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Effects of exercise training on cardiac function and myocardial remodeling in post myocardial infarction rats

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Abstract

Objective—To test the hypothesis that early exercise training after myocardial infarction (MI) could preserve cardiac function, alleviate left ventricular (LV) remodeling and induce a protective effect on morphology.

Methods—Male Sprague-Dawley rats underwent coronary ligation or sham operation, and were assigned to 3 groups: Sham, sedentary MI (SedMI), and exercise MI (ExMI). We measured the changes in collagen volume fraction, matrix metalloproteinase (MMP) 1, tissue inhibitor matrix metalloproteinase 1 (TIMP-1), angiotensin II receptor type 1 (AT1), and angiotensin converting enzyme (ACE) at gene and protein levels after 8 weeks of exercise training. Cardiac functions were determined by echocardiographic and hemodynamic measurements.

Results—Early exercise training after MI had no effect on LV wall thinning. Cardiac function was significantly preserved in the ExMI group in comparison to the SedMI group. The collagen volume fraction in the ExMI group was significantly lower than in the SedMI group. Compared to the SedMI group, the ExMI group showed a markedly decrease at both the gene and protein levels in TIMP-1 ($P < 0.05$). No significant differences were found in MMP-1 among the three groups. MMP-1/TIMP-1 ratio in the ExMI group was significantly higher than in the SedMI group. In addition, the expression of AT1 protein in the ExMI group was significantly lower than in the SedMI group. Furthermore, both ACE mRNA expression and ACE binding in the ExMI group are significantly decreased compared to the SedMI group.

Conclusions—Our results suggest that early exercise training after MI reduces TIMP-1 expression, improves the balance between MMPs and TIMPs, and mitigates the expressions of ACE and AT1 receptor. These improvements, in turn, attenuate myocardial fibrosis and preserve post-MI cardiac function.

Keywords

extracellular matrix; fibrosis; infarction; remodeling; exercise

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1. Introduction

Left ventricular (LV) remodeling following myocardial infarction (MI) consists of the process of cardiomyocyte loss, cardiomyocyte lengthening, LV wall thinning, infarct expansion, cardiomyocyte hypertrophy, and collagen accumulation. After MI, complex architectural alterations occur in both the infarcted and non-infarcted myocardium. Cardiac remodeling is initially an adaptive response to maintain normal cardiac function, however, it gradually becomes maladaptive and subsequently leads to progressive decompensation and congestive heart failure, which are associated with a poor prognosis [1]. Cardiac myocytes, cardiac fibroblasts, and extracellular matrix (ECM) play critical roles during the remodeling process.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteinases that are responsible for ECM degradation and remodeling under normal and pathological conditions [2–5]. MMPs are produced by fibroblasts, inflammatory cells, and myocytes in the heart [6]. Under ambient conditions, MMP activity is tightly controlled by the endogenous tissue inhibitors of metalloproteinases (TIMPs) [7]. The primary physiological role of TIMPs is thought to be regulation and maintenance of ECM homeostasis and integrity. The imbalance between MMP and TIMP expression appears to be responsible for the increased MMP activity observed in congestive heart failure, which is associated with myocardial matrix collagen disruption and ventricular remodeling [8,9].

Mediators of myocardial fibrosis post-MI have attracted considerable interest, and angiotensin (Ang) II has been implicated. Angiotensin converting enzyme (ACE) is markedly increased at sites of fibrosis and likely accounts for the elevated AngII concentration [10], while AngII receptors are also markedly increased and colocalized with ACE and fibrosis [11,12]. In addition, it is well known that transforming growth factor (TGF) β 1 is one of the major cytokines stimulating fibrosis process.

Post-MI exercise training studies have revealed several positive effects on the improvement of cardiac functions, such as, attenuated LV dilation and improved systolic function in patients with large anterior MI [13–16]. However, the factors responsible for the beneficial effects of exercise training on myocardial remodeling and function following MI remain to be elucidated. Accordingly, the current study was designed to evaluate the effect of post-MI exercise training on myocardial fibrosis, cardiac function, and factors inducing the adverse remodeling. For the first time, we investigated the changes caused by exercise training in type I and type III collagen, MMP-1, TIMP-1, TGF- β 1, and angiotensin II receptor type 1 (AT1), and ACE at both the gene and protein levels after MI. We hypothesized that early exercise training after MI is able to reduce the expression of TIMP-1, improve the balance between MMPs and TIMPs, reverse the MI-induced abnormalities in AT1 and ACE expressions, and decrease the collagen content, thereby attenuating deleterious cardiac remodeling and preserving post-MI cardiac function.

2. Materials and Methods

2.1. Animal model

Seven-week-old male Sprague-Dawley rats (190–200g) were used for this study. Rats were fed *ad libitum* on standard laboratory rat chow and had free access to tap water. To ensure the rats were accustomed to running, they were trained on a rodent treadmill for one week prior to surgery. The animals unable to adapt to running were excluded. MI was created by ligation of the left anterior descending coronary artery (LAD) [17]. Briefly, rats were anesthetized with 2% isoflurane mixed with oxygen. After left thoracotomy, the heart was exteriorized and the

LAD was ligated approximately 2 mm below the left atrium with a 6-0 silk suture. For the sham group, the suture was removed without tying and no infarction was created.

2.2. Experimental groups and exercise training

Echocardiography was performed on the surviving rats one week after surgery. Rats were matched by cardiac functions and randomly assigned to the following experimental groups: sham-operated control (n=8, Sham), sedentary MI (n=8, SedMI), exercise MI (n=8, ExMI). The rats assigned to the exercise group started exercising at 1 week post-MI using a motorized rodent treadmill, while the Sham and the SedMI rats remained sedentary throughout the experiment period. To allow gradual adaptation to exercise stress, training was initiated at 10 m/min, 5° incline for 10 min per session. The speed and duration were gradually increased to 16 m/min and 50 min per session (including a 5 min warm-up at 10 m/min) and maintained constant throughout the experiment. The exercise training was performed 5 days per week for 8 weeks. The determination of treadmill speed and exercise duration was based on the previous studies [18,19]. This exercise regimen was well tolerated by rats with MI. There were no mortalities during the eight weeks of exercise training.

2.3. Echocardiographic measurements

Echocardiographic measurements were performed the day before the initiation of post-MI exercise training and after 8 weeks of exercise training using an echocardiographic system equipped with a 10-MHz transducer (SonoHeart Elite, SonoSite Bothell, WA). Rats were anesthetized with 2% isoflurane mixed with oxygen, and a two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscle to record M-mode tracings. We measured the left ventricle anterior wall thickness, LV end-diastolic dimension (LVEDd), LV end-systolic dimension (LVESd), LV end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV). LV fractional shortening (FS) and LV ejection fraction (EF) were calculated as $FS(\%) = [(LVEDd - LVESd) / LVEDd] \times 100$ and $EF = (LVEDV - LVESV) / LVEDV$, respectively [20]. All measurements were averaged over three consecutive cardiac cycles.

2.4. In vivo hemodynamic measurement

Hemodynamic measurement was performed at the end of the experiment. Rats were anesthetized as mentioned above. A pressure transducer (Model SPR-838, Millar instruments) was inserted retrograde from the right carotid artery to the left ventricle cavity. The following hemodynamic parameters were measured: LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), aortic systolic pressure, aortic diastolic pressure and peak velocities of contraction and relaxation (dP/dt_{max}). After measuring, the hearts were harvested, frozen in isopentane with dry ice, and stored at -80°C until use.

2.5. Determination of infarct size and collagen content

Cryostat sections (6 µm) of the hearts were stained with collagen-specific picrosirius red (PSR) for fibrillar collagen measurements. Total infarcted epicardial and endocardial lengths were identified by PSR staining and measured using a computer software (Image Pro Plus, Media Cybernetics). The infarct size was calculated by dividing the sum of the planimetered endocardial and epicardial circumferences of the infarcted area by the sum of the total epicardial and endocardial circumferences of the left ventricle [21]. For collagen volume measurement, the PSR stained sections were imaged using polarized light [22] and digitized using a 10× objective in the non-infarcted myocardium. The collagen volume was measured using Image Pro Plus.

2.6. Immunohistochemical staining

Cryostat sections (6 μ m) were stained immunohistochemically with the following antibodies: anti- α -smooth muscle actin (α -SMA, Sigma) antibody, and anti-CD68 (Serotec) antibody. Briefly, the slides were treated with 0.3% H₂O₂ methanol and incubated with either anti- α -SMA (1:200 dilution) for myofibroblasts, or anti-CD68 (1:50 dilution) for macrophages. Following the incubation, slides were rinsed and incubated with a goat anti-mouse secondary antibody (Sigma, 1:150 dilution). The stain was developed using Fast 3.3'-diaminobenzidine tablet sets (D4293; Sigma). The sections were then counterstained with hematoxylin and examined by light microscopy.

2.7. Real-time polymerase chain reaction

Total RNA was extracted from non-infarcted LV with TRIzol. After DNase treatment, RNA samples were reverse-transcribed with oligo (dT) primers and MMLV reverse transcriptase (Promega, Madison, WI). Real-time polymerase chain reaction (RT-PCR) was used to quantify cardiac gene expressions. The relative expression of type I procollagen, type III procollagen, MMP-1, TIMP-1, TGF- β 1, AT1 and ACE mRNA was normalized to the amount of β -actin in the same cDNA using the standard curve method. The primers and probes used for type I procollagen and MMP-1 were as follows: 5'-CCAAGGAGAAAAAGCATGTCTG-3' (forward primer), 5'-CGCTTCCATACTCGAACTGGA-3' (reverse primer) and 5'-TTTGAGAGAGCATGACCGATGGA-3' (TaqMan probe) for type I procollagen α 1 chain (GenBank accession number Z78279). 5'-GAGGATATTAATCGCATCCAGGCTTT-3' (forward primer), 5'-CATGGATGTGGTGTGTTGCA-3' (reverse primer) and 5'-CCTTCCCCAAATCCCA-3' (TaqMan probe) for MMP-1 (GenBank accession number XM_001072423). Other primers and probes were Assay-on-Demand gene expression products by Applied Biosystems. Because of proprietary issues and the policy of Applied Biosystems, these exact primer sequences are not provided but can be requested from the company based on the information shown in Table 1.

2.8. Western blot

Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE), and transferred to PVDF membranes (Bio-Rad, Hercules, CA). The membranes were incubated with primary antibodies. The primary antibodies used were anti-type I collagen (Santa Cruz), anti-type III collagen (Santa Cruz), anti-MMP-1 (Chemicon), anti-TIMP-1 (Chemicon), anti-TGF- β 1 (Santa Cruz), anti-AT1 receptor (Santa Cruz) and GAPDH (Santa Cruz). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody. Subsequently, the protein bands were detected with enhanced chemiluminescence (Amersham) and exposed to an X-ray film. The protein band densities calculated using Quantity One software (Bio-Rad). GAPDH was used as an internal control.

2.9. ACE binding

[¹²⁵I]³⁵¹A, a tyrosyl derivative of lisinopril and potent competitive inhibitor of angiotensin converting enzyme (ACE) was used as the radioligand to label ACE. ³⁵¹A was iodinated by the chloramines T method [23]. Briefly, cryostat sections (16 μ m) were incubated in sodium phosphate buffer containing sodium chloride and bovine serum albumin with [¹²⁵I]³⁵¹A [0.3 μ Ci/ml (~300mmol)] for 1 h at 20°C. Non-specific binding was determined in parallel incubations containing either 1 mmol ethylenediamine tetracetate or 10 μ mol unlabeled lisinopril. After incubation, sections were washed and exposed to an X-ray film (Kodak NMB-6). Quantification of binding density was performed using a computer image analysis system (NTH Image 1.60).

2.10. Statistical analyses

Paired t-tests were used to analyze pre and post measurements within the groups. One way analyses of variance (ANOVA) were carried out to determine whether there were significant mean differences among the experiment groups. An ANOVA with significant F ratios ($P < 0.05$) was followed by Student-Newman-Keuls post hoc comparisons. A P value of less than 0.05 was considered statistically significant. Values are expressed as mean $\pm$ S.E.M.

3. Results

3.1. Infarct area and LV function

Table 2 displays the characteristics of the animals included in the study. The body weight was similar in all groups with an average range of 393g to 401g. The infarct size between the ExMI and the SedMI rats was comparable. The total heart weight and the ratio of heart weight to body weight between the two MI groups were also similar, but significantly higher than their sham-operated counterparts ($P < 0.05$). Yet, LVSP was significantly higher in the ExMI group than in the SedMI group ($P < 0.05$).

The echocardiographic measurement showed that the thickness of the anterior wall was similar between the ExMI and the SedMI groups (Figure 1A). Both LVEDd and LVESd in the ExMI and SedMI groups were significantly longer than in the Sham group at both week 1 and week 9 post-MI ($P < 0.001$) (Table 3). Nevertheless, LVESd in the ExMI group at week 9 post-MI was significantly shorter than in the SedMI group ($P < 0.05$). Furthermore, both FS and EF were well-maintained in the ExMI group after 8 weeks of exercise training, and deteriorated in the SedMI group over the same period of time (Figure 1B and C).

3.2. Effect of exercise training on collagen content

Figure 2 displays examples of PSR stained noninfarcted LV sections under brightfield and polarized light, as well as statistical analysis of PSR staining as the total collagen volume fraction. PSR-stained LV cross-sections showed that the collagen volume fraction in SedMI group was significantly ($P < 0.001$) higher than both the Sham and the ExMI group.

3.3. Macrophage infiltration and myofibroblast accumulation

As shown in Figure 3, the number of infiltrated macrophages was increased in the infarcted border area of the heart after MI, but did not differ between the SedMI and ExMI groups. The number of α -SMA-positive myofibroblasts was similar between the two MI groups. In the Sham group, positive α -SMA staining was less extensive and mainly limited to the subendocardium and vessel walls (data not shown).

3.4. Effects of exercise training on type I procollagen, type III procollagen, MMP-1, TIMP-1, TGF- β 1, AT1 receptor and ACE gene expressions

As seen in Figure 4, type I and type III procollagen mRNA did not reveal a significant difference between the ExMI group and the SedMI group. We were unable to detect measurable levels of MMP-1 mRNA in all the three groups. Interestingly, compared to the Sham group, TIMP-1 mRNA levels in the SedMI group increased robustly, whereas in the ExMI group the TIMP-1 mRNA levels were significantly lower ($P < 0.05$) in relation to the SedMI group levels. There were no significant differences in both AT1 receptor and TGF- β 1 mRNA levels between the ExMI group and the SedMI group. Additionally, ACE mRNA was markedly increased in the SedMI group compared to the ExMI group ($P < 0.05$).

3.5. Effects of exercise training on type I collagen, type III collagen, MMP-1, TIMP-1 and TGF- β 1 protein expressions

We did not find any significant differences in the expressions of type I collagen, type III collagen, TGF- β 1 or MMP-1 among the three groups (Figure 5 and 6). Consistent with their mRNA levels, TIMP-1 protein in the ExMI group was significantly lower than in the SedMI group ($P<0.05$) (Figure 6). Furthermore, the ratio of MMP-1/TIMP-1 in the ExMI group was similar to that of the Sham group, but was significantly higher than in the SedMI group ($P<0.05$). As shown in Figure 5B, the level of the AT1 receptor protein in the surviving LV myocardium of the SedMI rats was significantly higher than the ExMI rats ($P<0.05$).

3.6. ACE binding

As illustrated in Figure 7, ACE binding density is low in the sham-operated heart. In the infarcted rat heart, ACE binding is markedly increased at the site of MI, septum, non-infarcted LV, and right ventricle. Compared to the SedMI group, ACE binding density was significantly decreased at the site of noninfarcted LV and septum in the ExMI group ($P<0.05$). There was no significant difference at the site of MI between the two infarcted groups.

4. Discussion

The present study is centered on five major findings: first, exercise training significantly attenuated the expression of TIMP-1 at both gene and protein levels and improved the balance between MMP-1 and TIMP-1; second, exercise training attenuated the expression of AT1 receptor protein; third, exercise training reduced the ACE mRNA expression and ACE binding; fourth, exercise training significantly decreased collagen content and mitigated cardiac fibrosis; fifth, exercise training significantly preserved cardiac function.

Exercise training after MI has been controversial as it may aggravate infarct expansion [24]. However, some studies have demonstrated significant peripheral benefits from post-MI exercise training [25,26]. Orenstein *et al* [14] reported that exercise training increased noninfarct wall thickness, attenuate LV cavity and improve the adverse remodeling process by attenuating ventricular dilation and reducing wall tension. Likewise, a study by Jain and colleagues [15] showed that exercise training increased LV-developed pressure in both untreated and losartan treated MI hearts. However, in their study, exercise resulted in additional scar thinning in the untreated MI hearts, while no additional scar thinning was observed in rats receiving both losartan and exercise treatments. In our study, the thickness of the anterior wall was similar between the ExMI and SedMI groups. LVSP was significantly higher in the ExMI group than in the SedMI group. Both FS and EF were well maintained in the ExMI group after 8 weeks of exercise training, and deteriorated in the SedMI group over the same period of time. Thus, our data illustrate that early exercise training after MI preserves LV function without additional scar thinning.

The major structural proteins of the myocardial ECM are fibrillar type I and type III collagen, but the degree of stiffness in the myocardium is mainly determined by type I collagen. The accumulation of fibrillar collagen in the extracellular space is not only responsible for cardiac stiffness, but also for the disruption of electrical and mechanical properties of the heart. In the present study, collagen volume fraction was significantly lower in the ExMI group than in the SedMI group. This finding suggests that early exercise training may attenuate myocardial fibrosis in the infarcted heart.

MMPs and TIMPs regulate the ECM turnover and contribute to myocardial remodeling after MI. A hallmark of early cardiac remodeling post-MI is the expansion of the infarcted area as a result of the degradation of ECM molecules caused by an imbalance in the ratio of MMPs

and TIMPs [27]. Early amplification of MMP-1 synthesis and/or decrease of TIMP-1 in the infarcted heart coincides with collagen degradation in the necrotic myocardium, whereas the subsequent decrease of MMP-1 and/or increase of TIMP-1 in the infarcted heart might contribute to collagen accumulation at the late phase of post-MI remodeling [28]. Jayasankar and colleagues [9] demonstrated that the inhibition of MMPs was associated with significantly ameliorated ventricular geometry after MI in rats. In the present study, we did not find any detectable MMP-1 mRNA expression, which is consistent with the investigation by Li *et al* [29]. Additionally, we did not find any difference at the protein production level of MMP-1 among the three experimental groups at week nine post-MI, which is accordance with our previous study [30]. We speculate that MMP-1 expression is probably augmented temporarily after MI and returns to normal level thereafter. Webb and colleagues [31] demonstrated that TIMP-1 levels were higher at day 1 post-MI, and remained elevated through day 180 in patient with MI. Likewise, the present study demonstrated that TIMP-1 level increased significantly in the SedMI rats after 9 weeks post-MI. To our knowledge, the current study is the first to demonstrate that post-MI exercise training notably attenuates TIMP-1 expression at both gene and protein levels and subsequently improves MMP-1/TIMP-1 ratio. We speculate that these changes may lead to enhanced proteolytic activity, attenuate excessive LV fibrosis and cardiac stiffness, and thereby preserve cardiac function.

Macrophages are beneficial in the early stage of infarct healing but deleterious during the late phase of scar formation and LV remodeling [32]. In the present study, our data showed that the number of macrophages in the hearts of both the ExMI and SedMI groups were similar at week nine after MI. This finding suggests that exercise training did not exert any significant effect on the number of macrophages. Virag *et al* [33] reported that myofibroblast proliferation peaks day 4 post-MI and then declines progressively till week 4 post-MI. Accordingly, we did not detect any differences in myofibroblast among the three experimental groups. Presumably, myofibroblasts have disappeared due to apoptosis at week 9 post-MI since they express smooth muscle α -SMA transiently when activated during wound healing [34]. TGF- β 1 is well known as an important regulator of tissue fibrogenesis. The activation of TGF- β 1 leads to LV remodeling and failure after MI [35]. In our study, the expression of TGF- β 1 is similar in both the ExMI and SedMI groups 9 weeks after MI. This result indicates that exercise training does not affect cardiac TGF- β 1 expression.

Angiotensin II (Ang II) is one of the key factors regulating cardiac remodeling following MI. AT1-receptor appears to mediate many of the deleterious effects of chronic renin-angiotensin-aldosterone system activity. AT1 receptor blockade reduces LV collagen accumulation, decreases mortality, and attenuates overall LV remodeling [36] [15,37]. Interestingly, our data provides the first demonstration that exercise training significantly decreased the expression of AT1 receptor at protein levels 9 weeks after MI. Moreover, both ACE mRNA expression and ACE binding density in the noninfarcted LV is noticeably decreased in the ExMI group compared to the SedMI group. Accordingly, another possible mechanism for the beneficial effects of post-MI exercise training on myocardial remodeling and function is through the attenuation of ACE and AT1 receptor expressions.

Although the exact mechanism of post-MI exercise training-induced beneficial effects on myocardial remodeling is not fully understood, several studies [38–41] suggest that the effect of exercise training may be due to the increased baroreflex sensitivity, reduced sympathetic activity, and enhanced vagal tone. In animal studies [38,42], exercise training restored baroreflex sensitivity and reduced renal sympathetic nerve activity (RSNA) in rabbits with pacing-induced heart failure. The extent of augmented baroreflex and attenuated RSNA were well correlated with the reduction in plasma AngII level. Another mechanism is associated with the excitatory effects of circulating AngII. Plasma AngII was decreased after exercise training, which positively correlated with sympathetic nerve activity and conversely correlated

with arterial baroreflex function [38,43]. Furthermore, a reduction in circulating AngII by exercise training may act favorably on baroreflex control of sympathetic activity [44,45]. In addition, based on our study, it is conceivable to speculate that exercise-induced decrease in TIMP-1, ACE, and AT1 receptor expressions may contribute to the beneficial effects on cardiac remodeling and function.

In summary, this study demonstrates that early exercise training after MI is likely playing an important role in cardiac remodeling by attenuating the TIMP-1 expression, improving the balance between MMP-1 and TIMP-1, decreasing ACE and AT1 receptor expressions, and thereby decreasing the collagen content. These improvements, in turn, attenuate myocardial fibrosis and cardiac stiffness, and preserve post-MI cardiac function. Our data suggest that early exercise training is a positive clinically relevant option in post-MI rehabilitation.

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Figure 1. The results of echocardiographic measurements. A. Thickness of the anterior wall; B. Fractional shortening; C. Ejection fraction. PreDia, PreSys: the end-diastolic and systolic thickness of the anterior wall before exercise training; postDia, postSys: the end-diastolic and systolic thickness of the anterior wall after exercise training. ExMI (n=8), SedMI (n=8), Sham (n=8). Data are expressed as mean $\pm$ S.E.M. ** $P < 0.001$ vs the Sham group; ‡ $P < 0.05$ vs the ExMI group; ‡ $P < 0.001$ vs the ExMI group; # $P < 0.05$ vs corresponding baseline (pre), § $P < 0.001$ vs corresponding baseline.

Figure 2. Representative of Picrosirius red stained ventricular sections (A–C) and statistical analysis (D). Arrows indicate the collagen under brightfield (left) and polarized light (right). A. ExMI (n=8); B: SedMI (n=8); C: Sham (n=8). * $P < 0.05$, ** $P < 0.001$ vs the Sham group; ‡ $P < 0.001$ vs the ExMI group.

Figure 3.

Immunohistochemical results of myofibroblast proliferation and macrophages infiltration. Arrows indicate positive immunohistochemical staining for CD68 (A and B) and α -SMA (C and D) for hearts with exercise training (A, C) and sedentary MI groups (B, D). ExMI (n=8), SedMI (n=8), Sham (n=8). Original magnification $\times 400$. (E) Statistical analysis of macrophages. (F) Statistical analysis of myofibroblasts. MyoFb, myofibroblasts.

Figure 4.

Gene expression by real-time PCR. Data are expressed as ratios of target genes (type I procollagen, type III procollagen, TIMP-1, TGF- β 1, AT1 and ACE) to β -actin. ExMI (n=8), SedMI (n=8), Sham (n=8). [#] $P < 0.05$ vs the ExMI group.

Figure 5. The results of western blot for type I collagen, type III collagen, TGF-β1 (A) and AT1 receptor (B). The upper panel is a representative western blot. ExMI (n=8), SedMI (n=8), Sham (n=8). * $P < 0.05$ vs the sham group; # $P < 0.05$ vs the ExMI group.

Figure 6.

The results of western blot for MMP-1 and TIMP-1 (A) and the ratio of MMP-1/TIMP-1 (B). The upper panel is a representative western blot. ExMI (n=8), SedMI (n=8), Sham (n=8).

* $P < 0.05$ vs the Sham group; # $P < 0.05$ vs the ExMI group.

Figure 7. Autoradiographic ACE binding in the rat heart. LV, noninfarcted left ventricle; S, septum; RV, right ventricle; MI, myocardial infarction zone. ExMI (n=8), SedMI (n=8), Sham (n=8). * $P < 0.05$ vs the Sham group; ** $P < 0.001$ vs the Sham group; † $P < 0.05$ vs the ExMI group.

Table 1
Oligonucleotide primers used for real-time RT-PCR

Gene	Assay ID	Reference Sequence [*]	Amplicon Length	Exon Boundary
type III procollagen	Rn01437681_m1	NM_032085.1	71	48–49
TGF- β 1	Rn99999016_m1	NM_021578.1	76	4–5
TIMP-1	Rn00587558_m1	NM_053819.1	91	2–3
AT1	Rn00578456_m1	M74054.1	98	2–3
ACE	Rn00561094_m1	NM_012544.1	90	15–16
β -actin	Rn00667869_m1	NM_031144.2	91	4–5

* Accession numbers in GenBank for the sequence used in designing the primers.

Table 2*In-vivo* hemodynamics and general characteristics

Group	Sham (n=8)	ExMI (n=8)	SedMI (n=8)
Infarct size (%)	-	40.9±1.52	42.8±2.72
BW (g)	393±12	401±6	392±9
Ht Wt (g)	1.3±0.04 [*]	1.5±0.06	1.5±0.10
Ht Wt (g)/BW (kg)	3.4±0.06 [*]	3.8±0.12	3.9±0.18
LVSP (mmHg)	106±2.7	102±1.9 [#]	95±3.6 [†]
LVEDP (mmHg)	5±1	8±1	9±2 [†]
LV +dP/dt _{max} (mmHg/s)	6272±311	5278±203 [†]	4871±365 [‡]
LV -dP/dt _{max} (mmHg/s)	6246±461	5168±458	4205±406 [†]
Systolic aortic pressure (mmHg)	112±1.3 [*]	105±2.5	104±3.2
Diastolic aortic pressure (mmHg)	85±1.0 [*]	78±1.9	77±2.8

Values are expressed as mean ± S.E.M. BW, body weight at sacrificed; Ht Wt, heart weight; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, peak velocities of contraction and relaxation.

* $P < 0.05$ compared with the SedMI group or the ExMI group;

[†] $P < 0.05$ compared with the Sham group

[‡] $P < 0.01$ compared with the Sham group.

[#] $P < 0.05$ compared with the SedMI group.

Table 3

Doppler echocardiographic assessment of left ventricular geometry

	Sham (n=8)	ExMI (n=8)	SedMI (n=8)
1wk post-MI			
LVEDd (mm)	6.65±0.15	8.99±0.12 ^{**}	8.96±0.18 ^{**}
LVESd (mm)	3.75±0.18	7.45±0.16 ^{**}	7.38±0.26 ^{**}
9 wk post-MI			
LVEDd (mm)	7.45±0.20	11.37±0.15 ^{**}	11.39±0.19 ^{**}
LVESd (mm)	4.29±0.18	9.44±0.16 ^{**†}	10.10±0.25 ^{**}

LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension.

^{**} $P < 0.001$ compared with the Sham group

[†] $P < 0.05$ compared with the SedMI group.