ELSEVIER

Cardiovascular Research 46 (2000) 298–306

Cardiovascular Research

www.elsevier.com/locate/cardiores
www.elsevier.nl/locate/cardiores

MMP/TIMP expression in spontaneously hypertensive heart failure rats: the effect of ACE- and MMP-inhibition

Hua Li, Heather Simon, Thomas M.A. Bocan, J. Thomas Peterson*

Department of Cardiovascular Therapeutics, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA

Received 7 October 1999; accepted 28 January 2000

Abstract

Objective: Determine the effect of a matrix metalloproteinase inhibitor (MMPi) and angiotensin converting enzyme inhibitor (ACEi) on collagen, MMP, tissue inhibitors of MMPs (TIMPs) expression in the spontaneously hypertensive heart failure (SHHF) rat. **Methods:** Six groups were tested: normotensive 9- and 13-month-old Wistar–Furth (WF) rats, 9-month-old SHHFs (compensatory hypertrophy), 13-month-old SHHFs with HF, and 13-month-old SHHFs orally administered with either an MMPi (PD166793, 5 mg kg^{-1} day^{-1}) or ACEi (quinapril, 10 mg kg^{-1} day^{-1}) for 4 months. Collagen volume fraction was assessed histomorphometrically. Left ventricular (LV) mRNA [MMP-1,-2,-3,-7,-9,-11,-13,-14; TIMP-1,-2,-3,-4; and collagen α1(I) and α1(III)] and protein (MMP-2 and MMP-9 zymographic activity; Western blot analysis of MMP-13, and TIMP-1,-2,-4) levels could be quantified. **Results:** Collagen mRNA levels were elevated in SHHFs compared to age-matched controls, but collagen volume fraction was elevated only in 13-month-old SHHFs (~2×). Only MMP-2 mRNA levels increased significantly with HF. However, MMP-2 and MMP-9 zymographic activity, and MMP-13 protein levels increased. TIMP-1 and TIMP-2 mRNA and protein levels increased, and TIMP-4 protein levels decreased in SHHFs vs. controls. Both drug treatments reduced LV dilation; preserved systolic function; and normalized MMP/TIMP expression. Both drug treatments also reduced collagen volume fraction, but only quinapril reduced collagen mRNA levels and LV hypertrophy. **Conclusions:** The divergent effect of MMPi and ACEi on collagen mRNA levels and hypertrophy indicate that drug efficacy is mediated by different pathways in the SHHF rat.

Keywords: Heart failure; ACE inhibitors

1. Introduction

Left ventricular (LV) dilation, a common characteristic of remodeling in systolic heart failure (HF), has been shown to advance LV dysfunction, and to be related to the onset of morbidity and mortality in HF patients [7,22,28]. Dilation appears to have a causal role in the evolution of LV dysfunction. However, the molecular mechanisms mediating LV chamber dilation are still being elucidated. Matrix metalloproteinases (MMPs) have the predominant role in hydrolyzing extracellular matrix proteins, and have been proposed to mediate collagen degradation leading to LV dilation [24,26], and ultimately to heart failure (HF) [7,22,28]. MMPs are upregulated in the failing heart of both animals and man [16,29,34]. In addition, protein levels of the endogenous tissue inhibitors of matrix metalloproteases (TIMPs) are decreased in hearts from transplant patients which presumably also contributes to increased cardiac MMP activity [16]. MMP inhibition also reduces LV dilation in animal HF models [18,21,26] as well as myocardial infarction [10,24]. Angiotensin converting enzyme inhibitors (ACEi) also reduce LV dilation, but by a poorly understood mechanism(s).

We have previously reported that the MMP-inhibitor (MMPi), PD166793, blocks LV dilation and preserves LV systolic function in the spontaneously hypertensive heart failure (SHHF) rat [21], an inbred line derived from an initial cross between SHR and Zucker strains. MMP-1 has

*Corresponding author. Tel.: +1-732-622-7189; fax: +1-732-622-1480.

E-mail address: tom.peterson@wl.com (J.T. Peterson)

Time for primary review 40 days.

0008-6363/00/$ – see front matter
PII: S0008-6363(00)00028-6

been reported to be increased in the SHR [3] as well as MMP-2 and MMP-12 [20]. The protein level of a TIMP has also been reported to be decreased in SHRs [20], although given the technique used (reverse zymography) it is not clear which isoform. MMP/TIMP expression has not been characterized in the SHHF rat, nor has the effect of drug treatment on gene expression been characterized. Accordingly, we compared the expression of MMPs (-1,-2,-3,-7,-9,-11,-12,-13,-14), TIMPs (-1,-2,-3,-4), and collagen [α1(I),α1(III)] in SHHF vs. normotensive rats as well as the effect of MMPi and ACEi treatment on mRNA levels. Protein level determinations were made where antibodies which worked on rat samples were available for MMPs (-13) and TIMPs (-1,-2,-4). The lack of antibodies was not expected to be a major limitation because MMP expression has been reported to be transcriptionally mediated [32]. ACEi treatment has been reported to increase MMP-1 expression in SHRs thus reducing fibrosis [3]. Both ACEi and MMPi treatment have been reported to have no effect on MMP expression in the paced pig [18]. Therefore, it was expected that there would be: (1) a progressive increase in MMP expression in vehicle-treated SHHFs that paralleled LV dilation observed in this model; (2) a decrease in TIMP expression based on similar observations in the failing human heart [16]; (3) mRNA and protein level changes would parallel each other; and (4) MMPi treatment would not directly effect gene expression given that this drug inhibits the catalytic site of an extracellular enzyme.

2. Methods

2.1. Experimental animals

Normotensive male Wistar–Furth (WF) rats (Harlan, San Diego, CA) and obese male SHHF rats (Genetic Models, Indianapolis, IN) were used. Male normotensive and SHHF rats were assigned to one of six groups: 9-(N=12) and 13-month-old (N=13) WF rats, 9-month-old (N=11) SHHF rats with compensatory hypertrophy, 13-month-old (N=9) SHHF rats with overt HF, and 13-month-old SHHF rats treated with PD166793 (SHHF-793, N=12) or quinapril (SHHF-Q, N=10). PD166793 was administered as a 5 mg kg^{-1} day^{-1} dose, and quinapril as a 10 mg kg^{-1} day^{-1} dose in chow. All rats were maintained on a chow diet (Purina, No. 5002) for the duration of the study (4 months), and had ad libitum access to food and water. This study was approved by the Parke-Davis Animal Care and Use Committee, and the animal research protocol conformed to the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Cardiovascular assessment

Rats were anesthetized with sodium pentobarbital (25–50 mg kg^{-1}, IP) prior to testing, and ventilated with 50% oxygen. LV pressure was measured in closed-chest rats using a Millar pressure transducer (Houston, TX) inserted via the right carotid. Data was recorded at 500 Hz on a digital data acquisition system (Gould Po-Ne-Mah HD-4, Valley View, OH). A 30-s average was used for baseline hemodynamic measurements.

The heart was arrested with an intravenous administration of KCl, and rapidly excised. Cardiac dilation was measured by generating ex vivo left ventricular pressure volume (PV) curves. The PV curves were generated by evacuating the LV chamber of saline, and then filling the LV at a fixed rate using a programmable pump set at a 1 ml min^{-1} flow-rate. The LV was then frozen in liquid N_2 for subsequent biochemical analysis.

2.3. Northern analysis and RNA protection assay

Total RNA was extracted from frozen left ventricular tissues using Trizol, (Gibco-BRL, Gaithersburg, MD). Northern blots were made with 20 μg of total RNA using a technique described in detail elsewhere [13]. Autoradiography was performed by exposure to a Kodak film at −80°C for 24–48 h. To control the variations in sample loading, a mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment (Ambion, Austin, TX) was used to normalize all blots. The probes used for this study were the cDNA fragments flanking the coding sequence, including human MMP-1 (ATCC, MD); rat MMP-2 and MMP-13 (Dr. John Jeffery, Albany Medical College, Albany, NY); rat MMP-3 (Dr. Lynn M. Matrisian, Vanderbilt University, Nashville, TN); rat MMP-7 (Dr. Susan Abramson, University of Miami, Miami, FL); mouse MMP-9 (Dr. Steve Shapiro, Washington University, St. Louis, MO); mouse TIMP-1 and TIMP-2, and human TIMP-3 and TIMP-4 (Dr. Yi Sun, Department of Molecular Biology, Parke-Davis, Ann Arbor, MI); human collagen α1(I) and α1(III) (ATCC, MD).

RNA protection assays (RPA) were performed to detect low copy genes using a protocol described in detail elsewhere [13]. Total RNA (10 μg) was analyzed with an RPA II kit (Ambion, Austin, TX). Anti-sense RNA probes were generated from the restriction enzyme linearized plasmids with MAXIscript SP6/T7 kit (Ambion, Austin, TX). The hybridization between P^{32}-RNA probe and RNA was carried overnight at 45°C. RNase digestion was performed for 30 min at 37°C, after which protected RNA hybrids were extracted with pheno/chloroform, precipitated with ethanol. Ribonuclease protection products were electrophoresed, and the gel exposed to Kodak BioMax film to visualize product.

2.4. Gelatin and casein zymography

Protein samples containing MMP activity were extracted from the same set of samples used for RNA analysis.

Frozen LV tissues (~50 mg) were homogenized in 1 ml of ice-cold lysis buffer containing 150 mM NaCl, 20 mmol L^{-1} Hepes, 0.2 mmol L^{-1} EDTA, 25% glycerol and protease inhibitor (complete, Boehringer Mannheim). Homogenates were then aliquoted and stored at −80°C until use. MMPs activities were detected using a gelatin or casein containing substrate gel as previously described [19]. Extracts were thawed on ice, and the volume of 30 μg of protein was adjusted to 10 μl, then mixed with an equal volume of 2× SDS gel sample loading buffer. After electrophoresis, the gel was incubated with renaturing buffer and developing buffer at room temperature for 30 min each, then changed to fresh developing buffer once and incubated at 37°C for 18 h (gelatin), or 48 h (casein). The amount of LV protein loaded per lane for zymography (30 μg), and the development time used for gelatin zymography was within the linear range for measuring pro- and active-form gelatinase activity based on dose–response work done with recombinant MMPs (unpubl. obs.), and the results of Kleiner and Stetler-Stevenson [14]. Gelatin gels were stained with Coomassie Brilliant Blue (0.2%). Clear lytic zones indicated the presence of MMP enzymes. A separate set of gelatin and casein gels were developed in the presence of 20 mM EDTA; inhibition of lysis was used as a marker of MMP specific activity.

2.5. Western blot analysis

LV tissue extracts containing 40 μg total protein were electrophoresed on a 4–20% Tris–glycine gel, and then transferred onto a nitrocellulose filter. After blocking, the filter was incubated with diluted antibody and matched secondary antibody. Protein bands were visualized using an ECL mixture (NEN Life Science Products, Boston, MA). Blots were stripped, and this process repeated with a second antibody. All antibodies were obtained from Chemicon (Temecular, CA). Two sets of blots were prepared. The first set was used to measure MMP-13 (MAB3321, 1:2000) and TIMP-4 (AB816, 1:1000). The second set was used to measure TIMP-1 (AB800, 1:1000) and TIMP-2 (AB801, 1:1000).

2.6. LV morphometry

LV circumferential sections (2 mm) were maintained in fixative for 48 h and then embedded in paraffin. Five-micron sections were stained with picrosirius red for fibrillar collagen measurements. Stained sections were imaged using polarized light which causes clear demarcation of the fibrillar collagens [35]. LV cross-sections were divided into eight equal sections, digitized using a 10× objective, and analyzed (Image Pro Plus, Media Cybernetics, Silver Spring, MD). Percentage area of extracellular staining was computed from 15 to 24 random fields spanning the entire thickness of the LV wall thus providing a composite measure of interstitial, perivascular, and replacement fibrosis.

2.7. Data analysis

All the autograms and zymograms were scanned using a densitometer and analyzed by image quantitation software (Molecular Dynamics, Ver. 4.2a, Sunnyvale, CA). All data are expressed as the mean±S.E.M. Physiological data was analyzed using a one-way analysis of variance to test for treatment effects, and between-groups differences were assessed using a post hoc *t*-test (SAS v6.12, SAS Institute). RNA and zymography data was analyzed by ANOVA based on the ranks of the data because there was a significant departure from normality. Statistical significance was based on a P value≤0.05.

3. Results

In vivo hemodynamic and pressure–volume measurements are summarized in Table 1. There were no differences in LVESP, or LV function and geometry between the 9- and 13-month-old WF rats. LVESP was higher, but LV $+dP/dt$ was not significantly different in the 9-month SHHF rats compared to either WF group. LVESP was reduced in 13-month-old SHHFs from 9-month values. 13-month-old SHHFs showed a 50% reduction in LV

Table 1
Alterations of LV volume at a fixed pressure of 20 mm Hg (LVV), the rate of LV pressure increase ($+dP/dt$), LV weight (LVW), LV end-systolic pressure (LVESP), and LV gene expression during the development of heart failure in the SHHF rats as compared to normotensive WF rats, 13-month-old SHHFs treated with either PD166793 (SHHF-793), or quinapril (SHHF-Q) for 4 months[e]

	WF-9 (N=12)	WF-13 (N=10)	SHHF-9 (N=11)	SHHF-13 (N=9)	SHHF-793 (N=12)	SHHF-Q (N=6)
LVESP	143±4	142±4	209±8[a]	130±14[b]	211±10[a,c,d]	171±12
LV $+dP/dt$	6081±189	6040±149	6714±376[c]	3741±604[a]	7075±397[c]	6048±502[c]
LVW	776±27	790±28	1326±30[a]	1436±65[a]	1515±30[a,b,d]	1352±42[a]
LVV	521±16	514±11	705±25[a,c]	989±68[a]	804±31[a,c]	782±23[a,c]

[a] P<0.05 vs. age-matched WF control.
[b] P<0.05 vs. SHHF-9.
[c] P<0.05 vs. SHHF-13.
[d] P<0.05 vs. SHHF-Q.
[e] LVV, LV volume was measured at 20 mm Hg; LVW, LV weight; $+dP/dt$ (mm Hg s^{-1}): LV peak positive. Results presented as mean±S.E.M.

$+dP/dt$ compared to either 9-month-old SHHFs or WF rats. SHHFs treated with the MMPi, PD166793, from 9 to 13 months showed a maintenance of LVESP. LVESP was significantly lower in the quinapril-treated 13-month-old SHHFs compared to the MMPi-treated group. LV $+dP/dt$ in both drug treatment groups was comparable to 9-month-old SHHFs.

LV mass was elevated in all SHHF groups compared to both WF normotensive groups. LV weight was significantly greater in the 13-month-old MMPi-treated group compared to the 9-month-old SHHFs and 13-month-old quinapril-treated SHHF. This data indicates that progressive hypertrophy occurred in the MMPi group, but not in the quinapril-treated SHHFs.

LV volume at 20 mm Hg was used as a measure of dilation. LV volume did not change with age in normotensive WF rats. LV dilation was present in 9-month-old SHHF rats with normal LV function. This dilation increased further in untreated 13-month SHHFs, but was significantly reduced in both age-matched drug groups compared 13-month-old untreated SHHFs.

3.1. LV collagen mRNA and morphometry

Fig. 1 shows representative micrographs of picrosirius red stained LV cross-sections from a 9-month-old normotensive rat as well as each SHHF group. Collagen volume fraction was not different between 9- and 13-month-old normotensive rats, and 9-month-old SHHFs as shown in the top middle panel of Fig. 1. The collagen volume fraction increased about two-fold in untreated 13-month-old SHHFs. Total collagen volume fraction was reduced in both quinapril and MMPi treatment compared to 13-month-old vehicle-treated SHHFs. Both collagen α1(I and III) mRNA levels increased comparably in untreated 9- and 13-month-old SHHF rats compared to normotensive WF rats (Fig. 2). Quinapril significantly reduced collagen α1(I) mRNA levels while MMPi had no effect on mRNA levels of either collagen subtype.

3.2. MMP expression

All mRNA levels were normalized to GAPDH in this study. Some genes had LV mRNA expression levels too low for assessment by Northern (MMP-3,-7,-9,-11 and -13). Alterations in low copy gene expression were also assessed by RPA (MMP-3) or RT-PCR (MMP-7,-9,-11). The expression of the following MMPs was found to be unchanged: MMP-3,-7,-9,-11,-12,-13 and -14. No evidence was found that the rat heart expresses MMP-1 by Northern analysis, RPA, or RT-PCR. MMP-2 mRNA levels were significantly increased in 13-month-old (factor of 2.7 ± 0.2) but not 9-month-old (factor of 1.2 ± 0.2) SHHFs compared to age-matched normotensive controls (Table 2). MMPi and quinapril reduced MMP-2 mRNA levels to that of the SHHF-9 group. Fig. 3 shows Northern blots of MMPs using representative samples from each group illustrating that MMP-2 had a between-group elevation in mRNA levels.

MMP-2 and MMP-9 zymographic activity was upregulated in the compensatory hypertrophy (SHHF-9) and the failing (SHHF-13) LV (Table 2). Fig. 2 shows a gelatin zymogram of representative animals. MMP-2 activity is evident by the lytic bands at the 72-kD (pro-form) and the 66-kD (active-form) regions, and MMP-9 activity at the 92-kD region (pro-form). A lytic band representing the active form of MMP-9 (84-kD) was not observed in this study. There was a progressive increase in the active form (66-kD band) of MMP-2 in SHHF-9 (factor of 1.8 ± 0.2) and SHHF-13 (factor of 4.7 ± 0.5) rats compared to age-matched controls. Fig. 4 also shows that the basal level of MMP-9 protein is quite low in normal animals, and a progressive increase of MMP-9 activity occurred in SHHF-9 (factor of 1.7 ± 0.2) and SHHF-13 (factor of 3.9 ± 0.7) rats compared to age-matched controls. Lytic band density for pro- and active form of MMP-2 increased in SHHF-13 rats. Both drug treatments reduced MMP-2 active-form activity compared to age-matched vehicle controls. MMP-inhibition also reduced the pro-form of MMP-2 (72-kD band), and MMP-9 compared to SHHF-13. The MMP-2 results are consistent with the MMP-2 mRNA level changes. The disparity between MMP-9 mRNA and zymography data may be due to a greater sensitivity of zymography vs. Northern analysis, or to a post-transcriptional effect causing an increase in MMP-9 accumulation.

MMP-3,-7 and -13 protein levels were assessed using casein zymography, but no EDTA sensitive banding was observed. A Western blot analysis of MMP-13, however, revealed that 13-month-old untreated SHHF rats showed a significant increase in MMP-13 protein levels compared to normotensive WF rats or 9-month-old SHHFs (Fig. 5). Both PD166793 and quinapril reduced MMP-13 protein levels.

3.3. TIMP expression

All four TIMP mRNAs were quantifiable in the rat LV by Northern analysis (Fig. 6). TIMP-1 and TIMP-2 mRNA and protein levels were significantly higher in both 9- and 13-month-old SHHFs compared to age-matched normotensives (Table 3). TIMP-1 mRNA and protein levels increased progressively with age in SHHFs. No between group differences were observed for TIMP-3 and TIMP-4 mRNA levels. However, TIMP-4 protein levels in 9- and 13-month-old SHHFs was half that of age-matched normotensive controls (Table 3). Both MMPi and quinapril treatment significantly decreased TIMP-1 mRNA levels as well as TIMP-1 and TIMP-2 protein levels compared to 13-month-old non-drug-treated SHHFs. Both drug treatments normalized TIMP-4 protein levels.

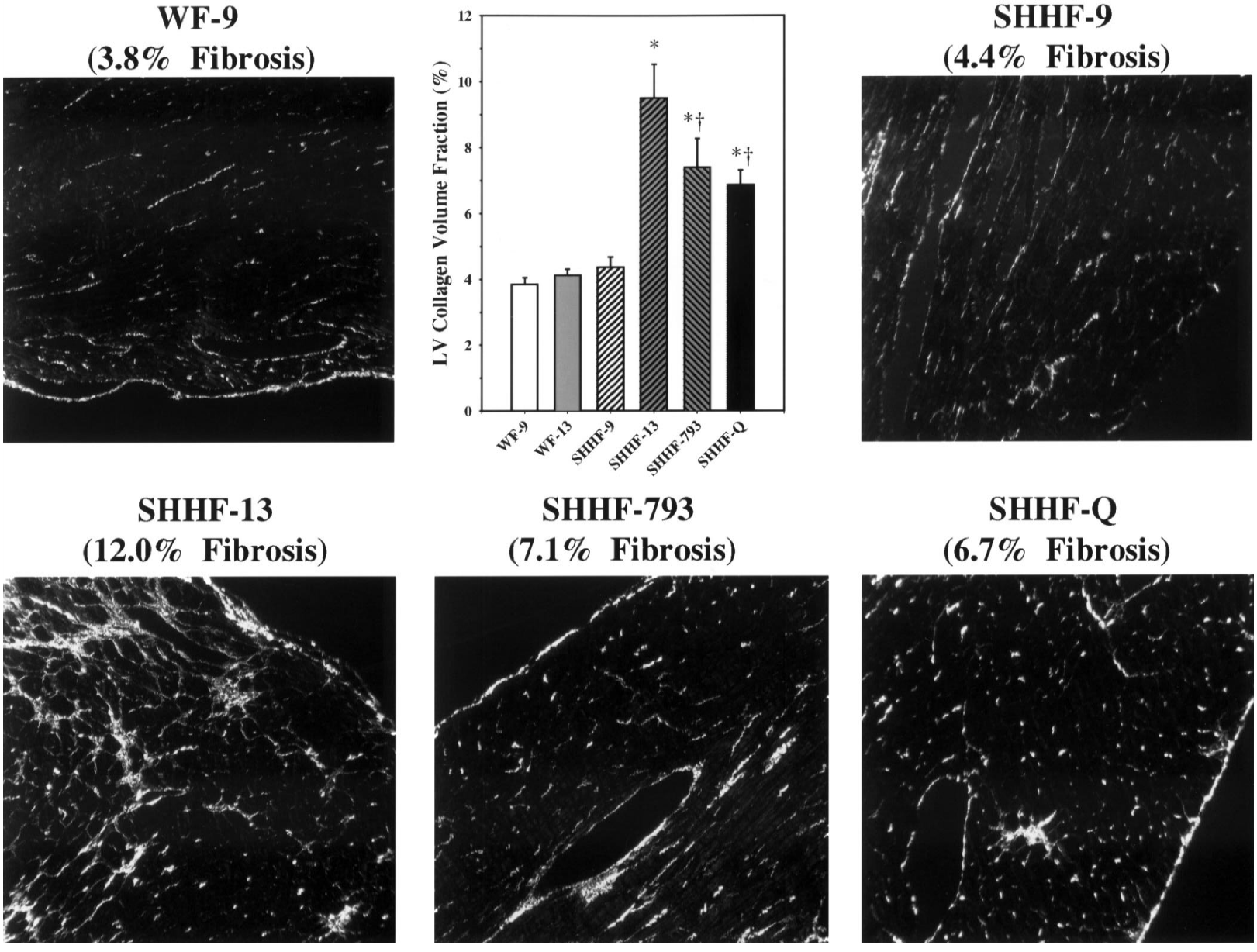

Fig. 1. Morphometric analysis of picrosirius red stained LV cross-sections. Representative left ventricular epicardial cross-sections from a 9-month-old normotensive WF rat (WF-9) and SHHF rat (SHHF-9) shown at top, and at bottom 13-month-old SHHF rats that were vehicle (SHHF-13), MMPi treated (SHHF-793), and quinapril treated (SHHF-Q). Drug administration was initiated at 9 months of age and continued for 4 months. Group means±S.E.M. are shown in the barchart in the top middle panel. *, $P \leq 0.05$ compared to WF groups. †, $P \leq 0.05$ compared to SHHF-13.

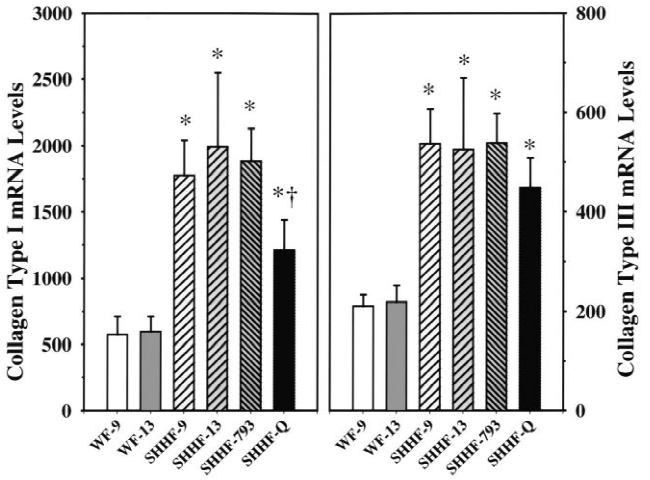

Fig. 2. Results of a Northern analysis expressed as arbitrary densitometric units comparing normotensive Wistar–Furth (WF) to spontaneously hypertensive heart failure (SHHF) for collagen α1(I) (left-panel) and collagen α1(III) (right-panel) mRNA levels normalized to GAPDH. Data presented as mean±S.E.M. *, $P \leq 0.05$ compared to age-matched WF group; †, $P \leq 0.05$ compared to SHHF-13.

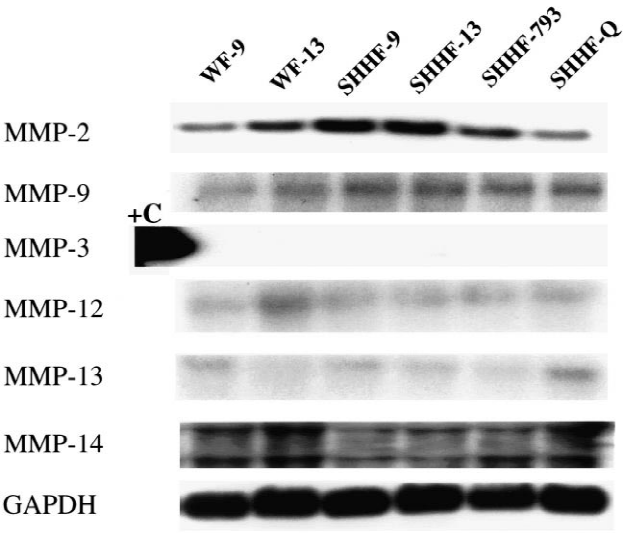

Fig. 3. Northern blots of MMPs (20 μg of total RNA per lane) from left ventricle of normotensive WF, SHHF-9 with compensatory hypertrophy, SHHF-13 with overt heart failure, and age-matched 13-month-old SHHF treated with MMPi (SHHF-793) or the ACE-inhibitor, quinapril (SHHF-Q). Samples from the same rat are shown in each blot. Only MMP-2 exhibited a between-group elevation in mRNA levels.

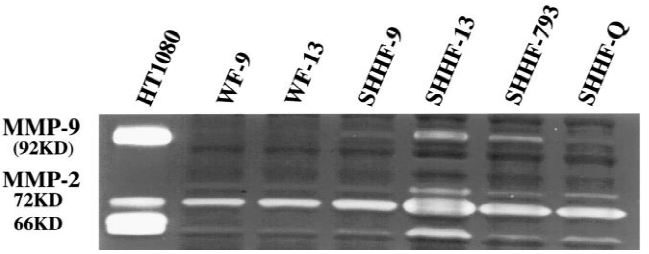

Fig. 4. MMP-2 and MMP-9 activity on gelatin zymogram SDS–PAGE gel. Protein samples were loaded onto gels at 30 μg per lane. Gels were incubated in developing buffer at 37°C for 48 h. Clear zones indicate the presence of enzyme.

4. Discussion

The important findings in the current study are four-fold: (1) MMPi as well as quinapril treatment reduced fibrosis; (2) we identified which MMPs increased (MMP-2,-9,-13) and which TIMPs decreased (TIMP-4) during compensatory hypertrophy and HF; (3) MMP/TIMP protein levels can be altered in the hypertrophied and failing heart without a significant change in mRNA levels (MMP-2,-9,-13 and TIMP-4); and (4) both drug treatments reduced LV dysfunction and remodeling as well as MMP/TIMP expression changes in SHHFs. LV dilation preceded LV dysfunction in this study as has been reported elsewhere [9]. This observation, and the efficacy of MMPi supports the role of MMP-mediated LV dilation in the transition between LV hypertrophy and HF.

4.1. Collagen expression and accumulation

Collagen type I mRNA and fibrosis reduction by quinapril in this study was not unexpected given previous demonstrations of this in SHRs [3,17] a parental line used to create the SHHF. Two other potential mechanisms mediating this ACE-inhibitor effect are decreased angiotensin type I receptor stimulated secretion of TGFβ [4],

Table 2
Alterations in MMP-2 based on Northern analysis, and MMP-2 and MMP-9 activity by zymography in SHHF rats as compared to normotensive WF rats, and 13-month-old SHHFs treated with either the MMPi PD166793 (SHHF-793), or quinapril (SHHF-Q) for 4 months[d]

	WF-9 (N=9)	WF-13 (N=5)	SHHF-9 (N=11)	SHHF-13 (N=9)	SHHF-793 (N=12)	SHHF-Q (N=12)
MMP-2 mRNA	1892±239	1326±173	2364±308	3518±307[a,b]	2022±285[c]	1432±259[b,c]
MMP-2 (66 kD)	36±8	24±2	64±8[a,c]	114±11[a,b]	41±5[c]	60±9[c]
MMP-2 (72 kD)	81±6	71±8	120±13[a]	200±44[a,b]	114±12[c]	135±17
MMP-9 (92 kD)	28±5	20±4	49±5[a]	78±14[a,b]	44±5[c]	59±7[a]

[a] $P<0.05$ vs. age-matched WF control.
[b] $P<0.05$ vs. SHHF-9.
[c] $P<0.05$ vs. SHHF-13.
[d] Both Northern and zymographic data are shown as arbitrary densitometric units. MMP-2 mRNA levels were normalized by GAPDH measurement from the same blot. Results presented as mean±S.E.M.

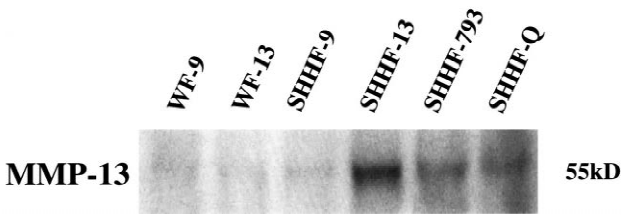

Fig. 5. Western blot analysis showing the abundance of type 3 collagenase (MMP-13) in normal and failing rat heart. Approximately 40 μg of total protein was separated on a 4–20% Tris–glycine gel under denaturing conditions. The size of MMP-13 bands was estimated based on the known size of protein markers.

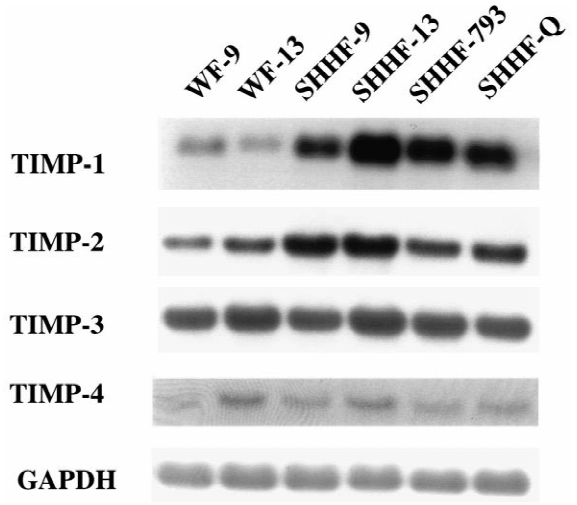

Fig. 6. Northern blots of four species of TIMP (20 μg of total RNA per lane) from the LV of a normotensive WF, SHHF-9 with compensatory hypertrophy, SHHF-13 with overt heart failure, and age-matched 13-month-old SHHFs treated with MMPi (SHHF-793), or quinapril (SHHF-Q).

and an upregulation of MMP-1 [3] in SHRs. Although the current study does not address the role of angiotensin or TGFβ, it does indicate that MMP-induced fibrinolysis does not account for lower LV collagen in quinapril-treated SHHFs.

Surprisingly, MMPi significantly lowered LV collagen compared to untreated 13-month-old SHHFs — despite a lack of an effect on collagen mRNA levels. Cardiac collagen deposition is controlled by non-MMP dependent post-translational system(s) such as lysosomal degradation, fibril secretion, and trimeric collagen assembly [2]. Collagen volume fraction in this study represented the sum of perivascular, interstitial, reactive and replacement fibrosis. Therefore, the lesser degree of fibrosis may reflect an indirect effect on the rate of HF development. Alternatively, MMP-inhibition may have inhibited myofibroblast migration and collagen deposition as shown elsewhere [10].

4.2. MMP upregulation

MMP-2 and MMP-9 zymographic activity, and MMP-13 protein levels were significantly greater in SHHFs compared to normotensive age-matched controls. MMP-2 and MMP-9 zymographic activity also increased progressively with age in SHHFs. The upregulation of MMP-2 activity in the current study agrees with results reported for SHRs [20] as well as human HF patients [16,29]. Several previous rat studies have reported that MMP-1 is upregulated during hypertension [3,23,25]. However, these studies either indirectly measured MMP-1 [3,23], or potentially measured rat collagenase (MMP-13) rather than MMP-1 [25]. In this study MMP-13 (collagenase-3) appears to act in concert with the gelatinases in the elevated turnover of extracellular matrix proteins during HF progression.

The increase in MMP protein levels without a corresponding significant elevation in mRNA levels is a novel observation. A recent study showed that extracellular matrix protein enhances MMP-2 enzyme stability as well as its activity [12]. Therefore, fibrosis may also initiate collagen degradation by causing MMP accumulation.

Three potential factors involved in increasing MMP mRNA levels in HF are cytokines, inflammatory cell infiltrate, and cytoskeletal remodeling. A variety of cytokines (e.g. TNFα, IL-1β) have been shown to increase MMP mRNA levels [31]. TNFα levels have been reported

Table 3
Alterations in LV TIMP-1 and TIMP-2 expression based on Northern and Western analysis, and TIMP-4 by Western[e]

	WF-9 (N=12)	WF-13 (N=10)	SHHF-9 (N=11)	SHHF-13 (N=9)	SHHF-793 (N=12)	SHHF-Q (N=6)
TIMP-1 mRNA	206±35	113±43	484±117[a]	1825±670[a,b]	455±180[a,c]	537±199[a,c]
TIMP-2 mRNA	1932±365	1798±349	3400±345[a]	4652±727[a]	3478±248[a]	3671±654[a]
TIMP-1 protein	109±20	114±15	195±26[a]	256±26[a]	180±22[a,c]	171±22[a,c]
TIMP-2 protein	131±8	165±15	155±27	256±22[a,b]	115±12[a,c,d]	171±17[c]
TIMP-4 protein	1116±58	946±20	645±90[a]	678±50[a]	961±47[c]	896±60[c]

[a] $P<0.05$ vs. age-matched WF control.
[b] $P<0.05$ vs. SHHF-9.
[c] $P<0.05$ vs. SHHF-13.
[d] $P<0.05$ vs. SHHF-Q.
[e] Both Northern and Western data are shown as arbitrary densitometric units. The mRNA levels were normalized by GAPDH measurement from the same blot. Results presented as mean±S.E.M.

to be elevated in the SHHF rat [1]. MMP-9 has typically been found to be associated with inflammatory rather than mesenchymal cells [30]. We have observed subendocardial necrosis in 13-month-old SHHF rats, and a modest inflammatory cell infiltrate (unpubl. obs.). The increase in MMP-9 observed in the SHHF-13 group may be due to a small number of inflammatory cells present at sites of reactive fibrosis heart.

4.3. TIMP upregulation

In this study, TIMP-1 and TIMP-2 increased, and TIMP-4 decreased in SHHFs. The progressive LV dilation that occurred in SHHFs indicates that MMP upregulation supercedes increased TIMP-1 and TIMP-2. TIMP upregulation in this study is similar to that reported by Thomas et al. [29]. Whether TIMP-1 and -2 upregulation produces a proportionate decrease in MMP activity is unclear. For example, the amount of MMP/TIMP complex is decreased in the failing human LV, and the ratio of free to bound TIMP increases [5]. Therefore, increased TIMP protein levels may not necessarily translate into reduced MMP activity.

Factor(s) involved in TIMP upregulation like that of MMPs in HF are ill-defined. TIMP-1 has been shown to be regulated primarily by cytokines and growth factors [6,15], and TNFα may be responsible for the induction of TIMP-1 observed in this study. MMP-2 and TIMP-2 share the AP-2 promoter, and this may explain why mRNA levels of both these genes paralleled each other. TIMP-2 binds to both the active and pro-form of MMP-2, having the greatest affinity among the TIMPs for MMP-2 [6,15]. Increased TIMP-2 may reflect a compensatory response directed against MMP-2 upregulation. Therefore, changes in TIMP-1 may reflect altered neuroendocrine activity while increased TIMP-2 may be a compensatory response.

TIMP-4 protein levels, but not mRNA levels were significantly lower in SHHF LV compared to age-matched normotensives corresponding to recent data from human HF patients [16]. TIMP-4 is of particular interest because of its predominant cardiac expression [8]. Decreased TIMP-4 protein levels presumably resulted in greater LV MMP activity. Restoration of TIMP-4 protein levels by both MMPi and quinapril may have lowered LV MMP activity.

4.4. Drug-induced alterations in gene expression

The effect of MMPi and ACEi on MMP/TIMP expression has not been clearly defined. MMPi treatment did not modify MMP expression in the paced pig [18]. The changes noted in cardiac MMP/TIMP expression of transplanted human hearts, a majority of which came from patients treated with ACE-inhibitors, suggests that ACEi does not modify MMP/TIMP expression in end-stage human HF. ACE-inhibitor treatment started within a few days post-MI significantly reduces LV dilation [27]. It is possible that this effect in humans is mediated by ACE-inhibitor-induced changes in MMPs as well as hypertrophy, or neuroendocrine activity.

The difference in the effect of ACEi vs. MMPi on collagen α1(I) mRNA levels and LV hypertrophy may reflect a difference between these two drugs on angiotensin II levels. Angiotensin II stimulates both myocardial collagen expression and hypertrophy [33]. High plasma levels of the same MMPi used in this study, PD166793, have recently been shown not to alter plasma renin activity in the paced pig [26]. The increase in plasma renin activity reported in SHHFs [11] suggests that quinapril, but not PD166793, reduced angiotensin levels in this study thus explaining why ACEi reduced collagen mRNA and LV hypertrophy while MMPi did not.

5. Conclusion

Both ACEi and MMPi treatment preserved LV systolic function, presumably by reducing LV remodeling. Increased MMP (-2,-9,-13) levels were not always associated with elevated mRNA levels which indicates that a post-translation process(es) modulate LV MMP levels. Both ACEi and MMPi reduced fibrosis and normalized MMP/TIMP expression. However, only ACEi reduced collagen α1(I) mRNA and LV hypertrophy. These results suggest that ACEi and MMPi efficacy involve different signaling pathways which converge to produce a similar physiological effect on LV geometry and function in the SHHF rat.

Acknowledgements

The authors wish to thank Wendy Rosebury and Andrew Robinson for their help in preparing the histological samples.

References

[1] Bergman MR, Kao RH, McCune SA, Holycross BJ. Myocardial tumor necrosis factor-alpha secretion in hypertensive and heart failure-prone rats. Am J Physiol 1999;277:H543–H550.
[2] Bishop JE, Rhodes S, Laurent GJ, Low RB, Stirewalt WS. Increased collagen synthesis and decreased collagen degradation in right ventricular hypertrophy induced by pressure overload. Cardiovasc Res 1994;28:1581–1585.
[3] Brilla CG, Matsubara L, Weber KT. Advanced hypertensive heart disease in spontaneously hypertensive rats. Lisinopril-mediated regression of myocardial fibrosis. Hypertension 1996;28:269–275.
[4] Brooks WW, Bing OH, Conrad CH et al. Captopril modifies gene expression in hypertrophied and failing hearts of aged spontaneously hypertensive rats. Hypertension 1997;30:1362–1368.
[5] Coker ML, Zellner JL, Crumbley AJ, Spinale FG. Defects in matrix metalloproteinase inhibitory stoichiometry and selective MMP induction in patients with nonischemic or ischemic dilated cardiomyopathy. Ann New York Acad Sci 1999;878:559–562.

[6] Docherty AJ, O'Connell J, Crabbe T, Angal S, Murphy G. The matrix metalloproteinases and their natural inhibitors: Prospects for treating degenerative tissue diseases. Trends Biotechnol 1992;10:200–207.
[7] Gaudron P, Eilles C, Ertl G, Kochsiek K. Compensatory and noncompensatory left ventricular dilatation after myocardial infarction: Time course and hemodynamic consequences at rest and during exercise. Am Heart J 1992;123:377–385.
[8] Greene J, Wang M, Liu YE et al. Molecular cloning and characterization of human tissue inhibitor of metalloproteinase 4. J Biol Chem 1996;271:30375–30380.
[9] Haas GJ, McCune SA, Brown DM, Cody RJ. Echocardiographic characterization of left ventricular adaptation in a genetically determined heart failure rat model. Am Heart J 1995;130:806–811.
[10] Heymans S, Luttun A, Nuyens D et al. Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat Med 1999;5:1135–1142, see comments.
[11] Holycross BJ, Summers BM, Dunn RB, McCune SA. Plasma renin activity in heart failure-prone SHHF/Mcc-facp rats. Am J Physiol 1997;273:H228–333.
[12] Itoh Y, Ito A, Iwata K et al. Plasma membrane-bound tissue inhibitor of metalloproteinases (TIMP)-2 specifically inhibits matrix metalloproteinase 2 (gelatinase A) activated on the cell surface. J Biol Chem 1998;273:24360–24367.
[13] Kingstone RE. In: Ausubel FM, Brent R, Kingstone RE, Moore DD, Seidman JG, Smith JA, Struhl K, editors, Current protocols in molecular biology, New York: John Wiley, 1994, pp. 421–429.
[14] Kleiner DE, Stetler Stevenson WG. Quantitative zymography: Detection of picogram quantities of gelatinases. Anal Biochem 1994;218:325–329.
[15] Leco KJ, Hayden LJ, Sharma RR et al. Differential regulation of TIMP-1 and TIMP-2 mRNA expression in normal and Ha-ras-transformed murine fibroblasts. Gene 1992;117:209–217.
[16] Li YY, Feldman AM, Sun Y, McTiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the failing human heart. Circulation 1998;98:1728–1734.
[17] Makino N, Sugano M, Otsuka S, Hata T. Molecular mechanism of angiotensin II type I and type II receptors in cardiac hypertrophy of spontaneously hypertensive rats. Hypertension 1997;30:796–802.
[18] McElmurray JH, Mukherjee R, New RB et al. Angiotensin-converting enzyme and matrix metalloproteinase inhibition with developing heart failure: Comparative effects on left ventricular function and geometry. JPET 1999;291:799–811.
[19] Moll UM, Youngleib GL, Rosinski KB, Quigley JP. Tumor promoter-stimulated Mr 92,000 gelatinase secreted by normal and malignant human cells: Isolation and characterization of the enzyme from HT1080 tumor cells. Cancer Res 1990;50:6162–6170.
[20] Mujumdar VS, Tyagi SC. Temporal regulation of extracellular matrix components in transition from compensatory hypertrophy to decompensatory heart failure. J Hypertens 1999;17:261–270.
[21] Peterson J, Rosebury W, Robertson A et al. Matrix metalloproteinase inhibition blocks progression of heart failure. Circ 1997;96:1520.
[22] Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81:1161–1172.
[23] Robert V, Besse S, Sabri A et al. Differential regulation of matrix metalloproteinases associated with aging and hypertension in the rat heart. Lab Invest 1997;76:729–738.
[24] Rohde LE, Ducharme A, Arroyo LH et al. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. Circulation 1999;99:3063–3070.
[25] Seccia TM, Bettini E, Vulpis V et al. Extracellular matrix gene expression in the left ventricular tissue of spontaneously hypertensive rats. Blood Press 1999;8:57–64.
[26] Spinale FG, Coker ML, Krombach SR et al. Matrix metalloproteinase inhibition during the development of congestive heart failure: Effects on left ventricular dimensions and function. Circ Res 1999;85:364–376.
[27] St. John Sutton M, Pfeffer MA, Moye L. Cardiovascular death and left ventricular remodeling two years after myocardial infarction: Baseline predictors and impact of long-term use of captopril: Information from the Survival and Ventricular Enlargement (SAVE) trial. Circulation 1997;96:3294–3299.
[28] St. John Sutton M, Pfeffer MA, Plappert T. Quantitative two-dimensional echocardiographic measurements are major predictors of adverse cardiovascular events after acute myocardial infarction. The protective effects of captopril. Circulation 1994;89:68–75.
[29] Thomas CV, Coker ML, Zellner JL et al. Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy. Circulation 1998;97:1708–1715.
[30] Ueno H, Yamashita K, Azumano I, Inoue M, Okada Y. Enhanced production and activation of matrix metalloproteinase-7 (matrilysin) in human endometrial carcinomas. Int J Cancer 1999;84:470–477.
[31] Vincenti MP, Coon CI, Brinckerhoff CE. Nuclear factor kappaB/p50 activates an element in the distal matrix metalloproteinase 1 promoter in interleukin-1beta-stimulated synovial fibroblasts. Arthritis Rheum 1998;41:1987–1994.
[32] Vincenti MP, White LA, Schroen DJ, Benbow U, Brinckerhoff CE. Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): Mechanisms that control enzyme activity, transcription, and mRNA stability. Crit Rev Eukaryot Gene Expr 1996;6:391–411.
[33] Weber KT, Sun Y, Guarda E. Structural remodeling in hypertensive heart disease and the role of hormones. Hypertension 1994;23:869–877.
[34] White AD, Bocan TMA, Boxer PA, Peterson JT, Schrier D. Emerging therapeutic advances for the development of second generation matrix metalloproteinase inhibitors. Curr Pharmaceutical Design 1997;3:45–58.
[35] Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic Res Cardiol 1994;89:397–410.