Exercise training combined with angiotensin II receptor blockade limits post-infarct ventricular remodelling in rats

Xiaohua Xu1, Wenhan Wan1, Lisa Ji2, Shunhua Lao1, Anthony S. Powers1, Weiyuan Zhao1, John M. Erikson3, and John Q. Zhang1*

1Laboratory of Cardiovascular Research, University of Texas at San Antonio, 1 UTSA Circle, San Antonio, TX 78249, USA; 2Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA; and 3Division of Cardiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

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Aims Our aim was to test the hypothesis that angiotensin II receptor blockade combined with exercise training after myocardial infarction (MI) could attenuate post-MI left ventricular remodelling and preserve cardiac function.

Methods and results Sprague-Dawley rats underwent ligation of the left descending coronary artery, resulting in MI, or a sham operation. Losartan treatment and exercise training were initiated 1 week after infarction and continued for 8 weeks, either as a single intervention or combined. Collagen volume fraction in the sedentary MI (MISed) group was significantly higher than other MI groups treated with exercise training and/or losartan. Compared with MISed group, hearts of rats receiving exercise and/or losartan treatment had lower tissue inhibitor of matrix metalloproteinase (TIMP) 1. Matrix metalloproteinase (MMP) 2 or MMP-9 did not differ among all groups. Additionally, the level of angiotensin II receptor type 1 (AT1) protein significantly decreased in response to exercise training. Furthermore, angiotensin converting enzyme (ACE) binding was markedly lower in hearts receiving exercise training than in the MISed hearts. Cardiac function was preserved in rats receiving exercise training, and the beneficial effect was further improved by exercise combined with losartan treatment in comparison to the MISed group.

Conclusion Our results suggest that post-MI exercise training and/or AngII receptor blockade reduces TIMP-1 expression and mitigates the expressions of ACE and AT1 receptor. These improvements, in turn, attenuate myocardial fibrosis and preserve post-MI cardiac function.

KEYWORDS
Myocardial infarction; Remodelling; Exercise; Angiotensin; Metalloproteinases

1. Introduction
Myocardial infarction (MI) results in structural and molecular alterations to both cardiac myocytes and the extracellular matrix (ECM).1 Left ventricular (LV) remodelling after MI originates as an adaptive process when the heart attempts to compensate for acute loss in contractile function. Over time, however, LV remodelling becomes maladaptive and a risk for the development of congestive heart failure (CHF).2

The cardiac renin-angiotensin system (RAS) is activated during the process of post-MI remodelling.3 Local generation of angiotensin II (AngII) and Ang II receptors have been reported to be increased in the infarcted hearts.3,4 In addition, many studies have demonstrated that inhibition of cardiac RAS with angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) improves LV function, prevents geometric remodelling, and prolongs survival, suggesting that AngII plays an important role in post-MI remodelling.5–8

The dynamic synthesis and breakdown of ECM proteins play a pivot role in post-MI LV remodelling. Matrix metalloproteinases (MMPs) are a family of extracellular proteases that are responsible for the ECM degradation during tissue remodelling under normal and pathological conditions, whereas MMP activity is tightly controlled by the endogenous tissue inhibitors of metalloproteinases (TIMPs).9–12 In particular, the imbalance between MMP and TIMP expression is associated with myocardial matrix collagen disruption and cardiac remodelling.13,14 Four types of TIMPs have been identified. Among these, TIMP-1 is produced by different cell types, including most types of connective tissue cells and those involved in the inflammatory processes. It has been demonstrated that AngII increases TIMP-1 expression
in rat heart endothelial cells and rat aortic smooth muscle cells, whereas TIMP-1 inhibits MMPs.  

Exercise training is emerging as an important complementary intervention in heart failure. Previous studies have demonstrated that exercise enhances aerobic capacity, attenuates LV dilation, regresses cellular hypertrophy, and improves cardiomyocyte contractility and myofilament function. These beneficial effects may be, in part, due to the exercise training-induced attenuation of RAS since it is well known that inhibition of RAS improves cardiac remodelling. Indeed, exercise training not only normalized circulating renin-angiotensin-aldosterone system in animals with MI, but also lowered plasma AngII in patients with heart failure. However, the impact of exercise training on factors contributing to myocardial fibrosis has not been elucidated. It seems plausible to hypothesize that the exercise training-induced improvement of cardiac function is due to the ameliorated myocardial fibrosis and remodelling. Therefore, the present study was designed to assess the effect of combination of AngII blockade and exercise training on myocardial remodelling and function after myocardial infarction. We investigated the changes in MMP-2, MMP-9, TIMP-1, ACE, and AT1 at both gene and protein levels after MI. Our aim was to test the hypothesis that exercise training would beneficially improve factors involving in post-MI fibrosis, reduce collagen content, thereby attenuating deleterious cardiac remodelling and preserving cardiac function. Such effects could be potentiated by addition of angiotensin II type 1 (AT1) receptor blocker, losartan, in conjunction with exercise training.

2. Methods

2.1 Animals and myocardial infarction

Animals used in these experiments were treated in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the study protocols were approved by the Institutional Animal Care and Use Committee at University of Texas at San Antonio. Seven-week-old male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA) were housed at constant temperature (22 ± 2 °C) on a 12 h light/dark cycle. They were fed ad libitum on standard laboratory rat chow and had free access to tap water. MI was made by ligation of the left anterior descending coronary artery as described previously.

2.2 Experimental groups

Echocardiography was performed on the surviving rats 1 week after surgery. Rats were matched by cardiac functions using echocardiography and randomly assigned to the following experimental groups: Sham-operated control (Sham), sedentary MI (MISed), MI plus exercise (MI + Ex), MI plus losartan (MISed + Los), and MI plus exercise and losartan (MI + Ex + Los).

2.3 Drug treatment and exercise training

Losartan treatment (20 mg/kg/day) was initiated 1 week post-MI. The determination of losartan dosage was based on the previous studies, which demonstrated positive effect in improving cardiac function and attenuating cardiac hypertrophy without symptoms of side effect. The drug administration was performed via gastric gavage twice a day for 8 weeks. Tap water was also given by gastric gavage to rats without losartan treatment to avoid the possible physiological alterations associated with gavage-induced stress. Rats assigned to the exercise groups started exercising at 1 week post-MI using a motorized rodent treadmill (Figure 1), whereas the sham and sedentary groups remained sedentary throughout the experiment period. To allow gradual adaptation to exercise stress, exercise 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. Exercise training was performed 5 days per week for 8 weeks.

2.4 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 (SonnoHeart Elite, SonoSite Bothell, WA, USA). Rats were anaesthetized with 2% isoflurane mixed with oxygen, and a two-dimensional short-axis view of the LV was obtained at the level of the papillary muscle to record M-mode tracings. We measured LV anterior wall thickness; LV end-diastolic dimension (LVEDd) and end-systolic LV dimension (LVESd); LV endocardial fractional shortening (FS) was calculated as (LVEDd – LVESd)/LVEDd and was expressed as a percentage. All measurements were averaged over three consecutive cardiac cycles.

2.5 In vivo haemodynamic measurements

Following 8 weeks of exercise training, all rats were anaesthetized as described in the previous section, and the right carotid artery was exposed. A pressure transducer (Model SPR-838, Millar Instruments) was inserted retrograde from the carotid artery to LV cavity. Haemodynamic parameters measured were LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), aortic systolic pressure, aortic diastolic pressure, and peak velocities of contraction and relaxation (dp/dtmax). After measuring, hearts were harvested, frozen in isopentane with dry ice, and stored at −80 °C until use.

2.6 Determination of infarct size and collagen content

Cryostat sections (6 μm) of the hearts were stained with collagen-specific picrosiriusred (PSR) for fibrillar collagen measurements at
four levels from the apex to the base. Infarct size was calculated by dividing the sum of the planimetered endocardial and epicardial circular circumferences of the infarcted area by the sum of the total epicardial and endocardial circumferences of the LV.28 Total epicardial and endocardial lengths occupied by the infarct as identified by PSR staining were measured using computer software (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA). For collagen volume measurement, the PSR stained cross-sections were imaged using polarized light which causes clear demarcation of the fibrillar collagen.29 The LV cross-sections were digitized using a 20× objective in the non-infarcted myocardium. Collagen volume in the non-infarcted myocardium was measured using Image Pro Plus program.

2.10 Statistical analyses
One-way analyses of variance (ANOVA) were carried out to determine whether there were significant mean differences among the experiment groups followed by Student-Newman-Keuls post hoc comparisons. A P-value of <0.05 was considered statistically significant. Values are expressed as mean ± SEM.

3. Results

3.1 General characteristics and post-infarct survival
The exercise training regimen was well tolerated by rats of both the MI + Ex and MI + Ex + Los groups. MI was associated with ~50% mortality during the first 48 h post-MI. All the animals used in our study survived the post-MI training period for 8 weeks accompanied by twice a day gastric gavage. Table 2 presents the characteristics of animals included in the studies. Only animals with an infarct size of more than 30% were included for further analysis. Thus, the population of the five groups was as follows: Sham (n = 10), MI (n = 9), MI + Ex (n = 10), MI + Ex + Los (n = 12), and MI + Ex + Los (n = 13). The infarct size was similar in all MI groups with an average range of 40.80–41.67% (Table 2). There was no significant difference for the body weight among the experimental groups (P > 0.05). The ratio of heart weight to body weight was significantly higher in the MI + Ex (P < 0.05) and MI + Ex + Los group compared with the Sham group, whereas the ratio was markedly decreased in the MI + Ex + Los group in comparison to both the MI + Ex (P < 0.05) and MI + Ex + Los group.

3.2 Echocardiography
Figure 2 shows the typical M-mode images of all groups. There is no significant difference among the MI groups in the anterior wall thickness either in systole (AWST) or in diastole (AWDT) at week 1 or week 9 post-MI (Table 3). As shown in Table 3, the posterior wall thickness in systole (PWST) and diastole (PWT) were similar in all MI groups. Nevertheless, exercise training significantly reduced LVESD compared with the sedentary infarcted group (P < 0.05), and this effect was amplified when exercise training combined with losartan treatment. Furthermore, exercise training significantly preserved FS compared with the sedentary MI rats (P < 0.05), whereas exercise training with losartan treatment showed a significant increased FS when comparing with the MI + Ex group (P < 0.05). These data revealed that exercise training...
appreciably preserved cardiac function after MI without causing scar thinning.

3.3 Haemodynamics

As shown in Table 2, MI caused a significant decrease in LVSP and dP/dt max, whereas exercise training and losartan treatment could maintain them at a higher level compared with the MISed hearts (P < 0.05). Both systolic aortic pressure and diastolic aortic pressure were significantly higher in the Sham group than in the MI groups (P < 0.05). Although exercise alone to some extent reduced LVDP in comparison to the MISed group, the most pronounced effect occurred when exercise training was combined with losartan treatment (P < 0.05). These results indicate that both systolic and diastolic functions were well preserved in the MI + Ex group after MI.

3.4 Collagen content in the non-infarcted LV myocardium

Figure 5 shows the representative examples of PSR stained non-infarcted LV sections under polarized light, as well as the statistical analysis of PSR staining as the total collagen volume fraction. The results showed that infarction caused an increase in cardiac fibrosis in the LV non-infarcted myocardium compared with the Sham group. Furthermore, the collagen volume fraction in the MISed group was significantly higher than other infarcted groups (P < 0.05). These data revealed that either exercise training or losartan treatment
attenuated post-MI myocardial fibrosis, and the combined treatment of exercise and losartan consolidates the effect.

3.5 Gene expressions in the non-infarcted left ventricular myocardium

As seen in Figure 4, we did not find any significant difference in the mRNA level of MMP-2, MMP-9, or AT1 among the infarcted groups. Interestingly, TIMP-1 mRNA levels decreased robustly in the MI + Ex, MI + Ex + Los and MISed + Los groups compared with the MISed group ($P < 0.05$). This indicates that both exercise training and losartan treatment lower TIMP-1 expression after 8 weeks of exercise training or losartan treatment. In addition, Figure 4 also showed that ACE expression was significantly lower in the MI + Ex group than in the MISed group ($P < 0.05$), whereas the expression significantly increased in response to losartan treatment compared with their corresponding groups ($P < 0.05$).

3.6 Protein expressions in the non-infarcted left ventricular myocardium

As shown in Figure 3, we did not find any significant difference in the protein expressions of MMP-2 or MMP-9 among all the experimental groups. Consistent with mRNA levels, there was significantly less TIMP-1 protein expression in the MI + Ex, MI + Ex + Los, and MISed + Los groups compared with the MISed group ($P < 0.05$). Thus, the results indicated that both exercise training and losartan treatment had TIMP-1 lowering effect. In addition, the expressions of AT1 receptor protein in the MISed and the MISed + Los groups were significantly higher than the Sham group ($P < 0.05$), whereas the expressions in the MI + Ex and MI + Ex + Los groups were significantly lower than in the MISed and MISed + Los groups ($P < 0.05$).

3.7 Angiotensin converting enzyme binding

As illustrated in Figure 6, ACE binding density is low in the sham-operated heart, whereas in the infarcted rat heart, it is tremendously increased ($P < 0.05$) at the site of MI, septum, non-infarcted LV, and right ventricle. When compared with other infarcted groups, ACE binding density in the MISed group was significantly increased in the non-infarcted LV and septum ($P < 0.05$). In addition, ACE binding density markedly higher in RV in the MISed + Los group than in the MI + Ex + Los group ($P < 0.05$). There was no significant difference in ACE binding density at the site of MI among the four infarcted groups.

4. Discussion

In the present study, we demonstrated that exercise training after MI provides beneficial effect on post-MI cardiac function and LV remodelling by altering the gene and protein expressions that regulate myocardial fibrosis, whereas such effects only slightly improved by the combination of exercise and losartan. These data provide further insights into the mechanisms underlying the improvement in morbidity and mortality produced by exercise training and ARB in patients with MI. There are five major findings in the current study. First, both ARB and exercise training significantly attenuated the expression of TIMP-1 at both gene and protein levels 9 weeks after MI. Secondly, exercise training after MI attenuated the expression of AT1 receptor protein. Thirdly, exercise training decreased the ACE binding. Fourthly, both ARB and exercise training after MI significantly decreased collagen content and mitigated cardiac fibrosis. Fifthly, exercise training with or without losartan significantly preserved cardiac function.

Previous studies have demonstrated significant benefits from exercise training post-MI \cite{31,32} Orenstein et al. \cite{33}
Figure 3  The results of western blot for TIMP-1, MMP-2, MMP-9, and AT1 receptor. The upper panel (A) shows a representative western blot and the lower panel (B) is statistical analysis. Data are expressed as mean ± SEM. *P < 0.05 vs. sham group; †P < 0.05 vs. MI + Ex group; ‡P < 0.05 vs. MISed group.

Figure 4  Real-time PCR for the expression of target genes (TIMP-1, MMP-2, MMP-9, AT1, and ACE) mRNA. The expression was normalized as a ratio using β-actin as a housekeeping gene. Data are expressed as mean ± SEM. *P < 0.05 vs. other MI groups; †P < 0.05 vs. MI + Ex group; ‡P < 0.01 vs. other MI groups.
reported that exercise increased non-infarct wall thickness, attenuated LV cavity, and improved the adverse remodelling process by attenuating ventricular dilation and reducing wall tension. Likewise, a study by Jain et al. showed that exercise training increased LV-developed pressure in both untreated and losartan-treated MI hearts. In the same study, these investigators also showed that exercise training resulted in additional scar thinning in the untreated MI hearts, whereas no additional scar thinning was observed in the post-infarct hearts receiving both losartan and exercise treatments. Yet, in the present study, the anterior scar thickness was similar in all the infarcted groups, indicating that there was no additional scar thinning caused by exercise training. The deviation from the previous exercise study may be due to the different exercise protocols. First, in our study, the rats began exercise session with 5 min of warm-up. Accumulating evidence has demonstrated that warm-up exercise improves endurance performance and attenuates myocardial ischaemia, as well as favours a better adaptation to metabolic demand, particularly in patients with coronary artery disease. Secondly, the exercise intensity and duration used in the present study were less strenuous than the ones used by Jain et al. (16 m/min, 50 min/session vs. 25 m/min, 60 min/session). The intense exercise regimen may induce excessive tension on the injured cardiac wall.
Our study showed that LVSP was markedly higher in the MI + Ex and MI + Ex + Los groups, whereas LVEDP was notably lower in the MI + Ex + Los group compared with that in the MISed group. FS was well maintained in the MI + Ex group after 8 weeks of exercise training and deteriorated in the MISed group over the same period of time. Furthermore, the beneficial effect was further improved by the joined treatment of exercise training with losartan. In addition, heart to body weight ratio in the MI + Ex + Los group was lower than both the MISed and MI + Ex groups. Thus, these data indicate that exercise training after MI significantly preserves LV function without additional scar thinning in this study, and AngII receptor blockade in conjunction with exercise training might make the latter more beneficial.

The ECM forms a structural network between adjoining cardiomyocytes, provides the mechanical supports, and maintains the structural alignment throughout the cardiac cycle. However, after MI, alterations in the collagen matrix cause cardiac muscle stiffness and impair myocyte re-lengthening, thus leading to progressive dysfunction and heart failure. In the present study, collagen volume fraction in the MISed group was significantly higher than in both exercise training and losartan treated groups. This finding suggests that exercise training as well as losartan treatment may attenuate myocardial fibrosis in the infarcted heart, which in turn improves the cardiac function.

Matrix turnover is crucial to tissue repair, while MMPs and TIMPs are the key elements involved in matrix degradation and contribute to myocardial remodelling after MI. Increased MMPs expression or decreased TIMPs expression could result in enhanced proteolytic activity and degradation of ECM molecules. Webb et al. demonstrated that TIMP-1 levels were higher at day 1 post-MI and remain substantially elevated through day 180 in patient with MI. The elevated TIMP-1 level may contribute to the accumulation of collagen content in the infarcted heart, leading to myocardial fibrosis. Similarly, the present study revealed that TIMP-1 level increased significantly in the MISed rats after 9 weeks post-MI. Interestingly, for the first time, our study revealed that both exercise training and losartan notably attenuates TIMP-1 expression at both gene and protein levels. However, no significant changes were detected in MMP-2 and MMP-9 among the five experimental groups, suggesting that it was just a basal expression despite exercise training and losartan treatment. Hence, based on our results, we speculate that exercise training and losartan treatment after MI may reduce the TIMP-1 expression, improve the balance between MMPs and TIMPs, and enhance the proteolytic activity 9 weeks post-MI, thus decrease the collagen accumulation, thereby reduce the cardiac stiffness, preserve LVSP and dP/dt max, and reduce LVEDP significantly in the late phase post-MI.

During post-MI remodelling, the circulating RAS is activated to maintain blood volume, blood pressure, and organ perfusion, but it also adversely affects the injured heart. Evidence has shown that RAS not only induces collagen accumulation globally in the infarct heart causing stiffness of myocardium, but also increases salt and water retention and total peripheral resistance, eventually leading to chronic heart failure. Similarly, ACE inhibition is well known to attenuate remodelling after MI, and its beneficial effects have been attributed, in part, to inhibition of an activated RAS. AngII is one of the key factors regulating cardiac remodelling following MI. Although AT1-receptor appears to mediate many of the deleterious effects of chronic RAS activity, attenuating the harmful effects of sustained AngII stimulation can be achieved by direct antagonism of AT receptors. Fraccarollo et al. reported that LV collagen expression and accumulation were comparably reduced by AT1 receptor blockade, thus the reduction of myocardial AT1 receptor expression may contribute to decrease cardiac fibrosis. The possible mechanisms by which losartan influenced LV remodelling post-MI include a reduction in afterload and preload, as well as direct cardiac effects. Losartan causes a reduction in preload, which, in turn, beneficially contributes to the improvement of LV function. Also, through the induction of natriuresis and diuresis, losartan has been shown to reduce in vivo LV diastolic pressure, thereby reducing preload in post-MI hearts and possibly causing some of the beneficial effects of AngII antagonism. In addition, some studies have reported that AT1 antagonists decrease mortality, decrease fibrosis in post-MI hearts to a similar extent as ACE inhibition therapy, and attenuate overall LV remodelling. It is worth noting that our data demonstrate that the expression of AT1 receptor significantly decreased at protein levels in response to exercise training independent of losartan treatment 9 weeks after MI. Furthermore, ACE mRNA expression significantly decreased in the MI + Ex group when compared with MISed group, whereas the expression markedly increased in the losartan treated groups compared with the corresponding untreated groups. The elevated ACE mRNA may be resulted from the tonic negative feedback regulation induced by ARBs. On the other hand, our results also showed that the ACE binding density is largely increased in the infarcted hearts compared with the Sham group, which is consistent with the investigation by Sun et al. However, the ACE binding density is significantly lower in the exercise training groups than in the MISed group. Accordingly, we provide additional mechanisms whereby exercise training may exert beneficial effects on post-MI myocardial remodelling through the attenuation of cardiac renin-angiotensin system. To our knowledge, the present study is the first to demonstrate the effect of post-MI exercise training in conjunction with losartan treatment on cardiac ACE and AT1 receptors.

Although the exact mechanisms of post-MI exercise training-induced beneficial effects on myocardial remodelling are not fully elucidated, several studies have suggested that the effect of exercise training may be due to increased baroreflex sensitivity, reduced sympathetic activity, and enhanced vagal tone. In addition, a reduction in circulating AngII by exercise training may act favourably on baroreflex control of sympathetic activity. Furthermore, early decrease of TIMP-1 in the infarcted heart coincides with collagen degradation in the necrotic myocardium, whereas the subsequent increase of TIMP-1 in the infarcted heart might contribute to collagen accumulation at the late phase of post-MI remodelling. Losartan has been shown to decrease neurohormonal factors, such as AngII, norepinephrine, aldosterone, atrial natriuretic peptide, brain natriuretic peptide (BNP), and ET-1. Therefore, it exerts protective effects on myocardial remodelling by blocking RAS. Although we did not measure the BNP in this study, a significant decrease in plasma BNP along with...
an increase in LV ejection fraction has been observed by Kinugawa et al. in chronic heart failure patients treated with Losartan and subjected to exercise. In addition, based on our study, it is conceivable to speculate that decreased expressions of TIMP-1, ACE, and AT1 receptor are involved in the mechanisms by which Losartan and exercise would attenuate fibrosis and preserve cardiac function.

In summary, this study demonstrates that exercise training and/or AngII receptor blockade after MI is likely playing an important role in cardiac remodelling by attenuating TIMP-1 expression, improving the balance between MMPs and TIMPs, decreasing ACE and AT1 receptor expression, and thereby decreasing the collagen content. These improvements, in turn, attenuate myocardial fibrosis and cardiac stiffness, and preserve post-MI cardiac function. Exercise training and AngII receptor blockade both have strong beneficial effects, although the benefit of combining exercise and AngII receptor blockade appears to be very modest.

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