Original Article

Transgenic Expression of Matrix Metalloproteinase-1 Inhibits Myocardial Fibrosis and Prevents the Transition to Heart Failure in a Pressure Overload Mouse Model

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Hypertension induces dysfunctional matrix remodeling that results in the development of myocardial fibrosis. Myocardial fibrosis adversely affects compliance, electrical activity and cardiac function in patients with hypertensive heart disease. Matrix metalloproteinases (MMPs) are a class of enzymes that regulate the remodeling of the matrix in response to pressure overload. Several studies have shown that the MMP-1/TIMP (tissue inhibitor of matrix metalloproteinase) ratio is decreased in hypertensive heart disease. However, the exact role that MMP-1 has in modulating the fibrotic response to hypertension is largely unknown. We hypothesized that cardiac expression of MMP-1 in mice would protect against the development of dysfunctional matrix remodeling during pressure overload. To investigate this, a suprarenal aortic banding model was utilized. Banded and unbanded MMP-1 transgenic mice were compared with appropriately matched wild-type mice. The banded mice were examined at 2 and 5 weeks after banding. MMP-1 attenuated the development of cardiac fibrosis, prevented left ventricular dilation and preserved cardiac function in mice that were exposed to pressure overload. Thus, MMP-1 protected the heart from the dysfunctional remodeling that occurs in response to chronic hypertension. In conclusion, these results suggest that strategies aimed at improving the MMP-1/TIMP balance in the myocardium may help to prevent the onset and progression of hypertensive heart disease. (*Hypertens Res* 2008; 31: 725–735)

***Key Words*: hypertension, collagen, matrix metalloproteinase, fibrosis**

Introduction

Hypertension is a primary cause of heart disease. Untreated, chronic hypertension can cause cardiac hypertrophy and the subsequent development of congestive heart failure (*1*). While the association between hypertension and congestive heart failure is firmly established, the molecular mechanisms by which hypertension mediates these effects have not been fully defined. It is known that chronic pressure overload can lead to deleterious changes in the structure of the cardiac extracellular matrix (*2*). Myocardial fibrosis occurs as a result of the mechanical stress induced by hypertension (*3*). The global accumulation of collagen leads to the development of a stiff, non-compliant left ventricle (*4*). This disturbance in the cardiac matrix can alter the regulation of cardiomyocyte function and behavior, ultimately causing depressed cardiac performance (*5*). Indeed, the remodeling of the extracellular matrix is a central factor in the development of hypertensive cardiomyopathy (*6*).

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The primary structural element of the cardiac matrix is fibrillar collagen (*7*). This component maintains the proper geometrical arrangement of myocytes to ensure that their contraction generates the optimal force (*8*). Fibrillar collagen assumes a triple helical conformation that is resistant to degradation from most proteinases (*9*). Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are capable of degrading the components of the extracellular matrix (*10*). MMP-1 is a collagenase that degrades fibrillar collagen by attacking a site from the N-terminal domain that cleaves collagen into 3/4 and 1/4 fragments(*11*). The expression and activity of MMPs are increased in the myocardium in response to pressure overload (*12*). This is critical since MMPs are important regulators of the adaptive changes that occur to the matrix as a result of chronic hypertension (*13*, *14*). However, the impact of the expression of individual MMPs on the progression of disease and the alteration of the cardiac matrix remains to be determined.

Our laboratory has previously demonstrated that cardiac-specific expression of MMP-1 in transgenic mice leads to the loss of collagen and the development of left ventricular (LV) dysfunction at 1 year of age (*15*). This clarified the result of chronic MMP-1 expression under basal conditions in the heart. The effect of an MMP, however, may be altered by the local stimuli to which the heart is exposed (*16*). To determine the impact of MMP-1 expression on the heart during pressure overload, a mouse suprarenal aortic banding model was used in the present study. Two-month-old banded and unbanded MMP-1 transgenic mice were compared with appropriately matched wild-type mice. This time point was chosen as the transgenic mice are phenotypically normal at this age. The goal of this study was to determine how MMP-1 affected the development of cardiac hypertrophy, collagen accumulation and LV dysfunction that occurs in response to hypertension. This information is expected to provide insight into the potential role of MMP-1 in the development of hypertensive cardiomyopathy.

Methods

Generation of α-MHC-Collagenase

The generation of the α-MHC–collagenase mice has been previously described (*15*). Briefly, a 9.3-kb genomic MMP-1 gene fragment was ligated to the MHC promoter at the site immediately preceding the translational initiation site. The resulting plasmid, pJ1360, was confirmed by restriction enzyme analysis and DNA sequencing. The translation start site and the reading frame of the MMP-1 gene were found to be intact, and Kozak's rules for translational efficiency were maintained (*17*). Transcription initiation occurs within the α-MHC promoter.

DNA Fragment Preparation and Microinjection

To remove prokaryotic sequences that sometimes inhibit transgene expression (*18*), the 15.0-kb transgene was isolated from pJ1360 by Not1/Sal1 enzymatic digestion (12 bp and 17 bp of the prokaryotic sequence remained, respectively), purified by CsCl centrifugation, and microinjected into fertilized mouse eggs (F1[C57BL/6 × CBA/J] × F1 [C57BL/6 × CBA/J]).

Southern Blot Analysis of DNA

Approximately 3 weeks after birth, DNA from the tails of pups was prepared and Southern blot analysis was performed as previously described (*15*).

Animal Use and Surgical Procedure

Ten- to twelve-week-old male transgenic mice (C57BL × CBA) and wild-type littermates underwent suprarenal aortic banding. The transgenic mice were heterozygotes (+/–) and they were compared with age-matched wild-type littermates (–/–). This age group was chosen because the hearts of the transgenic mice are phenotypically normal at this time period (*15*). To expose the aorta, a midline abdominal incision was made under the guidance of a dissecting microscope. The abdominal aorta was banded with 6-0 silk thread between the right and left renal arteries using a 26-gauge needle (outside diameter, 0.45 mm) to standardize the diameter of the ligation (*19*). A sham procedure was performed in an identical manner in the controls with the exception that these mice did not undergo ligation of the aorta. All procedures were approved by Columbia University's Institutional Animal Care and Use Committee.

Echocardiography

Anesthetized animals were placed on a mouse bed in a shallow left lateral decubitus position. Transthoracic echocardiography was performed using a pediatric broad band 6–15 MHz linear array ultrasound transducer (Agilent Sonas 5500; Agilent Technologies, Palo Alto, USA). The ultrasound beam depth was set at 2 cm and frame rate at 150 frames/s. The two-dimensional parasternal short-axis views were obtained at the level of LV papillary muscles.

L-Hydroxyproline Assays

The L-hydroxyproline concentration in the hearts of the transgenic and wild-type mice was determined using a modified Woessner protocol (*20*). The heart tissue was lyophilized for 12 h, and the left ventricle was isolated by shaving away the great vessels, the right ventricle, and both atria. The left ventricle was then minced, weighed, and hydrolyzed with 4 mL 6 Eq/L HCl at 125°C at 200 psi in an autoclave for 3 h. One

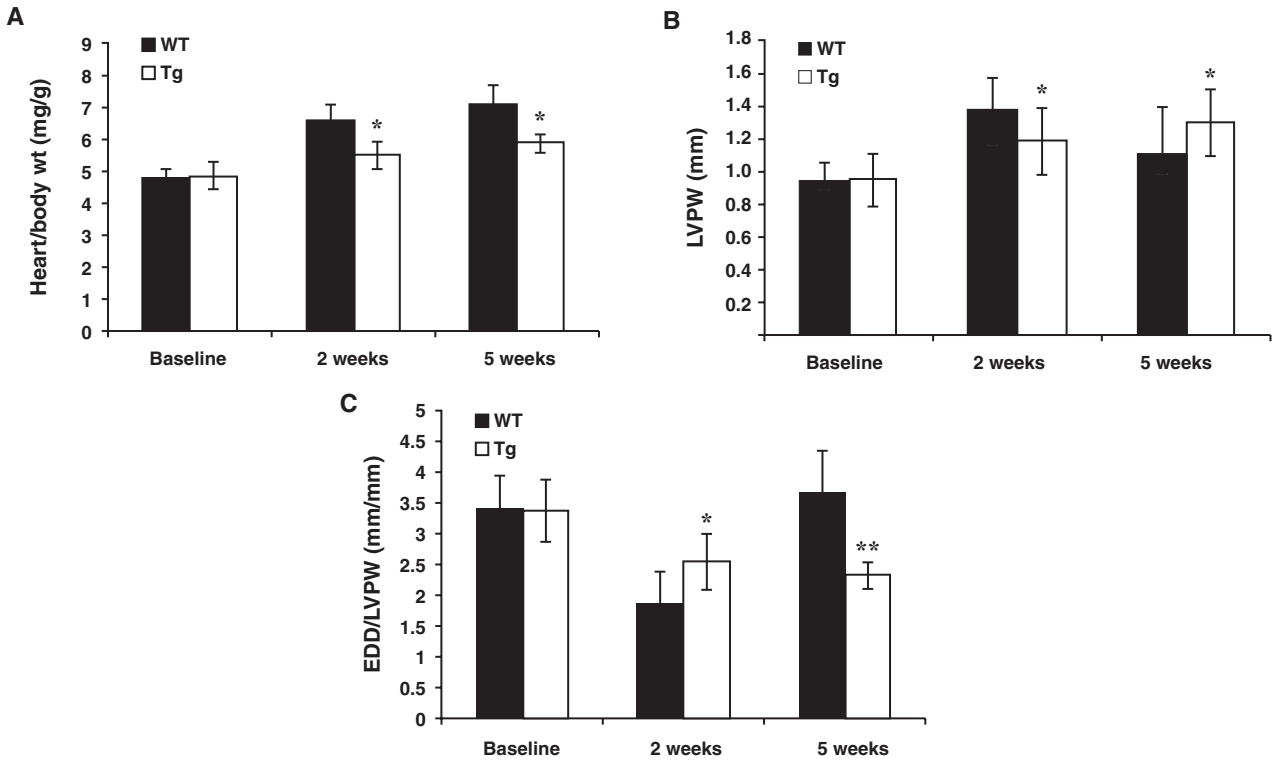

Fig. 1. *MMP-1 attenuates hypertrophic changes post-banding. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice (WT) and white bars those for transgenic mice (Tg). A: The heart/body weight ratio was significantly higher in the wild-type mice at 2 and 5 weeks post-banding. *p < 0.05 for wild-type vs. transgenic mice at 2 and 5 weeks post-banding. B: The hearts of the wild-type mice had a significantly higher left ventricular posterior wall thickness at the 2 week time point post-banding compared to transgenic mice. By 5 weeks, however, the ventricles of the wild-type mice had begun to dilate and the transgenic mice had a significantly greater left ventricular posterior wall (LVPW) thickness. *p < 0.05 for wild-type vs. transgenic mice at 2 and 5 weeks post-banding. C: The ratio of the end-diastolic diameter (EDD) to the LVPW thickness decreased significantly in the wild-type mice at 2 weeks. This decrease was greater than that seen in the transgenic mice, demonstrating that MMP-1 attenuated the hypertrophic response at this time point. At 5 weeks, the wild-type mice had transitioned to a dilated phase. This was manifested by a marked increase in the EDD/LVPW ratio. This transition did not occur in the transgenic mice. *p < 0.05 for wild-type vs. transgenic mice at 2 weeks post-banding; **p < 0.01 for wild-type vs. transgenic mice at 5 weeks post-banding.*

Table 1. Echocardiographic Data

	Control			Cardiac MMP-1		
	Baseline	2 weeks	5 weeks	Baseline	2 weeks	5 weeks
Number (*n*)	10	10	10	10	10	10
BW (g)	31.8±3.6	32.3±4.0	32.3±3.4	30.1±2.9	32.8±3.6	32.8±3.6
LVPW (cm)	0.094±0.012	0.14±0.2	0.11±0.017	0.095±0.016	0.12±0.02*	0.13±0.02*
LVEDD (cm)	0.32±0.013	0.29±0.055	0.38±0.036	0.31±0.035	0.30±0.035	0.31±0.025*
LVESD (cm)	0.23±0.024	0.19±0.03	0.29±0.03	0.21±0.029	0.20±0.029	0.2±0.029*
LVEDA (cm^2)	0.093±0.014	0.088±0.017	0.13±0.012	0.087±0.016	0.09±0.012	0.092±0.011*
LVESA (cm^2)	0.048±0.01	0.043±0.01	0.072±0.012	0.043±0.01	0.04±0.01	0.053±0.01*

Values shown are mean±SEM. **p*<0.05, cardiac MMP-1 *vs*. control mice at the same time point. MMP-1, matrix metalloproteinase-1; BW, body weight; LVPW, left ventricular (LV) posterior wall thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; LVEDA, LV end-diastolic area; LVESA, LV end-systolic area.

milliliter of the hydrolysate was evaporated and reconstituted with 1 mL distilled H_2O and then re-evaporated. Reconstitution was then done with 5 mL of distilled H_2O. Hydroxyproline standard solutions of 0, 1, 2, 4, 6, 8, and 10 mg/mL were made. A sample solution (2 mL) was oxidized with 1 mL of Chloramine-T (Sigma, St. Louis, USA) solution for 20 min. The Chloramine-T was then destroyed with 1 mL of 3.15 mol/L perchloric acid. After 5 min, 1 mL of *p*-dimethylaminobenzaldehyde solution was added. The sample was vortexed, incubated in a 60°C bath, and then cooled under tap water for 5 min. The absorbency of the solutions was determined at 557 mm. The hydroxyproline concentration was determined directly from the standard curve.

Histological Analysis

Hearts were arrested in diastole with phosphate buffered saline (PBS)/20 mmol/L KCl solution and pressure fixed at 20 mmHg with 4% paraformaldehyde or 10% neutral buffered formalin. Paraffin-embedded tissues were sectioned (4-μm thick) and stained with hematoxylin and eosin for light microscopy. Paraffin sections were also stained with picrosirius red and analyzed with a polarized light microscope to evaluate the distribution of myocardial collagen. For immunohistochemical analysis of TGF-β staining, lung sections were deparaffinized with xylene and rehydrated. Protease type XXIV (Sigma) was utilized for antigen retrieval in this experiment. After blocking endogenous peroxidase with 0.3% H_2O_2 in methanol for 30 min, the sections were treated with 0.005% protease type XXIV in 0.05 mol/L Tris-HCl buffer, pH 7.6, for 30 min at 37°C and rinsed in PBS. They were then incubated for 30 min with normal goat serum and reacted for 12 h at 4°C with rabbit polyclonal antibodies (20 μg/mL) against TGF-β1 (MBL International, Woburn, USA) or rabbit non-immune immunoglobulin (25 μg/mL) as a control. This was followed by incubation with a 1:200 dilution of horseradish peroxidase–conjugated goat anti-rabbit secondary antibody (Pierce, Rockford, USA) for 1 h. After washing in PBS, color was developed with 0.03% 3,3′-diaminobenzidine tetrahydrochloride in 50-mmol/L Tris-HCl buffer, pH 7.6, and counterstaining was performed with hematoxylin ($n=4$ animals for the immunohistochemistry studies).

Hemodynamic Analysis

In vivo intraventricular hemodynamic analysis was performed on transgenic and wild-type littermate mice at baseline and at 2 and 5 weeks after aortic banding. Two-month-old mice were utilized for this experiment. A total of 60 mice (baseline: 10 wild-type and 10 transgenic mice; 2 weeks: 10 wild-type and 10 transgenic mice; and 5 weeks: 10 wild-type and 10 transgenic mice) were anesthetized with 2.5% Avertin at 0.015 mL/g body weight. A midline incision in the neck exposed the trachea, and the mouse was intubated intratracheally with a 22-gauge angiocatheter (Becton Dickinson, Franklin Lakes, USA) which was secured with a 3-0 silk suture (USSC, Princeton, USA). The mouse was mechanically ventilated with a 0.5 mL ambient air tidal volume at 110 breaths/min using a small animal respirator/ventilator (Columbus Instruments, Columbus, USA). A median sternotomy was

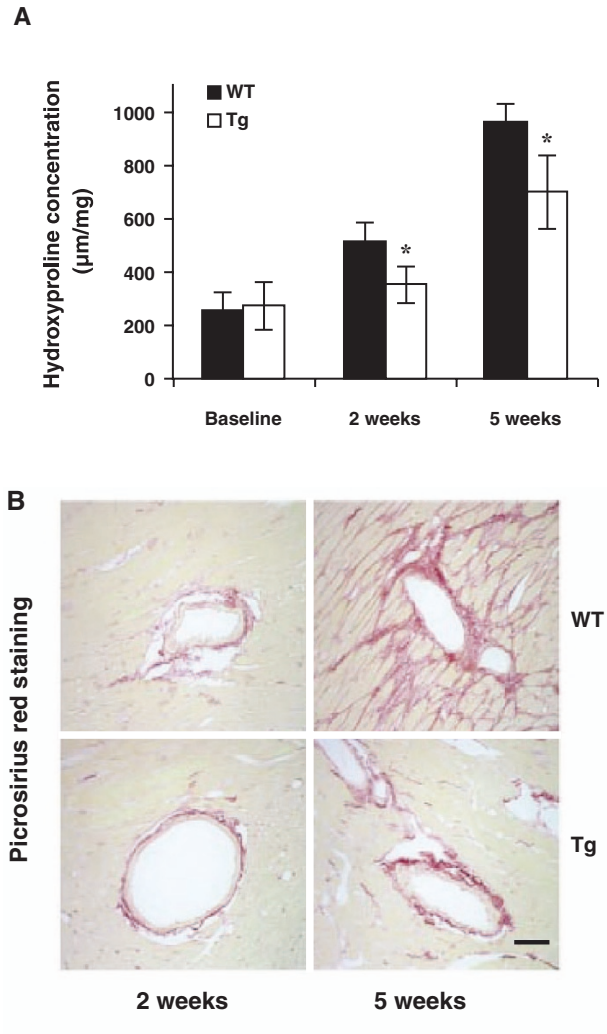

Fig. 2. *MMP-1 decreased the accumulation of heart collagen in banded mice. A: The hydroxyproline concentration increased both at 2 and 5 weeks post-banding in the wild-type mice. MMP-1 expression in the heart attenuated collagen accumulation at both these time points. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice (WT) and white bars those for transgenic mice (Tg). *$p<0.05$ for wild-type vs. transgenic mice at 2 and 5 weeks post-banding. B: Picrosirius red staining of heart sections revealed increased collagen deposition in the banded wild-type mice at 2 (top left) and 5 weeks (top right). The transgenic mice, however, did not demonstrate this intense fibrotic response within the tissue. Collagen staining was decreased in this group at 2 (bottom left) and 5 (bottom right) weeks (scale bar = 10 μm).*

performed, and the heart was exposed. Digitized intraventricular hemodynamic measurements were obtained *via* a left ventricle apical puncture with a 26-gauge fluid-filled angiocatheter (Becton Dickinson) attached to a high-fidelity pressure transducer that was connected to an eight-channel chart recorder set at 1,000 Hz (MacLab 8s; ADInstruments, Mountain View, USA). The data were stored on a computer for subsequent analysis (PowerMac 5300C; Apple Computer Inc., Cupertino, USA).

Statistical Analysis

Data are expressed as the means±SEM. Two-way analysis of variance measures (ANOVA) were conducted using commercially available software (Microsoft Excel; Microsoft, Seattle, USA). A difference was considered statistically significant at $p=0.05$.

Results

Effect of MMP-1 on Hypertrophic Responses after Abdominal Aortic Banding

There were no significant differences between the 2-month-old wild-type and transgenic mice at baseline. Aortic banding induced hypertrophic changes at the 2-week measurement point in the wild-type mice. These changes, however, were markedly attenuated in the MMP-1 transgenic mice. In contrast to the MMP-1 transgenic mice, the wild-type mice exhibited a significant increase in heart/body weight ratio (Fig. 1A; $n=10$, $*p<0.05$ at 2 weeks and 5 weeks) and LV posterior wall thickness (LVPW) (Table 1 and Fig. 1B; $n=10$, $*p<0.05$ at 2 weeks and 5 weeks). In addition, the wild-type mice developed a concentric hypertrophy manifested by a discernible decrease in the ratio of LV end-diastolic dimension over posterior wall thickness (LVEDD/PW) after 2 weeks of banding. The hypertrophic changes at that time point were significantly greater than those seen in the MMP-1 transgenic mice (Fig. 1C; $n=10$, $*p<0.05$). At 5 weeks after banding, the LVEDD/PW ratio was normalized in the wild-type mice as they developed evidence of ventricular dilation. However, in the MMP-1 transgenic mice, the LVEDD/PW ratio remained significantly lower than in the wild-type mice at this time point ($n=10$, $**p<0.01$). In fact, none of the transgenic hearts developed the ventricular dilation that was observed in the wild-type animals. Morphometric analysis of cross-sections of LV tissue revealed a lower cardiomyocyte cross-sectional area in the hearts of the transgenic animals compared to the wild-type animals (transgenic banded mice 175±18 μm^2 *vs*. wild-type banded mice 245±21 μm^2, $n=3$ in each group, $p<0.001$).

Effect of MMP-1 Expression on the Accumulation of Collagen Post-Aortic Banding

Aortic banding induced a significant increase in heart collagen in the wild-type mice but not in the MMP-1 transgenic mice. Compared to the MMP-1 transgenic mice, the wild-type mice had an increased heart hydroxyproline concentration both at 2 and 5 weeks post-banding (Fig. 2A, $n=10$ in each group, $*p<0.05$ at 2 weeks and 5 weeks post-banding). In addition, picrosirius red staining demonstrated severe perivascular and interstitial fibrosis within the hearts of wild-type banded mice while the fibrotic response was clearly reduced in the hearts of the MMP-1 transgenic mice post-banding (Fig. 2B).

Effects of MMP-1 on LV Remodeling and Pump Function at 5 Weeks after Aortic Banding

After 5 weeks of banding, signs of cardiac dysfunction developed in the wild-type mice but not in the MMP-1 transgenic mice. In contrast to the MMP-1 transgenic mice, the left ventricle of the wild-type mice became significantly dilated as manifested by an increased LV end-diastolic dimension (LVEDD) (Table 1) (Fig. 3A; $n=10$, $*p<0.05$) and area (LVEDA) (Fig. 3B; $n=10$, $*p<0.05$) measured by echocardiography and histology (Fig. 3C). Both the wild-type and MMP-1 transgenic mice had a significant increase in LV systolic pressure (LVSP), dP/dt max and absolute value of dP/dt min (Table 2 and Fig. 4) at 2 weeks after banding. At this early time point, the wild-type mice had already begun to develop signs of diastolic dysfunction as evidenced by an increased LVEDP compared to the MMP-1 transgenic mice (Fig. 5; $n=10$, $*p<0.05$). In contrast to the wild-type mice, the hearts of the transgenic mice maintained an augmented LVSP at 5 weeks post-banding (Fig. 4A; $n=10$, $*p<0.05$) and had increased fractional shortening and preserved systolic and diastolic function up to 5 weeks post-banding (Fig. 4D, B and C; $n=10$, $*p<0.05$ and Fig. 5).

Effect of Banding and MMP-1 Expression on TGF-β Levels in the Heart

Given the differences in hydroxyproline accumulation that were noted between the banded wild-type and MMP-1 transgenic mice, the TGF-β protein levels were analyzed. For this purpose, TGF-β1 immunostaining was performed on control and banded hearts of wild-type and transgenic mice. In wild-type mice, minimal TGF-β was detected under control conditions (Fig. 6, top left). After banding, however, intense staining was noted in the cardiomyocytes of the banded mice (Fig. 6, top right). In contrast, the MMP-1 transgenic mice had more staining in the heart at baseline compared to the wild-type control mice (Fig. 6, bottom left). Unlike the wild-type mice, TGF-β staining did not increase significantly in the MMP-1 transgenic mice following aortic banding (Fig. 6, bottom right).

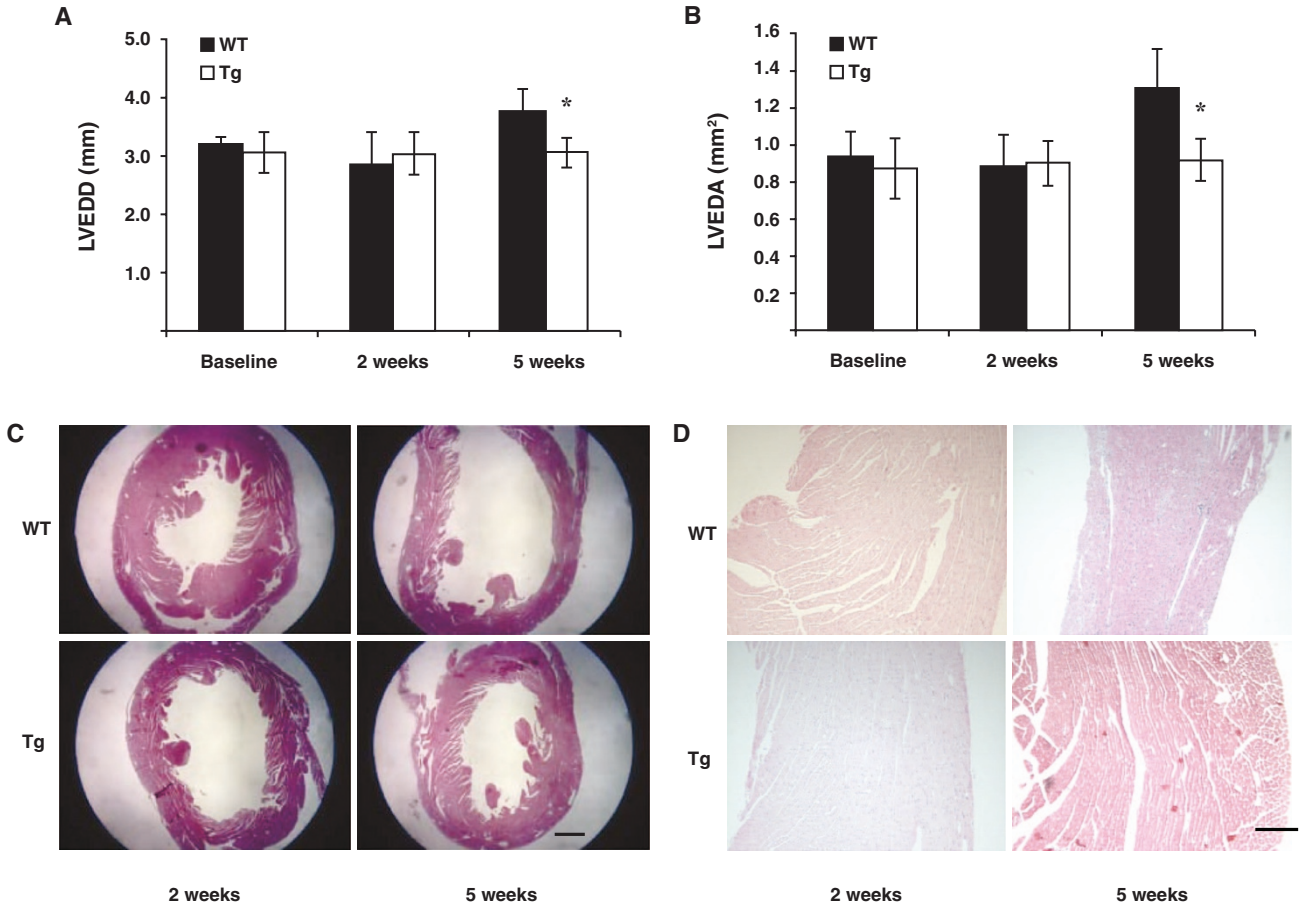

Fig. 3. *MMP-1 prevents dysfunctional LV remodeling in response to pressure overload. A: Beginning at 5 weeks, the wild-type mice demonstrated a significant increase in left ventricular end-diastolic dimension (LVEDD). In contrast, the LVEDD remained at normal levels in the transgenic mice. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice (WT) and white bars those for transgenic mice (Tg). *$p < 0.05$ for wild-type vs. transgenic mice at 5 weeks post-banding. B: Ventricular dilation was evident in the wild-type mice 5 weeks after banding. These mice had a significantly greater left ventricular end-diastolic area (LVEDA) compared to baseline non-banded mice and compared to banded transgenic mice. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice and white bars those for transgenic mice. *$p < 0.05$ for wild-type vs. transgenic mice at 5 weeks post-banding. C: Histologic analysis of heart sections (4× magnification) demonstrated marked hypertrophy in the wild-type mice at 2 weeks post-banding (top left). The transgenic mice demonstrated normal morphology in the early time period post-banding (bottom left). At 5 weeks, marked dilation is evident in the wild-type mice (top right). The transgenic mice demonstrated hypertrophic changes; however, the overall cardiac morphology was well preserved (bottom right). Scale bar = 1 mm. D: Histologic analysis of heart sections (10× magnification) demonstrated marked hypertrophy in the wild-type mice at 2 weeks post-banding (top left). The transgenic mice demonstrated normal morphology in the early time period post-banding (bottom left). At 5 weeks, marked dilation is evident in the wild-type mice (top right). The transgenic mice demonstrated hypertrophic changes; however, the overall cardiac morphology was well preserved (bottom right). Scale bar = 25 μm.*

Discussion

This study demonstrates that the heart-specific expression of MMP-1 attenuates the development of cardiac fibrosis post-aortic banding in mice. Most notably, MMP-1 expression prevented LV dilation and preserved cardiac function in mice that were exposed to pressure overload. Five weeks post-banding, the wild-type mice developed globally enlarged and dilated hearts. Thus, their hearts increased in weight and had a much higher LVEDD/PW ratio. In contrast, the transgenic mice developed mild hypertrophic changes but had no signif-

Table 2. Hemodynamic Parameters

	Control			Cardiac MMP-1		
	Baseline	2 weeks	5 weeks	Baseline	2 weeks	5 weeks
Number (*n*)	10	10	10	10	10	10
BW (g)	30.6±3.2	30.8±3.0	32.8±4.9	30.6±4.5	28.9±1.9	32.7±2.9
HR (bpm)	458±66	515±29	513±52	478±69	489±33	544±31
LVSP (mmHg)	98±8.6	146±14	114±21	106±13	139±12	139±11
LV d*P*/d*t* max (mmHg/s)	7,802±2,303	12,396±2,071	7,961±1,371	8,651±2,551	11,294±1,658	10,834±2,065
LV d*P*/d*t* min (mmHg/s)	−7,299±2,164	−8,880±1,661	−7,454±1,65	−7,386±1,897	−10,029±1,755	−10,057±1,464

Values shown are mean±SEM. *$p<0.05$. MMP-1, matrix metalloproteinase-1; BW, body weight; HR, heart rate; LVSP, left ventricular (LV) systolic pressure; LV d*P*/d*t* max, maximum first derivative of LV pressure, LV d*P*/d*t* min, minimum first derivative of LV pressure.

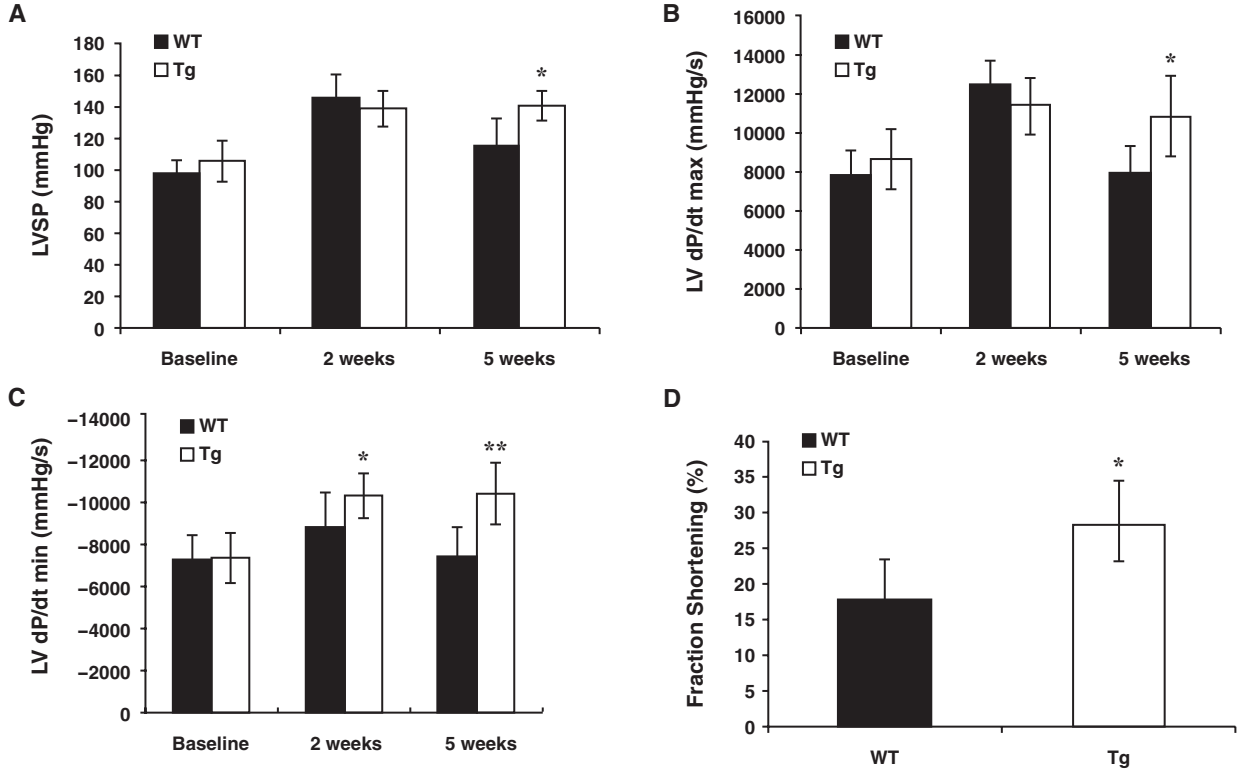

Fig. 4. *MMP-1 preserves systolic performance during pressure overload in mice. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice (WT) and white bars those for transgenic mice (Tg). A: Left ventricular systolic pressure (LVSP) increased similarly at 2 weeks post-banding in the wild-type and transgenic mice in response to banding. By 5 weeks, the wild-type mice demonstrated signs of systolic dysfunction with a decrease in LVSP. The transgenic mice had preserved LVSP and systolic function at this time point. *$p<0.05$ for wild-type vs. transgenic mice at 5 weeks post-banding. B: Contractility as determined by the maximum first derivative of left ventricular pressure (LV dP/dt max) was well preserved in both groups of mice at the 2-week measurement point. By 5 weeks, however, the wild-type mice had a significant decrease in contractility while contractility remained well preserved in the transgenic mice. *$p<0.05$ for wild-type vs. transgenic mice at 5 weeks post-banding. C: The absolute value of LV dP/dt max remained high in both groups at the 2-week measurement point. However, by 5 weeks, the absolute value markedly decreased in the wild-type mice while remaining unchanged in the transgenic mice. *$p<0.05$ for wild-type vs. transgenic mice at 2 weeks post-banding; **$p<0.01$ for wild-type vs. transgenic mice at 5 weeks post-banding. D: Left ventricular function as determined by fractional shortening was significantly greater in the MMP-1 transgenic mice at 5 weeks post-banding. *$p<0.05$.*

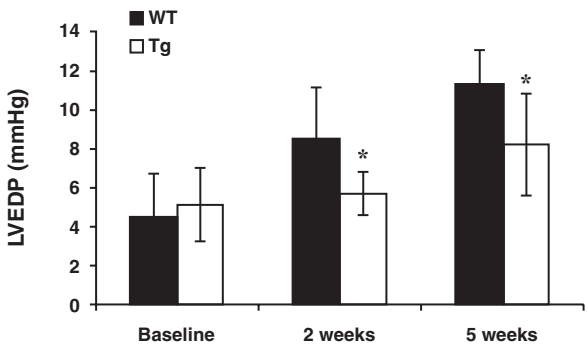

Fig. 5. *MMP-1 prevents diastolic dysfunction post-aortic banding. A progressive increase in left ventricular end diastolic pressure was seen in the wild-type mice after banding. This increase was significantly greater than that seen in the transgenic mice both at 2 and 5 weeks post-banding. Error bars indicate the standard deviation. Black bars represent the findings for wild-type mice (WT) and white bars those for transgenic mice (Tg). *$p < 0.05$ for wild-type vs. transgenic mice at 2 and 5 weeks post-banding.*

icant ventricular dilation. Thus, their hearts weighed less but had a much lower LVEDD/PW ratio compared to the banded wild-type mice. These results suggest that MMP-1 is able to protect the heart from the dysfunctional remodeling of the cardiac matrix that occurs in response to chronic hypertension.

Myocardial fibrosis is one of the pathological features of chronic pressure overload (*3*). Biopsy studies have shown a consistent increase in myocardial collagen content in patients with hypertensive heart disease compared to normotensive controls (*21*, *22*). In normal hearts, equilibrium is reached between collagen synthesis and degradation. However, in hearts exposed to chronic pressure overload, humoral factors that promote collagen synthesis and impair its degradation may result in increased collagen deposition (*23–25*). This interstitial fibrosis decreases myocardial compliance, impairs diastolic relaxation and, over time, may lead to ventricular dilation and a decrease in systolic performance (*26*).

There is significant evidence to suggest that alterations in the MMP/TIMP (tissue inhibitor of matrix metalloproteinase) balance may contribute to the myocardial fibrosis that is seen in patients with hypertensive heart disease. Clinical studies have shown a decrease in serum MMP-1 (*27*, *28*) and an increase in serum TIMP-1 (*28*, *29*) in patients with hypertensive heart disease. In dogs subjected to chronic pressure overload, myocardial MMP-1 levels were significantly decreased while TIMP-1 levels were unchanged (*16*). Recently, it was demonstrated that the expression of TIMP-1 and TIMP-2 positively correlated with the degree of interstitial fibrosis and diastolic dysfunction that occurs in chronic pressure overload in human hearts (*30*). Importantly, local factors that mediate hypertension may also disrupt the MMP/TIMP balance in the heart. Angiotensin II promotes collagen accumulation by stimulating fibroblasts to produce plasminogen activator inhibitor-1, which can prevent MMP-1 activation (*31*). Furthermore, angiotensin II induces TGF-β expression in cardiac fibroblasts (*32*), which decreases the expression of MMP-1 while increasing the expression of TIMP-1 (*33*, *34*), thereby promoting fibrosis by altering the MMP/TIMP balance. This mechanism, however, does not appear to be what was occurring in the MMP-1 transgenic mice described in this study. RNase expression analyses conducted on the hearts revealed that the TIMP-1, -2 and -3 levels were not altered by banding or the presence of the transgene (data not shown). Thus, the difference in fibrosis between the wild-type and transgenic mice that we observed post-aortic banding was unrelated to the levels of cardiac TIMPs.

While cardiac TIMPs did not appear to influence fibrosis in this model, immunostaining studies for TGF-β suggest that MMP-1 modulates cardiac collagen content by altering the expression of this profibrotic peptide within the cardiomyocytes. At baseline, the MMP-1 transgenic mice had increased TGF-β protein levels compared to the wild-type mice, which is consistent with the development of hypertrophy and fibrosis over time in these mice (*15*). After aortic banding, TGF-β staining significantly increased within the cardiomyocytes of the wild-type mice, and this change coincided with a marked increase in cardiac collagen content. However, in the MMP-1 transgenic mice, TGF-β staining and collagen accumulation were notably attenuated by the expression of MMP-1 in response to pressure overload. TGF-β is known to play an important regulatory role in MMP-1 expression (*35*) and the deposition of collagen. These findings indicate that a complex interplay between MMP-1, TGF-β and biomechanical forces exerts a determining effect on the development of cardiac fibrosis and hypertrophy.

This study demonstrated that MMP-1 expression attenuates pressure-induced remodeling of the cardiac matrix. This is critical, as the matrix plays a vital role in organizing and directing the contractile forces that are generated by cardiomyocytes during systole (*36*). The collagen fibrils that traverse the heart provide a direct physical connection between all the contractile elements of the myocardium (*37*). As a result of these interconnections, the heart is able to generate a significantly greater force than would otherwise be possible. Thus, despite the fact that isolated cardiomyocytes are only capable of 15% shortening, the heart as a whole is able to eject 60% of the volume present in the left ventricle at end diastole. Given the role that these fibrillar elements have in coordinating contractile forces, disturbances in the cardiac matrix can have profound effects on myocardial function (*38*, *39*). MMPs are secreted as proenzymes and this MMP-1 transgene was generated with the full-length proform of the gene. The transgenic mice express the proform of MMP-1 within the myocardium and collagenase activity is detected in the hearts of the mice (*15*). We assert that this collagenase can be activated in this model by plasminogen (*40*) or by reactive

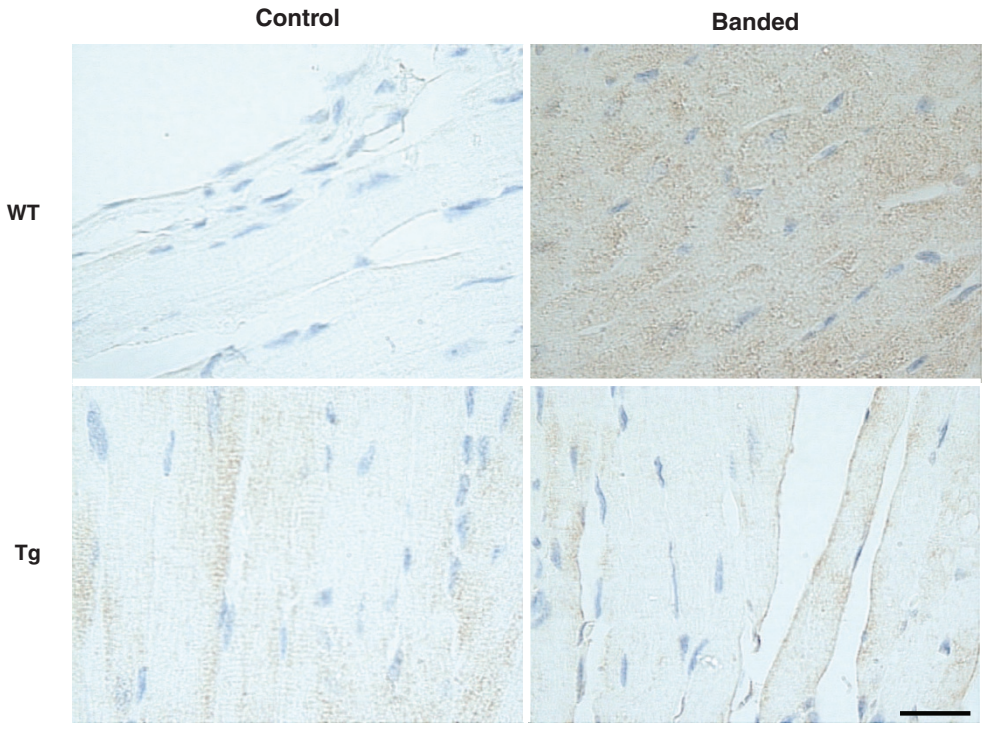

Fig. 6. *MMP-1 decreases the levels of TGF-β in the heart post-aortic banding. Immunostaining for TGF-β demonstrated low levels at baseline in the hearts of wild-type mice (WT, top left). The intensity of staining in the hearts of the wild-type mice significantly increased at 2 weeks post-aortic banding (Tg, top right). Staining for TGF-β within the cardiomyocytes was more intense at baseline in the transgenic mice (bottom left). Unlike the wild-type mice, the intensity of staining was not augmented post-aortic banding (bottom right). Representative images are displayed (n = 4 for each group). No signal was detected within the cardiomyocytes of sections treated with non-immune serum (images not shown). Scale bar = 40 μm.*

oxygen species (*41*) which are elevated in the heart under conditions of pressure overload (*42*). While it is known that the expression of MMPs is upregulated in disease states of the heart (*43*, *44*), the impact of this expression has not been fully determined. Numerous studies have suggested that MMPs may have a detrimental impact on cardiac structure and performance. Inhibition of MMP-2 has been shown to prevent cardiac rupture after myocardial infarction in mice (*45*, *46*), while the loss of TIMP-1, a natural inhibitor of MMPs, was shown to exacerbate post-infarct remodeling (*47*). Similarly, the loss of MMP-9 expression had a protective effect on LV remodeling and function in the acute pressure overload model (*48*). These results have generated considerable interest in the use of MMP inhibitors as a potential treatment for heart diseases (*49*, *50*). The results from this study, however, demonstrate that MMPs have a complex role in modulating cardiac function. As we have seen with MMP-1, the effect of these proteases may be highly dependent on the physiologic stimuli to which the heart is exposed. Thus, inhibition of collagenase activity during increased afterload may actually exacerbate pressure-induced remodeling and accelerate the transition to heart failure. Further investigation will be needed to better understand the mechanisms that affect the MMP-1/TIMP balance in the hypertensive myocardium. Factors that could normalize this balance may lead to effective strategies to prevent myocardial fibrosis and inhibit the transition to heart failure in patients with arterial hypertension.

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