staining appeared similar to controls. Scanning electron microscopy revealed a disruption of the fibrillar collagen weave in the pacing CHF group (Figure 6). In the selective MMP inhibition group, the relative content the fibrillar collagen weave was similar to control values (5.8±0.05%). Maceration digestion revealed a highly structured fibrillar weave with clear profiles for individual myocytes (Figure 6). In marked contrast, cellular digestion of pacing CHF samples revealed a disorganized, collapsed, and emphysematous fibrillar matrix. In the selective MMP inhibition group, the organization of the collagen matrix appeared similar to normal control myocardium. Myocardial hydroxyproline content was 0.28±0.05 mg/g wet weight in the control group and was unchanged either pacing group. However, the salt extractable fraction of hydroxyproline was significantly increased in the pacing CHF group compared with control (10.1±1.8 versus 4.96±0.94%; P<0.05) and remained increased in the selective MMP inhibition group (14.9±4.8%; P<0.05).

LV Myocardial MMP Activity and Content
MMP zymographic activity was increased in myocardial samples from the pacing CHF group when compared with
control (13,390±1,604 versus 8,912±1,443 pixels; *P<0.05). However, in the selective MMP inhibition group, trypsin-activated MMP zymographic activity was similar to control values (8,291±917 pixels). By ELISA, LV myocardial MMP-2 concentration was 1578±205 ng/g in control samples and was increased in the pacing CHF and selective MMP inhibition groups (2180±270 and 3178±548 ng/g, respectively; *P<0.05).

Discussion

Increased myocardial expression and activation of the MMPs have been demonstrated in patients with CHF and thereby have been implicated to contribute to the LV remodeling process.2,6,13 Broad spectrum MMP inhibition has been demonstrated to modify the progression of LV remodeling and slow the CHF process in several animal model systems.5,7–9 Thus, selective targeting of myocardial MMPs that directly contribute to the LV remodeling process constitutes an important avenue of investigation. In past clinical reports of end-stage CHF, MMP-1 levels were reduced, whereas increased levels of the gelatinases (MMP-2, MMP-9) and stromelysin (MMP-3) were observed.6 The present study instituted MMP inhibition during the pacing pig model of CHF, which would achieve plasma levels necessary to inhibit a portfolio of MMP species, but would not directly affect MMP-1 activity. Using this selective MMP inhibition strategy, the important findings of the present study were 2-fold. First, selective MMP inhibition improved LV function and systemic hemodynamics and reduced the degree of neurohormonal activation. Second, the structural basis for the effects of MMP inhibition on LV geometry and function included LV myocyte growth and improved integrity of the collagen matrix.

A recent study demonstrated that MMP-1–sparing inhibition favorably affected the early remodeling process in rabbits after myocardial infarction.19 The present study demonstrated that MMP-1 inhibition is not a fundamental requirement for influencing the LV myocardial remodeling process in developing CHF. Selective MMP inhibition significantly affected the LV myocardial remodeling process as evidenced by increased posterior wall thickness and reduced chamber dimensions when compared with untreated pacing values. Because important determinants of LV wall stress are wall thickness and radius, LV peak wall stress was reduced in the MMP inhibition group when compared with untreated pacing CHF values. Thus, a contributory factor for the increased LV pump function observed with selective MMP inhibition was the reduction in LV wall stress. Two indices of LV ejection performance were evaluated: the preload recruitable stroke work relation and the rate corrected velocity of circumferential fiber shortening, corrected for heart rate (Vcf/c) and end-systolic wall stress relation was reduced after 3 weeks of rapid pacing. Slope of this relation was increased from untreated pacing values in the MMPi group, but remained reduced from control values. *P<0.05 vs control; ++P<0.05 vs rapid pacing only.
tial fiber shortening-end-systolic stress relation. These indices of LV ejection performance were reduced with pacing CHF and improved selective MMP inhibition. With selective MMP inhibition, the continuity of the myocardial fibrillar collagen weave was improved from the untreated pacing CHF. Therefore, a structural mechanism for the improved LV systolic function with selective MMP inhibition was a stabilization of intermyocyte collagen that in turn would improve the transduction of myocyte shortening into an overall LV ejection.

Another contributory factor for the improved LV function with selective MMP inhibition was the effects on neurohormonal system activity. Plasma norepinephrine and endothelin levels were reduced with selective MMP inhibition relative to untreated pacing CHF. The reduction in these bioactive molecules suggests that selective MMP inhibition during rapid pacing reduced the degree of neurohormonal stimulation, which in turn would provide favorable effects on vascular resistance and LV myocardial function. Moreover, this reduced neurohormonal activity with selective MMP inhibition would produce a secondary favorable effect on the LV remodeling process. A contributory factor for the reduced plasma norepinephrine and endothelin levels with selective MMP inhibition was the attenuation in the degree of hemodynamic compromise that invariably occurs in the pacing model of CHF.

Because changes in the myocardial collagen matrix have been implicated to influence LV compliance, an important objective of the present study was to examine LV stiffness characteristics after selective MMP inhibition. Consistent
with past reports, there were no significant changes in LV chamber or myocardial stiffness with pacing CHF. A number of factors influence LV chamber stiffness such as loading conditions, myocardial active relaxation processes, as well as myocardial stiffness. In the present study, despite the increased LV myocardial stiffness, LV chamber stiffness was not increased in the selective MMP inhibition group. This finding was likely due to the fact that the increased LV myocardial stiffness was offset by a reduction in LV loading conditions (reduced wall stress) and a normalization of active relaxation. These results suggest that selective MMP inhibition in this model of pacing CHF improved indices of LV ejection performance without significant effects on LV chamber compliance. In the present study, the institution of MMP inhibition in this pacing model resulted in significant LV myocardial growth as evidenced by increased LV mass. Whether this increase in LV mass may adversely affect LV diastolic function over a longer period of time warrants further study.

Past reports have demonstrated that LV myocyte geometry is significantly altered with pacing CHF. Specifically, LV isolated myocyte length is increased and cross-sectional area decreased with pacing CHF. These changes in LV geometry resulted in an overall increase in computed LV myocyte volume. Consistent with these past reports, the present study demonstrated a reduction in LV myocyte cross-sectional area with pacing CHF. In contrast, selective MMP inhibition with rapid pacing increased LV myocyte cross-sectional area. These results suggest that a distinctly different pattern of LV myocyte growth response occurred with selective MMP inhibition. It has been demonstrated previously that a reduction in the normal LV myocyte cross-sectional area to length ratio was associated with the transition to maladaptive LV remodeling and the progression to CHF. The increased LV myocyte cross-sectional area with selective MMP inhibition may represent a favorable adaptive response. The increased LV myocyte cross-sectional area was a likely structural basis for the increase in LV wall thickness with selective MMP inhibition, which in turn was translated into a reduction in LV circumferential wall stress.

In the present study, pacing CHF resulted in changes in myocardial fibrillar collagen structure and physical characteristics. Specifically, the continuity of the fibrillar collagen weave was significantly disrupted with pacing CHF and the

**Figure 6.** Top, Representative photomicrographs of LV myocardial sections stained with picro-Sirius from control, with pacing CHF, and rapid pacing with concomitant MMP inhibition (CHF-MMPi). In the pacing CHF group, the fibrillar collagen weave surrounding individual myocytes appeared reduced and disrupted. In the pacing CHF and MMP inhibition group, the fibrillar collagen weave appeared increased between adjoining myocytes. Original magnification of all photomicrographs: ×100. Middle, Using scanning electron microscopy, a fine fibrillar collagen weave was observed within the interstitium of control LV myocardium and evenly surrounded individual myocytes. With pacing CHF, disruption of collagen fibrils between adjacent myocytes could be readily observed. Relative distribution of the fibrillar collagen weave appeared increased and slightly thickened in the MMPi group. Bottom, Using maceration digestion, the fibrillar collagen weave surrounding individual myocyte profiles could be readily appreciated in control myocardial sections. In the pacing CHF myocardium, the fibrillar collagen matrix appeared collapsed and highly disorganized. In the pacing and MMPi group, the structure and organization of the fibrillar collagen matrix was similar to controls.

**Figure 7.** Immunohistochemical staining for laminin was performed in LV sections taken from control, pacing CHF, and selective MMP inhibition (MMPi). In control sections, a uniform linear staining pattern along myocyte fascicles was observed (a, d, and g). A continuous staining pattern could be observed for laminin in control sections between the extracellular space and myocytes (arrow, panel d). In pacing CHF sections, disruption of the laminin staining pattern was observed (b, e, and h). Specifically, a discontinuity in laminin staining along myocyte fascicles was observed (arrows, e). At higher power, a fragmented staining pattern for laminin was observed in the pacing CHF sections (arrow, h). In the selective MMP inhibition group, laminin staining appeared similar to controls (c, f, and i). Original magnification of panels a through f: 400×; g through i: 600×.
degree of collagen solubility, reflecting the degree of collagen cross-linking was reduced. Non-cross-linked collagen fibrils are more susceptible to proteolytic cleavage by MMPs. With selective MMP inhibition, the degree of collagen cross-linking was similar to pacing CHF values, suggesting that MMP inhibition did not influence posttranslational events in collagen biosynthesis. Instead, selective MMP inhibition improved fibrillar collagen structure and architecture. These observations suggest that MMP inhibition prevented proteolysis of the more susceptible collagen matrix and thereby maintained a more normal extracellular architecture. Whereas the structure of the collagen matrix appeared improved with selective MMP inhibition, the collagen fibrils appeared thickened when compared with normal myocardial samples. The physiological significance of this observation remains to be established. In a recent report using an MMP-1–sparing inhibitor in a rabbit model of myocardial infarction, it was demonstrated that collagen turnover rates were not affected. Consistent with this past report, the present study demonstrated that in this pacing model, the institution of selective MMP inhibition was not associated with a fibrotic response or significant collagen accumulation.

The dosing strategy used in this study resulted in the inhibition of MMP-2 and MMP-9, both of which degrade extracellular components associated with the LV myocyte basement membrane. Specifically, these MMPs degrade laminin, collagen IV, and fibronectin; all fundamental components necessary for maintaining the myocyte-fibrillar collagen interface. Using immunohistochemistry, the present study demonstrated a disrupted staining pattern for laminin with pacing CHF. Laminin is a large glycoprotein, is a major component of the extracellular matrix, and contributes to cell attachment and growth response. Thus, a loss of normal laminin continuity within the myocardial interstitium would likely affect myocyte adhesion and alignment to the extracellular matrix. Significant alterations in extracellular myocyte support and basement membrane adhesion capacity have been demonstrated with pacing CHF. In the present study, selective MMP inhibition attenuated the degree of focal disruptions to the laminin architecture, which in turn would potentially improve myocyte engagement to the extracellular matrix. The attachment of the myocyte to the extracellular matrix is achieved through the class of transmembrane proteins called integrins, and an important binding domain for these integrins is laminin. Engagement of integrins to the extracellular matrix results in an important series of intracellular events, which regulate cell behavior. Thus, integrin engagement to the extracellular matrix likely constitutes an important pathway by which physical stimuli are transduced into intracellular signaling molecules and in turn influence myocyte growth. While remaining speculative, one potential mechanism by which selective MMP inhibition increased LV mass in the chronic pacing model was through a stabilization of the extracellular matrix-integrimplex complex.

Preactivation of LV myocardial extracts with the serine protease trypsin resulted in much higher MMP zymographic activity in the untreated pacing group when compared with normal values and is consistent with past reports. Using a quantitative immunoassay, MMP-2 myocardial levels were increased in the pacing CHF group. In the selective MMP inhibition group, MMP-2 myocardial levels were increased from normal values and were similar to untreated pacing values. In contrast, MMP zymographic activity after serine protease activation was reduced to within normal values in the selective MMP inhibition group. In this assay, protease activation is performed before detergent treatment and electrophoretic separation. Thus, the reduction in recruitable MMP zymographic activity was likely due, at least in part, to the presence of the MMP inhibitor in the myocardial extract. In vivo plasma levels of the MMP inhibitor were sufficient for obtaining the desired profile of MMP species inhibition. In addition, the release of MMP-2 into the plasma was decreased with selective MMP inhibition, suggesting reduced activation of this MMP species. Taken together, these in vivo and in vivo studies provide documentation that the desired pharmacological effect of selective MMP inhibition was achieved. However, it must be recognized that these are indirect measurements of the degree of in-vivo MMP inhibition achieved at the myocardial level.

Although the etiologies of clinical CHF are diverse, a common end-point is LV remodeling and pump dysfunction. Chronic pacing in animals causes well defined, predictable, and progressive LV dilation, contractile dysfunction, and neurohormonal activation and therefore was chosen for the present study. However, extrapolation of the findings from this project to clinical forms of CHF should be done with caution. Nevertheless, the present study demonstrated that selective control of myocardial MMP activity favorably influenced the LV myocardial remodeling process in developing CHF. The use of broad-spectrum MMP inhibition in patients with metastatic cancer have reported severe musculoskeletal pain necessitating treatment discontinuation. In contrast, initial clinical reports suggest that more selective MMP inhibition, in particular sparing MMP-1, was not associated with this musculoskeletal syndrome. Taken together, these past observations and the results from the present study suggest that a selective MMP inhibition strategy can be developed that will effectively retard the LV remodeling process in developing CHF without adversely affecting normal tissue structure and function. Thus, the development of strategies that selectively inhibit myocardial MMPs in CHF represent a potentially novel therapeutic target.

Acknowledgments
This study was supported in part, by NIH grants HL59165, PO1 HL48788-08, and a basic research grant from Procter and Gamble.

References
18. King et al Selective MMP Inhibition in Heart Failure
Selective Matrix Metalloproteinase Inhibition With Developing Heart Failure: Effects on Left Ventricular Function and Structure

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*Circ Res.* 2003;92:177-185; originally published online December 19, 2002;
doi: 10.1161/01.RES.0000052312.41419.55

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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