Simvastatin Improved Matrix Metalloproteinase Mediating Ventricular Remodeling in Rats after Myocardial Infarction

Jinying Zhang[1], Xiang Cheng[2], Yuhua Liao[2], Baojun Lu[1]

1. Department of Cardiology, First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan 450052, China; jyzhang@zzu.edu.cn

2. Institute of Cardiology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei 430022, China

Abstract: Objectives. It was observed that effect of simvastatin on both matrix metalloproteinase (MMP)-2,9 and type I collagen mediating ventricular remodeling in rats after acute myocardial infarction (AMI). **Methods.** The AMI model of rat was made by ligature of left anterior descending coronary artery, and the animals were divided into three groups: simvastatin treatment group (MI-S), myocardial infarction control group (MI-C) and sham group (Sham). The animals were feed four weeks. The mRNA expression of MMP-2 and MMP-9 in noninfarcted zone of left ventricle (LV) were determined by reverse transcription-polymerase chain reaction (RT-PCR), and cardiac type I collagen in noninfarcted zone were measured by immunohistochemistry, cardiac function was determined by echocardiography and hemodynamics analysis. **Results.** After four weeks, mRNA expression of MMP-2 and -9 and type I collagen in LV post-myocardial infarction (MI) groups were more than Sham group ($P<0.05$), and the indices in MI-S group were significantly lowered than those in MI-C group ($P<0.05$), while higher than Sham group ($P<0.05$). Compared with Sham group, hemodynamics analysis showed that left ventricular end-diastolic pressure (LVEDP) significantly increased ($P<0.01$), while systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular systolic pressure (LVSP) and LV pressure $\pm dp/dt_{max}$ significantly decreased ($P<0.05$), and there was no change for heart rate (HR) in MI-C group. Compared with MI-C group, the LVEDP significantly lowered ($P<0.05$), and LV pressure $\pm dp/dt_{max}$ obviously increased ($P<0.05$), while HR, SBP, DBP and LVSP did not alter in MI-S group. Compared with Sham group, echocardiography showed that left ventricular end-diastolic diameter (LVEDd) significantly increased, and both fractional shortening (FS) and ejection fraction (EF) significantly reduced ($P<0.05$, respectively) in MI-C group. Compared with MI-C group, simvastatin significantly decreased LV dilatation and improved LV function ($P<0.05$). **Conclusion.** Simvastatin could attenuate mRNA expression of MMP-2 and MMP-9 in LV 4 weeks after MI-rats, reduced collagen synthesis, and improved cardiac function. [Life Science Journal. 2005;2(1):72-76] (ISSN: 1097-8135).

Keywords: simvastatin; acute myocardial infarction; matrix metalloproteinase-2,9; RT-PCR; immunohistochemistry; hemodynamics; echocardiography; rat

1 Introduction

The extent of primary ischemic necrosis as well as the later effects of distending forces and the cardiac tissue healing process influenced ventricular dilatation after myocardial infarction (MI)[1-3]. The activity and dynamic expression of matrix metalloproteinases (MMPs) may affect lots of the morphological changes that happen after MI at both infracted and peri-infarcted zones[4,5]. MMPs are members of a family of enzymes that degrade specific extracellular matrix (ECM) components; the activity of MMPs is increased in both experimental MI and clinical dilated cardiomyopathy[6]. As extracellular matrix degradation may play an important role in LV remodeling, MMP inhibiting has emerged as a potential therapeutic strategy for patients at risk for development of congestive heart failure. Preliminary data proposed that administration of an MMP inhibitor may decrease left ventricular enlargement in pacing-induced models of congestive heart failure and in spontaneous heart failure in rats[7]. The effects of MMP inhibition in the post-MI period are incompletely understood. The present study evaluated the effects of administration

of oral simvastatin in early left ventricular remodeling as assessed by transthoracic echocardiography and hemodynamics after experimental MI in rats.

2 Materials and Methods

2.1 Infarction model and experimental groups

Wistar rats weighing 200 − 240 g were anesthetized by intraperitoneally injection of sodium pentobarbital (30 mg/kg) intubated, and ventilated with a small-animal respirator. The left anterior descending coronary artery was ligated proximately with a 7 − 0 silk suture after a left anterior thoracotomy. Sham-operated rats underwent the identical procedure without ligation of coronary artery. The following experimental groups were studied: ① Sham-operated (Sham) ($n = 10$); ②MI control (MI-C) ($n = 12$); ③MI simvastatin (MI-S) ($n = 12$). Simvastatin (40 mg/kg body weight) was given by gastric gavage 24 hours after the anesthesia, then continued for 4 weeks (40 mg/kg per day). An equal amount of normal saline was given to the other two groups every day.

2.2 Measurement of MMP-2, 9 mRNA expression by RT-PCR

The infarcted heart was sectioned into noninfarcted zone by visual inspections. Total RNA was reversely transcribed into first-strand cDNA after isolation by using TRIzol reagent. MMP-2, 9 and GAPDH gene expression were analyzed in noninfarcted area. The sense primer (S) and the antisense primer (A) for MMP-2, 9 were as follows:
MMP-2 S, 5′-ACCATCGCCCATCATCAAGT -3′, A, 5′-CGAGCAAAAGCATCATCCAC -3′(348bp-production).
MMP-9 S, 5′-AACTTTGTAGGGTCGGTTCTG-3′, A, 5′-CCCTGTGAGTGGGTTGGATT-3′ (469bp-production).
GAPDH S, 5′-TATGATGACATCAAGAAGGTGG -3′, A 5′-CACCACCCTGTTGCTGTA-3′ (213bp-production).

PCR amplification was performed by adding each cDNA sample 2 μl to 20.5 μl of reaction mixture. Each cycle consisted of denaturation at 94°C for 40 seconds, annealing for 40 seconds (MMP − 2, 9 at 60°C, GAPDH at 56°C), extension at 72°C for 1 min, and final extension at 72°C for 5 min. Each PCR product was separated by electrophoresis on a 1.5% agarose gel and tested by a digital image analysis system (GSD8000, UVP, England). Each amplified cDNA fragment was counted for semiquantitative evaluation by normalization with the GAPDH band.

2.3 Measurement of type I collagen by immunohistochemistry

Paraffin-embedded myocardium specimens in noninfarcted zone were serial sectioned into a thickness of 3 μm. The section was incubated with primary antibodies [anti-rat type I collagen (Monoson) (1 : 100)] which were stored at 4℃ overnight. Incubation with biotinylated second antibody was performed at room temperature for 30 min. Immunoreactivity was evaluated under the microscope using the HPIAS-2000 software.

2.4 Echocardiography and hemodynamics analysis

Two-dimensional echocardiography was performed on each rat before surgery and 4 weeks after surgery with a 10 MHz (short focus) transducer. Long-axis, short-axis, and apical four-chamber images were measured. The LV end-diastolic and end-systolic volumes (EDV and ESV, respectively) were calculated by the modified Simpson's method[8]. Cardiac output was calculated as (EDV-ESV)/1000 × heart rate; the LV ejection fraction (LVEF) was determined as (EDV-ESV)/EDV × 100%. The fractional shortening (FS) was measured at the short-axis image. Hemodynamic studies were performed after the animals were anesthetized with an intraperitoneal injection of 1.0 g/kg urethan. Through the right common carotid artery, a catheter filled with heparin solution was inserted into LV and hemodynamic data recorded.

2.5 Statistic analyses

The data are given as mean ± SD. The multivariate ANOVA was used to determine the overall difference between the three independent groups. The statistical significance between groups was determined using a post hoc Bonferroni/Dunn test. A value of $P<0.05$ was considered significant.

3 Results

3.1 RT-PCR analysis of MMP-2, 9 gene

Compared with Sham operation group, the mRNA expression of MMP-2 and MMP-9 significantly increased in noninfarcted zones of MI-C group ($P<0.05$). MMP-2 and MMP-9 expression were obviously decreased in MI-S group ($P<0.05$), but still higher than those in Sham operation group ($P<0.05$) (Figure 1).

3.2 Immunohistochemistry analysis of type I collagen

Compared with Sham operation group, type I collagen was markedly increased in noninfarcted zone of MI-C group ($P<0.01$). Compared with MI-C group, type I collagen was significantly lowered in MI-S group ($P<0.05$) (Figure 2).

3.3 Hemodynamics analysis

Table 1 displays hemodynamic data at 4 weeks after operation. In the MI group, LV end-diastolic

pressure (LVEDP) significantly increased ($P<0.01$), systolic blood pressure (SBP), diastolic blood pressure (DBP), LV systolic pressure (LVSP) and LV pressure maximal rate of rise and fall ($\pm dp/dt_{max}$) noticeably decreased ($P<0.05$), while heart rate (HR) did not change compared with the Sham operation group. LVEDP decreased ($P<0.05$) and $\pm dp/dt_{max}$ significantly increased ($P<0.05$) in the MI-S group compared with the MI-C group. Other variables were not different between both MI groups.

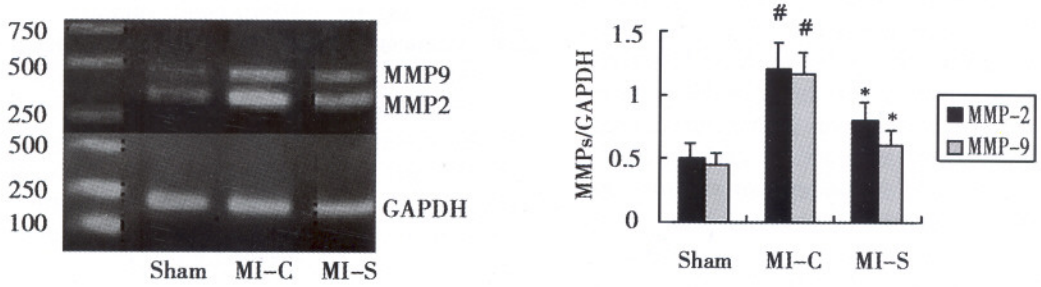

Figure 1. Effect of simvastatin treatment on mRNA expression of MMP-2,9 in Sham group, non-infacted LV myocardium of MI-C group and MI-S group 4 weeks post-MI. Quantitative analyses mRNA of MMP-2,9, * $P<0.01$ vs. Sham group, # $P<0.05$ vs. MI-C group.

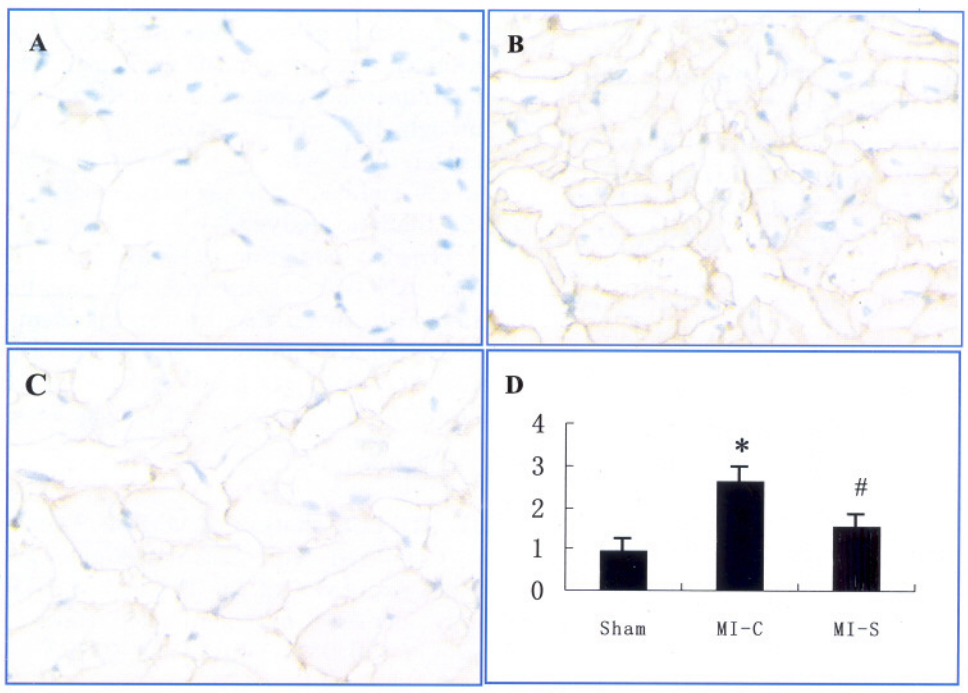

Figure 2. Effect of simvastatin greatment on protein production of type I collagen in Sham group (A) non-infacted LV myocardium of MI-C group; (B) and MI-S group; (C) 4 weeks post-MI (magnification × 200); (D) Quantitative image analyses of type I collagen production, * $P<0.01$ vs. Sham group, # $P<0.05$ vs. MI-C group.

Table 1. The effects of simvastatin treatment on hemodynamics

Groups	*n*	HR (bpm)	SBP (mmHg)	DBP (mmHg)	LVSP (mmHg)	LVEDP (mmHg)	$+dp/dt_{max}$ (mmHg/s)	$-dp/dt_{max}$ (mmHg/s)
Sham	10	361 ± 23	125.7 ± 8.3	99.1 ± 9.0	131.9 ± 8.3	2.2 ± .0.3	6546 ± 631	5477 ± 485
MI − C	12	383 ± 25	109.4 ± 9.1*	89.7 ± 7.8*	113.3 ± 10.5*	22.5 ± 4.7*	4084 ± 449*	2837 ± 251*
MI − S	12	377 ± 19	110.1 ± 9.6*	90.3 ± 7.1*	112.8 ± 11.1*	12.6 ± 1.5*#	4951 ± 381*#	3435 ± 358*#

Compared to Sham group, * $P<0.05$; Compared to MI-C group, # $P<0.05$

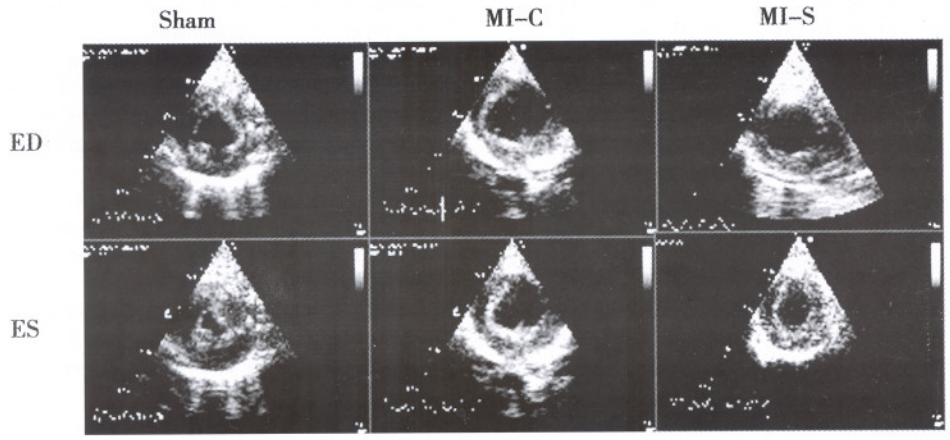

Figure 3. Echocardiographic vews of LV from a sham-operated rat (Sham), an untreated MI rat (MI－C) and a simvastatin-treated MI rat (MI-S) at end-diastolic (ED) and end-systolic (ES) dimension. The MI-S rat has decreased left ventricular diastolic dimensions compared with the untreated MI-C rat.

3.4 Echocardiographic studies

Figure 3 shows representative short-axis echocardiographic images from a Sham-operated animal, an untreated MI rat and a simvastatin-treated MI rat. Compared with Sham operation group, there were significantly increased LVEDd ($P<0.01$), markedly decreased FS ($P<0.01$) and EF ($P<0.01$) in the MI-C group. Although these parameters also differed from the Sham operation group in the MI-S group, simvastatin significantly attenuated LV dilatation and improved LV function compared with the MI-C group ($P<0.05$) (Table 2).

Table 2. The effects of simvastatin treatment on echocardiographic measurements

Groups	n	LVEDd (mm)	EF (%)	FS (%)
Sham	10	4.3±0.2	87.2±3.5	53.4±3.3
MI－C	12	7.8±0.4*	42.1±3.9*	21.2±2.1*
MI－S	12	5.6±0.3#	49.7±4.1#	27.5±2.2#

Compared to Sham group, * $P<0.05$; Compared to MI-C group, # $P<0.05$

4 Discussions

MMPs are a family of zinc-depending endoproteinases that specifically degrade ECM components. They are important enzymes which degrade matrix in the cardiac remodeling process after MI. The activity increase of MMPs leads to reduction of ECM, destruction of cardiac supporting structure and ventricular dilatation[9]. MMPs modulate synthesis of collagen, the rise of MMPs activity enhances fibrosis, and fall of MMPs activity decreases fibrosis[10]. Clinical research confirmed[11] that MMPs were obviously associated with stability of atherosclerotic plaque, the plasm MMPs markedly rose in patients with coronary heart disease, and inhibition of MMPs activity may increase plaque stability. The experimental study proposed that MMPs participated in the remodeling process after MI, and were activated 1 day after MI. Activity and gene expression of MMP-2, 9 markedly increased during remodeling process after MI[12,13], and application of MMPs inhibitors could decrease ventricular dilatation in rats after MI and improve cardiac performance[14－17]. It was further showed that the rat with MMP-9 deficiency existed residual necrosis expansion, the healing process retard after MI[17,18].

Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, which are capable of lowering the serum cholesterol level and are successfully used to treat hypercholesterolemia and atherosclerosis. Moreover, the ability of statins to lower the mortality and morbidity of cardiovascular diseases has been ascribed not only to their cholesterol-lowering activities but also to a number of additional effects, including improving endothelial cell function, enhancing fibrinolysis, and antithrombotic activity. In addition, a number of important anti-inflammatory effects of statins have been reported.

This study confirmed that mRNA expression of MMP-2, 9 was significantly higher in MI rats, and simvastatin could obviously decrease mRNA expression of MMP-2, 9. It was assumed that simvastatin could not only stabilize atherosclerotic plaque, lower the incidence of unstable angina and MI, but also alleviate cardiac fibrosis, restrict development of ventricular remodeling and heart fail-

ure and improve prognosis patient with MI by inhibiting expression of MMPs. However, it is unknown whether statins directly or indirectly inhibited expression of MMPs.

Acknowledgments

National Natural Science Foundation (No. 30370574); Henan Province's Creation Talent Projection of Medical Science & Technology, China (No. 2002116); Zhengzhou University's Scientific Research Development Fund, China (No. 2004021).

Correspondence to:
Jinying Zhang
Department of Cardiology
First Affiliated Hospital,
Zhengzhou University
Zhengzhou, Henan 450052, China
Telephone: 01186-371-6516-9392
01186-135-0383-0283
Email: jyzhang@zzu.edu.cn

References

1. Pfeffer JM, Pfeffer MA, Fletcher PJ, et al. Progressive ventricular remodeling in rat with myocardial infarction. Am J Physiol 1991;260(5 pt 2):H1406 – 14.
2. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. Circulation 1990;81(4):1161 – 72.
3. Rumberger JA. Ventricular dilatation and remodeling after myocardial infarction. Mayo Clin Proc 1994;69(7):664 – 74.
4. Tyagi SC, Ratajska A, Weber KT. Myocardial matrix metalloproteinases: localization and activation. Mol Cell Biochem 1993;126(1):49 – 59.
5. Cleutjens JPM, Kandala JC, Guarda E, et al. Regulation of collagen degradation in the rat myocardium after infarction. J Mol Cell Cardiol 1995;27(6):1281 – 92.
6. 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(17):1708 – 15.
7. Spinale FG, Krombach RS, Coker ML, et al. Matrix metalloproteinase inhibition with congestive heart failure improves left ventricular geometry and pump function. Circulation 1997;96(Suppl I):I – 520.
8. Schiller N B, Shah P N, Crawford M. Recommadations for quantitation of the left ventricle by two-dimensional echocardiography. J Am Soc Echocardiogr 1989;2(5):358 – 67.
9. Spinale FG, Coker ML, Bond BR, et al. Myocardial matrix degradation and metalloproteinase activation in the failing heart: a potential therapeutic target. Cardiovasc Res 2000;46(2):225 – 38.
10. Li YY, Feng YQ, Kadokami T, et al. Modulation of matrix metalloproteinase activities remodels myocardial extracellular matrix in TNF-a transgenic mice. Circulation 1999;100(Suppl):1752.
11. Brown DL, Desai KK, Vakili BA, et al. Clinical and biochemical results of the metalloproteinase inhibition with subantimicrobial doses of doxycycline to prevent acute coronary syndromes (MIDAS) pilot trial. Arterioscler Thromb Vasc Biol 2004;24(4):733 – 8.
12. Romanic AM, Burns-Kurtis CL, Gout B, et al. Matrix metalloproteinase expression in cardiac myocytes following myocardial infarction in the rabbit. Life Sci 2001;68(7):799 – 814.
13. Hojo Y, Ikeda U, Ueno S, et al. Expression of matrix metalloproteinase in patients with acute myocardial infarction. Jpn Circ J 2001;65(2):71 – 5.
14. 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(23):3063 – 70.
15. Villarreal FJ, Griffin M, Omens J. Early short-term treatment with doxycycline modulates postinfarction left ventricular remolding. Circulation 2003;108(12):1487 – 92.
16. Podesser BK, Siwik DA, Eberli FR, et al, ET(A)-receptor blockade prevents matrix metalloproteinase activation late postmyocardial infarction in the rat. Am J Physiol Heart Circ Physiol 2001;280(3):H984 – H991.
17. Heymans S, Luttun A, Nuyens D, et al. Inhibition of plasminogen activators or matrix metalloproteinase prevents cardiac rupture but impaires therapeutic angiogenesis and causes cardiac failure. Nat Med 1999;5(10):1135 – 42.
18. Ducharme A, Frantz S, Aikawa M, et al. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. J Clin Invest 2000;106(1):55 – 62.