Selective matrix metalloproteinase inhibition attenuates progression of left ventricular dysfunction and remodeling in dogs with chronic heart failure

Hideaki Morita, Sanjaya Khanal, Sharad Rastogi, George Suzuki, Makoto Imai, Anastassia Todor, Victor G. Sharov, Sidney Goldstein, Timothy P. O’Neill, and Hani N. Sabbah

Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Heart and Vascular Institute, Detroit, Michigan; and Procter and Gamble, Cincinnati, Ohio

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Matrix metalloproteinases (MMPs) are enzymes responsible for extracellular matrix degradation. They play a key role in maintaining left ventricular (LV) geometry and function. The extracellular matrix provides structural support for myocardial fibroblast and blood vessel interactions. MMPs are synthesized as inactive zymogens and secreted into the extracellular space. MMPs have been shown to be involved in a variety of pathological and healing processes, such as tumor growth and metastasis, rheumatoid arthritis, and periodicontal disease. In the cardiovascular field, it is now becoming clear that extracellular matrix degradation is involved in the pathogenesis of atherosclerosis, restenosis, dilated cardiomyopathy, and myocardial infarction.

Expression and activity of MMPs are elevated in both patients and experimental animal models with heart failure (HF) (1–3, 15, 21–23, 25). Moreover, deletion of MMPs in mice attenuates LV remodeling and cardiac rupture after myocardial infarction (5, 7, 8). Thus MMP inhibition has emerged as a potential therapeutic target for prevention and treatment of HF. Nonselective MMP inhibition has been shown to attenuate LV remodeling and progression in several experimental models, such as mice and rats with induced myocardial infarction and rapidly paced pigs (16, 17, 22). However, nonselective MMP inhibitors can also be associated with undesirable systemic side effects (9). Therefore, selective MMP inhibition may be a more ideal therapeutic strategy for the treatment of HF. In this study, we examined the effects of selective MMP inhibition on the progression of LV dysfunction and remodeling in dogs with moderate HF induced by multiple sequential intracoronary embolizations.

METHODS

Animal model. The canine model of chronic HF used in the present study has been previously described in detail (18). In this experimental preparation, chronic LV dysfunction is produced by multiple sequential intracoronary microembolizations with polystyrene Latex microspheres (70–102 mm in diameter). In the present study, 24 healthy mongrel dogs, weighing between 20 and 30 kg, underwent serial coronary microembolizations to produce HF. Embolizations were performed 1 to 3 wk apart and were discontinued when LV ejection fraction (EF) decreased by 25% or more from baseline. The study was approved by the Henry Ford Hospital Institutional Animal Use and Care Committee and conformed to the “Position Statement of the American Heart Association on Research Animal Use.”

Determination of doses of PG-530742. The high and low doses of PG-530742 used in this study were selected based on a plasma pharmacokinetics study performed on three microsphere-induced HF dogs. Blood samples were collected from each animal via a superficial leg vein using blood collection tubes. Blood samples were collected before oral drug administration and at the following times: day 1, predose and post-first dose at 0.5, 1, 2, 3, 4, 6, 8, and 24 h; and...
day 3, predose and post-first dose at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h. The average plasma concentrations were 14 and 150 ng/ml for the low-dose and the high-dose drug administration, respectively. On the basis of pharmacokinetic studies, the high-dose PG-530742 regimen was chosen to produce complete inhibition of MMP-2, -3, -9, and -13 (average plasma concentrations ≥10-fold above the IC50 for these enzymes) while sparing MMP-1 and -7 (average plasma concentrations ≥10-fold below the IC50 for these enzymes). The low-dose PG-530742 regimen was chosen to also partially spare MMP-3 (average plasma concentrations approximately equal to the IC50 for this enzyme).

Study protocol. The study was a randomized, double-blind trial. Two weeks after the last microembolization procedure, all dogs underwent a prerandomization left and right heart catheterization. The 2-wk period ensured that all infarctions produced by the last microembolization session were completely healed. One day after cardiac catheterization, dogs were randomized to 3 mo of therapy with low-dose PG-530742 (0.2 mg/kg, n = 8), high-dose PG-530742 (3.5 mg/kg, n = 8), or matched placebo (n = 8). No other drugs were used in any of the study arms during the 3 mo of follow-up. Hemodynamic, angiographic, and neurohormonal measurements were made at baseline, before initiation of therapy (pretreatment), and after 3 mo of therapy (posttreatment). After a final hemodynamic and angiographic measurement performed at the end of 3 mo of therapy, dogs were anesthetized and the heart was rapidly harvested for histological evaluation.

Hemodynamic and angiographic measurements. Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments, Houston, TX) during cardiac catheterization. Peak LV rate of change in pressure during isovolumic contraction (dP/dt) and relaxation (dP/dt) and end-diastolic pressure were measured from the LV waveform. Single-plane left ventriculograms were obtained during each catheterization after completion of the hemodynamic measurements with the dog placed on its right side. Ventriculograms (~60 right anterior oblique projection) were recorded on 35-mm cinefilm at 30 frames/s during the injection of 20 ml of contrast material (RenO-M-60, Squibb, Princeton, NJ). Correction for image magnification was made with a radiopaque-calibrated grid placed at the level of the LV. LV end-diastolic volume and end-systolic volume were calculated from ventricular silhouettes using the area-length method as described previously (4). Global indexes of LV shape were used to quantify changes in chamber sphericity. LV shape was quantified from angiographic silhouettes as the ratio of the major-to-minor axes at end diastole and end systole (12).

Echocardiographic measurements. Echocardiographic studies were performed as previously described (11) by using a Hewlett-Packard model 77020A ultrasound system with a 3.5-MHz transducer and recorded on a Panasonic 6300 VHS recorder for off-line analysis. A LV short-axis view at midpapillary muscle level was recorded. This view was used to calculate the percent LV area fractional shortening, defined as the difference between the end-diastolic area and the end-systolic area divided by the end-diastolic area times 100. LV end-diastolic circumferential wall stress was calculated as described previously (6). Thickness of the intraventricular septum and the LV wall as measured from M-mode echocardiograms and averaged to obtain a single representative value for LV wall thickness.

Histological and morphometric assessments. From each heart, three transverse slices (~3 mm thick), one each from basal, middle, and apical thirds of the LV, were obtained. For comparison, tissue samples obtained from seven normal dogs were prepared in an identical manner. From each slice, transmural tissue blocks were obtained and embedded in paraffin blocks. From each block, 6-μm-thick sections were prepared and stained with Gomori trichrome to identify fibrous tissue. The volume fraction of replacement fibrosis, namely, the proportion of scar tissue to viable tissue in all three transverse LV slices, was calculated as the percent total surface area occupied by fibrous tissue using computer-based video densitometry (MOCHA, Jandel Scientific, Corte Madera, CA). Transmural tissue blocks were obtained from the free wall segment of the slice, mounted on cork using Tissue-Tek embedding medium (Sakura, Torrance, CA), and rapidly frozen in isopentane precooled in liquid nitrogen and stored at ~70°C until used. Cryostat sections were prepared and stained with fluorescein-labeled peanut agglutinin (Vector, Burlingame, CA) after pretreatment with 3.5 U/ml neuraminidase type V (Sigma Chemical, St. Louis, MO) to delineate the myocyte border and the interstitial space, including capillaries, as previously described (13). Sections were double stained with rhodamine-labeled Griffonia simplicifolia lectin I to identify capillaries. Ten radially oriented microscopic fields (magnification, ×100; objective, ×40; and ocular, ×2.5) were selected at random from each section and photographed using 35-mm color film. Fields containing scar tissue (infarcts) were excluded. Average cross-sectional area of each myocyte was measured by using computer-based planimetry. The volume fraction of interstitial collagen (interstitial fibrosis) was calculated as the percent total surface area occupied by interstitial space minus the percent total area occupied by capillaries (13). Capillary density was calculated as the number of capillaries per square millimeter. The oxygen diffusion distance was measured as half the distance between two adjoining capillaries.

Measurements of mRNA gene expression. Total RNA from LV myocardium of all three study groups, as well as from six normal dogs, was isolated by extraction with RNA Stat-60 (Tel-Test, Friendswood, TX) using the guanidium thiocyanate phenol-chloroform method according to the manufacturer’s instructions. Tissues were homogenized in RNA Stat-60 solution (150 mg tissue/1.5 ml RNA Stat 60) with a Omni mixer homogenizer (Omni International, Marietta, GA), which was followed by extraction with chloroform, precipitation with isopropanol, and finally a washing of the precipitated RNA with 75% (vol/vol) ethanol. The obtained RNA was dissolved in aquanase free water. The concentration of RNA was determined by using a spectrophotometer (Beckman DU-640) at 260 nm. Total RNA was diluted to 0.1 mg/ml concentration and denatured at 95°C for 5 min, followed by rapid cooling in an ice bath. Approximately 10 μg of total RNA were primed with 0.5 μg of oligo-dT15 primer. Total RNA was reverse transcribed by reverse transcriptase using a cDNA synthesis kit (Promega, Madison, WI). The samples were incubated at 42°C for 1 h, after which the reaction was terminated at 95°C for 5 min. PCR was carried out in a thin-walled, 0.5-ml autoclave tube with an assay volume of 50 μl, including 2 μl of cDNA, using a cDNA amplification kit (In vitrogen). The gene-specific primers for GAPDH, MMP-1, -2, -3, -9, and -13 were synthesized. The PCR mixture was amplified by a thermal cycler (Bio-Rad). A hot start was given at 94°C for 3 min, and amplification was performed for 30 cycles according to the following program: denaturing at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min. After 30 cycles, the mixture was kept at 72°C for 3 min and then held at 4°C. All PCR reaction products were run on 1% agarose gels and visualized by ethidium bromide staining. Gel photographs were taken by a digital camera and analyzed by computer software (Gel-Doc) while the gels were illuminated by ultraviolet light from below. Quantification was performed by scanning the bands and analyzing them densitometrically. Fluorescent bands were quantified in densitometric units.

Data analysis. To ensure that all study measures were similar at baseline, comparisons were made between the three study groups before any embolizations and at the time of randomization before initiation of therapy. The measurements and data analysis were done with the investigators blinded to the treatment groups. To assess treatment effect, the change in each measure from pretreatment to posttreatment was calculated for each of the three study groups. For these comparisons, the data were examined by one-way ANOVA with the investigators blinded to the treatment groups. To assess treatment effect, the change in each measure from pretreatment to posttreatment was calculated for each of the three study groups.
tween pretreatment and posttreatment measures were made by using a Student’s paired t-test with \( P < 0.05 \) considered significant. Histomorphometric findings were examined among the three study groups and 7 normal dogs using one-way ANOVA with significance set at \( \alpha \leq 0.05 \). If significance was attained, then pairwise comparisons were performed using the Student-Newman-Keuls test with significance set at \( P \leq 0.05 \). An identical ANOVA-based statistical analysis was used for mRNA expression of all MMP genes measured in the study. All data are reported as means \( \pm SE \).

RESULTS

At baseline, all dogs that entered into the study had hemodynamic, angiographic, and echocardiographic findings that were within the normal limits for mongrel dogs in our laboratory. There were no significant differences in the baseline hemodynamic, echocardiographic, and angiographic measurements done before induction of HF between the dogs in the high-dose, low-dose, or the placebo groups.

Findings in dogs treated with placebo. In the placebo-treated dogs, EF significantly decreased (36 ± 1 to 33 ± 1\%, \( P = 0.003 \)) during the 3-mo follow-up period. Similarly, fractional area shortening decreased significantly (38 ± 2 vs. 33 ± 2\%, \( P = 0.03 \)). This was accompanied by significant increases in both end-diastolic volume and end-systolic volume (57 ± 2 vs. 63 ± 2 ml, \( P = 0.0004 \); and 36 ± 1 vs. 42 ± 2 ml, \( P = 0.002 \), respectively). LV end-diastolic and end-systolic axis ratio also decreased significantly, indicating that the shape of the LV was approaching that of a sphere. There were also significant decreases in peak LV +dP/dt and −dP/dt and increases in LV end-diastolic wall stress. Changes in the heart rate and mean aortic pressures were not significant at 3-mo follow-up (Table 1).

Findings in dogs treated with low dose. As in the placebo-treated dogs, the EF (35 ± 1 vs. 31 ± 1\%, \( P = 0.0034 \)) and fractional area shortening (37 ± 2 vs. 35 ± 2\%, \( P = 0.039 \)) decreased significantly during the 3-mo follow-up period in the dogs treated with low dose PG-530742. The end-diastolic volume and end-systolic volume increased significantly (58 ± 2 vs. 63 ± 2 ml, \( P = 0.002 \); and 38 ± 2 vs. 43 ± 2 ml, \( P = 0.0007 \), respectively), which was accompanied by increases of both LV end-diastolic and -systolic axis ratios. LV +dP/dt and −dP/dt decreased significantly, and LV wall stress increased. Heart rate and mean aortic pressure were not significantly different in the 3-mo follow-up period (Table 1).

Findings in dogs treated with high dose. In dogs treated with high-dose PG-530742, EF (36 ± 1 vs. 40 ± 1\%, \( P = 0.0034 \)) and fractional area shortening (39 ± 2 vs. 42 ± 2\%, \( P = 0.039 \)) increased significantly after 3 mo of therapy. The end-diastolic volume and end-systolic volume decreased significantly (59 ± 4 vs. 57 ± 4 ml, \( P = 0.016 \); and 38 ± 2 vs. 34 ± 2 ml, \( P < 0.001 \), respectively). The LV axis ratio was not altered, indicating that LV shape did not change. There was a decrease of end-diastolic wall stress and a trend toward increasing LV +dP/dt and −dP/dt. Heart rate and mean aortic pressure were not significantly changed (Table 1).

Comparison of treatment effects. The changes in hemodynamic, angiographic, and neurohormonal data from pretreatment to posttreatment measurements were compared between the treatment groups (Table 1). The EF and fractional area shortening were significantly increased in dogs treated with high dose but not with low dose compared with placebo. Similarly, end-diastolic volume and end-systolic volume were significantly lower in dogs treated with high dose but not with low dose compared with placebo. Peak LV +dP/dt and peak LV −dP/dt were significantly higher in the high-dose group compared with the placebo group. The LV axis ratios were higher in the high-dose group compared with the other groups, which indicates that LV shape was more preserved.

Histomorphometric findings. Histomorphometric results are shown in Table 2. Volume fraction of replacement fibrosis, volume fraction of interstitial fibrosis, and cardiomyocyte cross-sectional area were significantly higher in placebo-treated dogs compared with normal dogs. Volume fraction of replacement fibrosis was reduced by 30% in dogs treated with high dose than in placebo-treated dogs and reduced by 44% compared with dogs treated with low dose. Similarly, the volume fraction of interstitial fibrosis was reduced by 29% in the dogs treated with high dose compared with placebo-treated dogs and reduced by 33% compared with those treated with low dose. Similarly, average cardiomyocyte cross-sectional area was 28% smaller in dogs treated with high dose compared with placebo-treated dogs and 32% smaller compared with those treated with low dose. There were no statistically significant differences in volume fraction of replacement fibrosis, volume fraction of interstitial fibrosis, and cardiomyocyte cross-sectional area between dogs treated with low dose and dogs treated with placebo.

mRNA expression. The results of mRNA expression in LV tissue are shown in Table 3 and Fig. 1. There were no differences in expression of the housekeeping gene GAPDH among normal dogs, placebo-treated HF dogs, and dogs treated with low-dose or high-dose PG-530742. mRNA expression of MMP-1, -2, -3, -9, and -13 increased significantly in LV tissue of normal dogs compared with placebo-treated HF dogs. Low-dose PG-530742 did not have a significant effect on mRNA expression of any of the MMPs measured. In contrast, high-dose PG-530742 significantly decreased mRNA expression of MMP-2, -3, -9, and -12 but did not have any effect on mRNA expression of MMP-1 (Table 3 and Fig. 1).

Table 1. Comparison of the change from pretreatment to posttreatment in hemodynamic, echocardiographic, and angiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>High Dose</th>
<th>Low Dose</th>
<th>Placebo</th>
</tr>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>−7±4*†</td>
<td>12±6</td>
<td>15±7</td>
</tr>
<tr>
<td>Mean aortic pressure, mmHg</td>
<td>−5±6</td>
<td>−8±4</td>
<td>0±5</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>−4±1*†</td>
<td>−3±1*</td>
<td>0±0</td>
</tr>
<tr>
<td>EDV, ml</td>
<td>−2±1*†</td>
<td>5±1</td>
<td>6±1</td>
</tr>
<tr>
<td>ESV, ml</td>
<td>−4±0*†</td>
<td>5±1</td>
<td>6±1</td>
</tr>
<tr>
<td>Ejection Fraction, %</td>
<td>3±1*†</td>
<td>−4±1</td>
<td>−4±1</td>
</tr>
<tr>
<td>Peak LV +dP/dt, mmHg/s</td>
<td>230±113*†</td>
<td>−380±89</td>
<td>−344±105</td>
</tr>
<tr>
<td>Peak LV −dP/dt, mmHg/s</td>
<td>314±135*†</td>
<td>−288±68</td>
<td>−203±110</td>
</tr>
<tr>
<td>LV ED axis ratio</td>
<td>0.01±0.02*†</td>
<td>−0.09±0.03</td>
<td>−0.07±0.02</td>
</tr>
<tr>
<td>LV ES axis ratio</td>
<td>0.02±0.04*†</td>
<td>−0.16±0.04</td>
<td>−0.14±0.04</td>
</tr>
<tr>
<td>FAS, %</td>
<td>3±1*†</td>
<td>−2±1</td>
<td>5±2</td>
</tr>
<tr>
<td>LV ED wall stress, g/cm²</td>
<td>−8±2*†</td>
<td>13±2</td>
<td>15±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVEDP, left ventricular (LV) end-diastolic (ED) pressure (EDP); EDV and ESV, ED and end-systolic (ES) volume, respectively; +dP/dt and −dP/dt, peak LV rate of change in pressure during isovolumic contraction and relaxation, respectively; FAS, fractional area shortening. \* \( P < 0.05 \) vs. placebo; \* \( P < 0.05 \) vs. low dose.
sphericity, is associated with downregulation of stretch previously shown that reverse global LV remodeling in this dog cardiomyocyte hypertrophy. Sabbah’s laboratory (19) has pre-decreased cardiomyocyte cross-sectional area, a measure of magnitude difference. netic studies in a limited number of animals showed average possibility that the low dose of the compound had high-remodeling, or mRNA expression of MMPs may be due to the -13 decreased compared with placebo-treated HF dogs. The not the low-dose mRNA, the expression of MMP-2, -3, -9, and 530742 are consistent with the observation that at this dose and dogs (19, 20, 24). The beneficial effects of high-dose PG-blockers, eplerenone, and devices in this model of HF in/\text{s}/H9252

dosing in dogs with moderate HF. The effects seen with the high dose are modest but in line with studies done earlier with-13 decreased compared with placebo-treated HF dogs. The fact that low-dose PG-530742 had no effect on LV function, remodeling, or mRNA expression of MMPs may be due to the possibility that the low dose of the compound had high-clearance kinetics and the high-dose probably provided longer plasma levels and, therefore, imparted an effect. Pharmacokinetic studies in a limited number of animals showed average plasma concentrations over 24 h of 14 ng/ml for the low dose compared with 150 ng/ml for the high dose, a one order of magnitude difference.

In the present study, therapy with high-dose PG-530742 also decreased cardiomyocyte cross-sectional area, a measure of cardiomyocyte hypertrophy. Sabbah’s laboratory (19) has previously shown that reverse global LV remodeling in this dog model of HF, as evidenced by decreased LV size and chamber sparsity, is associated with downregulation of stretch response proteins. The latter are typically elevated in HF and have been implicated in triggering the process of LV hypertrophy (19). In the present study, high-dose PG-530742 caused a reduction of both LV size and sphericity, conditions that could have led to decreased stretch response proteins and, therefore, attenuation of cardiomyocyte hypertrophy.

The present study did not examine the effects of PG-530742 on LV diastolic function in detail. Nonetheless, we observed a significant reduction in LV end-diastolic pressure and peak LV \(-dP/dt\) after therapy with high-dose PG-530742. We also observed a reduction in the volume fraction of interstitial fibros is after therapy with high-dose PG-530742. Increased interstitial fibrosis is typically associated with increased LV diastolic stiffness. High-dose PG-530742 also decreased LV end-dia-stolic circumferential wall stress. These observations, when considered in concert, support the notion that high-dose PG-530742 also has a beneficial effect on LV diastolic function.

Several studies have examined the effects of nonsel ective MMP inhibitors in animal models of HF. Rohde et al. (17) first demonstrated that administration of nonsel ective MMP inhibitor attenuates early LV enlargement 4 days after experimental myocardial infarction. Spinale et al. (22) also have shown that nonsel ective MMP inhibition limited LV dilatation and reduced LV wall stress in pigs with tachycardia-induced HF. However, nonsel ective MMP inhibitors can be associated with undesirable systemic side effects, such as polyarthritis that is dose dependent (9).}

Recently, several studies have examined the role of each MMP using genetically modified mice. MMP-9-deficient mice were almost completely protected against cardiac rupture 4 days after acute myocardial infarction, and healing after myocardial infarction was delayed, as evidenced by reduced leukocyte infiltration into the infarct region (8). Targeted deletion of MMP-9 also attenuated LV enlargement and collagen accumulation after experimental myocardial infarction in mice (5). In addition, targeted deletion of MMP-2 also attenuated early LV rupture and late remodeling after experimental myocardial infarction in mice (7). MMP-3 is increased in patients with nonischemic dilated cardiomyopathy (8). The increase in MMP-3 has also been shown to degrade a wide portfolio of extracellular matrix substrates and activate other MMPs, which would enhance net collagenase activity (16, 28). Therefore,

### Table 2. Histomorphometric measurements

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Placebo</th>
<th>High Dose</th>
<th>Low Dose</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>VFRF, %</td>
<td>12.0 ± 1.21</td>
<td>8.4 ± 0.67†</td>
<td>15.0 ± 1.38</td>
<td></td>
</tr>
<tr>
<td>VFF, %</td>
<td>3.5 ± 0.3</td>
<td>13.9 ± 2.62*</td>
<td>9.9 ± 0.55</td>
<td>14.8 ± 0.54</td>
</tr>
<tr>
<td>MCSA, μm²</td>
<td>616 ± 18</td>
<td>688 ± 26*</td>
<td>498 ± 22†</td>
<td>737 ± 28</td>
</tr>
<tr>
<td>CD, Cap/mm²</td>
<td>1.952 ± 0.52</td>
<td>1.777 ± 0.56*</td>
<td>2.024 ± 0.76†</td>
<td>1.660 ± 0.66</td>
</tr>
<tr>
<td>ODD, μm</td>
<td>11.8 ± 0.1</td>
<td>11.4 ± 0.16*</td>
<td>9.6 ± 0.19</td>
<td>12.1 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of dogs. VFRF, volume fraction of replacement fibrosis; VFF, volume fraction of interstitial fibrosis; MCSA, myocyte cross-sectional area; CD, capillary (Cap) density; ODD, oxygen diffusion distance. *P < 0.05 vs. normal; †P < 0.05 vs. placebo.

### DISCUSSION

Consistent with earlier studies from our laboratory (18, 20, 24), results of the present study indicate that, in the absence of any therapeutic intervention, progressive deterioration of LV systolic function and chamber remodeling occur in dogs with moderate HF, secondary to loss of viable myocardium. The present study demonstrates that long-term monotherapy with high-dose but low-dose selective MMP inhibitor PG-530742 prevents progressive LV dysfunction and LV chamber remodeling in dogs with moderate HF. The effects seen with the high dose are modest but in line with studies done earlier with β-blockers, eplerenone, and devices in this model of HF in dogs (19, 20, 24). The beneficial effects of high-dose PG-530742 are consistent with the observation that at this dose and not the low-dose mRNA, the expression of MMP-2, -3, -9, and -13 decreased compared with placebo-treated HF dogs. The fact that low-dose PG-530742 had no effect on LV function, remodeling, or mRNA expression of MMPs may be due to the possibility that the low dose of the compound had high-clearance kinetics and the high-dose probably provided longer plasma levels and, therefore, imparted an effect. Pharmacokinetic studies in a limited number of animals showed average plasma concentrations over 24 h of 14 ng/ml for the low dose compared with 150 ng/ml for the high dose, a one order of magnitude difference.

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### Table 3. Comparison of mRNA expression for MMPs in the LV myocardium between normal dogs, placebo-treated dogs, and low-dose- and high-dose-treated dogs

<table>
<thead>
<tr>
<th>MMP</th>
<th>Normal</th>
<th>Placebo</th>
<th>Low Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>MMP-1, du</td>
<td>802 ± 52</td>
<td>1,626 ± 42*</td>
<td>1,584 ± 54*</td>
<td>1,582 ± 32*</td>
</tr>
<tr>
<td>MMP-2, du</td>
<td>430 ± 32</td>
<td>1,436 ± 24*</td>
<td>1,276 ± 48*</td>
<td>509 ± 18†</td>
</tr>
<tr>
<td>MMP-3, du</td>
<td>588 ± 58</td>
<td>1,745 ± 43*</td>
<td>1,642 ± 50*</td>
<td>1,138 ± 37†</td>
</tr>
<tr>
<td>MMP-9, du</td>
<td>504 ± 22</td>
<td>1,696 ± 97*</td>
<td>1,508 ± 98*</td>
<td>752 ± 41†</td>
</tr>
<tr>
<td>MMP-13, du</td>
<td>449 ± 38</td>
<td>1,474 ± 17*</td>
<td>1,335 ± 55*</td>
<td>868 ± 19†</td>
</tr>
<tr>
<td>GAPDH, du</td>
<td>344 ± 4</td>
<td>347 ± 4</td>
<td>341 ± 2</td>
<td>342 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of dogs. MMP, matrix metalloproteinase; du, densitometric units. *P < 0.05 vs. normal; †P < 0.05 vs. placebo.
MMP-3 inhibition could be important in preventing progressive LV dysfunction and remodeling in HF.

The selective MMP-inhibitor PG-530742 has been shown to inhibit early postmyocardial infarction remodeling in pigs (29). Similar results have been reported with another selective MMP inhibitor in a pig model of pacing-induced cardiomyopathy (10). The current study extends this positive finding in a dog model of ischemic cardiomyopathy. In pharmacokinetic studies, both low- and high-dose PG-530742 inhibited MMP-2, -9, and -13 in plasma, but, unlike the high dose, the low dose produced only modest inhibition of MMP-3. mRNA expression measured in LV tissue in the current study, however, did not show significant myocardial MMP inhibition with low-dose PG-530742 compared with placebo.

In our study, selective MMP inhibition also reduced volume fraction of replacement fibrosis, volume fraction of interstitial fibrosis, and cardiomyocyte hypertrophy and increased capillary density and improved oxygen diffusion distance compared with placebo. These findings may favor cellular remodeling to improve oxygen delivery to cardiomyocytes and, in doing so, enhance energetics of the failing heart and, ultimately, intrinsic contractile function of the failing cardiomyocytes. It has been demonstrated that fibrillar collagen architecture and not necessarily volume is altered with selective MMP inhibition (10).

Therefore, the histomorphometric changes seen in this study may reflect improved myocardial geometric remodeling in the treated dogs rather than direct effect of the MMP inhibition. The model used in the current study is unique from prior reports studying MMP inhibition. In this model of ischemic cardiomyopathy, selective MMP inhibition with a high dose resulted in significant attenuation of LV chamber remodeling.

In conclusion, the results of this study indicate that early, long-term monotherapy with high-dose selective MMP inhibitor PG-530742 decreases mRNA expression of the drug-targeted MMPs and prevents progressive LV dysfunction and chamber remodeling in dogs with experimentally induced ischemic cardiomyopathy. Treatment with high-dose PG-530742 also decreases cardiomyocyte hypertrophy and myocardial interstitial fibrosis and preserves global LV geometry.

GRANTS

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