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Research paper

Effect of Atorvastatin on Serum MMP-2, MMP-9 and TIMP-1 in Rabbits with Chronic Heart Failure

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Abstract

Objective: To observe the effects of atorvastatin on serum matrix metalloproteinase-2(MMP-2), matrix metalloproteinase-9(MMP-9), and the tissue inhibitor of metalloproteinase-1(TIMP-1) in the development of chronic heart failure. To investigate the role of atorvastatin in the therapy of chronic heart failure and determine its possible mechanism of action. **Methods:** Thirty Japanese Big Ear rabbits were randomly selected and divided into 3 groups: sham-operated group(SO group), heart failure control group(HC group) and heart failure atorvastatin therapy group(HA group), with 6, 12 and 12 animals in the respective groups. Volume overloading was produced in the HC group and HA group animals by creating an aortic insufficiency, induced by damaging the aortic valve with a catheter introduced through the carotid artery. After 14 days, abdominal aorta constriction was performed in order to obtain a pressure overload. Six weeks later rabbits in the HA group were administered atorvastatin 3mg. $Kg^{-1}.d^{-1}$ for 4 weeks, at which time the experiment was terminated. Arterial blood was drawn and serum levels of MMP-2, MMP-9 and TIMP-1 were measured in all groups at the same time using an ELISA method. **Results:** Structural and functional indicators of chronic heart failure(CHF) were seen in both the HC and HA groups, but atorvastatin significantly reduced the observed effects. The serum concentrations of MMP-2, MMP-9 and TIMP-1 were at low levels in all three groups at the start of the study, with no difference between them($P < 0.05$). At the end of 6th week concentrations were significantly increased in the HC and HA groups compared with the SO group($P < 0.05$), but there were no differences between the HC group and HA group($P > 0.05$). The increased concentrations in HC group continued to the end of the experiment, but values in the HA group were all lower than those in the HC group by the end of the experiment($P < 0.05$). **Conclusion:** Serum concentrations of MMP-2, MMP-9 and TIMP-1 increase significantly during the course of CHF, paralleling the pathological progress of CHF. Atorvastatin benefits CHF, and the decreased serum levels of MMP-2, MMP-9 and TIMP-1 may be one of the drug's mechanism of action.

Key words: chronic heart failure; matrix metalloproteinases; statins; animal model

INTRODUCTION

Chronic heart failure(CHF) is a common clinical disease, often the end result of various organic or functional heart diseases. It is one of the main reasons for cardiac death. Ventricular remodeling is a basic mechanism of chronic heat failure[1]. In recent years, it has been concluded that an inflammatory response induced by the cytokine network and degradation of extracellular matrix is the molecular basis of the reconfiguration process. Chronic heart failure is caused by an interaction of a complicated network composed of various inflammatory cytokines and relevant molecules, instead of the action of a few inflammatory factors. It has been called a "Network-based Cooperative Crime"[2]. Thus, a new approach for treating heart failure in recent years has been intervening with the inflammatory reaction.

Among the more commonly used drugs, studies have found statins have the effect of improving the ventricular remodeling. Their effect appears to be related to their ability to inhibit cardiac hypertrophy, interstitial fibrosis, and inflammation, and further studies suggest they improve endothelial function[3]. In mid 1970s, statins were first studied and successfully developed in Japan, and they were subsequently used

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clinically as lipid-lowering drugs. In recent years these 3-hydroxy-3methylglutaryl coenzyme A(HMG-CoA) reductase inhibitors were found to have multiple uses, including having a therapeutic effect in chronic heart failure[4,5]. However, their specific mechanism of action in heart failure remains undefined. It has been shown that matrix metalloproteinases and their inhibitory factor play an important role in ventricular remodeling associated with heart failure. In particular, matrix metalloprotein-ase 2, matrix metalloproteinase 9 and the tissue inhibitor 1 of matrix metalloproteinase have important effects in the course of chronic heart failure[6]. Increasingly, studies at home and abroad have focused on the effect of statins and matrix metalloproteinase on left ventricular remodeling, although these studies are usually only limited to one or two protein factors. In the present report, atorvastatin has been used to intervene in the course of heart failure. Changes in serum MMP-2, MMP-9 and TIMP-1 during the course of experimentally induced heart failure were observed in order to explore the effect of atorvastatin on these factors and provide theoretical support for its use in the treatment of chronic heart failure, and improve prognosis and quality of life in patients with heart failure.

MATERIALS AND METHODS

Experimental animals

Thirty healthy common Japanese Big Ear rabbits of either sex, weighing between 2.5-3.0 Kg were used in the experiments. Animals were provided by the Experimental Animal Center of Nantong University.

Equipment

A BL-420E biological function experimental system, manufactured by Chengdu TME Technology Co, Ltd, was used in all cardiovascular function studies. A HP-SONOS5500 Multi-function Cardiac Colour Ultrasonic Scanner manufactured by Hewlwtt-Pacard Co. USA, was provided by the Echocardiography Room of the First People's Hospital of Nantong, and was used to assess both cardiac morphology and performance indices.

Establishment of the rabbit heart failure model

Thirty rabbits were assigned to two groups at random using a random number table, Six animals were placed in a sham-operated(SO) group and 24 rabbits were assigned to the heart failure model group. Rabbits in the model group were further divided into two groups at random, namely heart failure control(HC) group and heart failure atorvastatin therapy(HA) group, each with 12 rabbits.

In the two model groups 30 mg/kg 3% pentobarbital sodium was injected via marginal ear vein to induce surgical anesthesia. The ECG was recorded using lead Ⅱ and a sterile field was produced in the neck region after shaving, using iodine and alcohol. The right common carotid artery was exposed and a 4F catheter was inserted centrally, sheath and ductus. After fixation the 4F ductus was connected to the biological function experimental system via a pressure transducer. Heart rate (HR), aortic systolic pressure(ASP) and aortic diastolic pressure(ADP) were recorded. The ductus was passed into the left ventricle with the steel wire inducer. A large number of ventricular premature beats and pressure fluctuations were observed. When the recording had stabilized the left ventricular end diastolic pressure (LVEDP) was measured. The ductus was then withdrawn to the aortic valvular orifice and HR, ASP and ADP again measured. If these values gave indication that aortic insufficiency had not been induced the valve was poked 2-3 times forcefully with the ductus. The success criteria used were: aorta pulse pressure(APP) increased by 40% and diastolic pressure decreased by 40%. When the criteria had been attained the sheath was withdrawn and the vessel ligated. Penicillin, 800 000 units per day, was injected intramuscularly for three days for infection prevention. Aortic stenosis was produced in rabbits in the model groups 2 weeks after the aortic valve damage. An incision was made at the left side of the ventral median line, exposing the abdominal aorta above the left renal artery. A 4F or 5F ductus was selected, depending on the aorta circumference, placed parallel to the aorta and ligated with #4 thread. The ductus was used to narrow the aorta by 50%. Penicillin was used for infection prevention as above. The sham-operated(SO) group animals received the same surgery as animals in the model groups, except for the aortic valve damage and stenosis of the abdominal aorta. Echocardiography examination of rabbits in the model groups took place at the end of the 6th week after the experiment This further confirmed the success of the heart failure model by assessing the degree of reflux and the degree of aortic valve damage and the structural and functional changes of left ventricle.

Drug dosage regimen

Atorvastatin tablets were obtained from Pfeizer Pharmaceuticals Ltd. USA. Tablets were ground and the powder mixed with saline and administered by gavage (3 mg,Kg-1. d-1) daily to HA group animals for 4 weeks. SO and HC group animals were administered an equal volume of saline by gavage.

Specimen collection and analysis

Ten ml arterial blood was obtained from the central ear artery from rabbits in each group at the beginning of the experiment, at the end of the 6th week and at the

end of the 10th week of the experiment. After clotting, samples were centrifuged and serum removed and stored at -80℃. Serum MMP-2, MMP-9 and TIMP-1 concentrations were measured using ELISA kits at the end of the experiment, as recommended in the manufacturers instructions. Kits were purchased from Wuhan Zhongxin Company.

Statistical treatment

Statistical analyses of data by were performed using SPSS11.5 statistical software. Data are expressed as mean ± standard deviation values($\bar{x} \pm s$), and one-way ANOVA tests were used to compare means of several groups. Least significant difference values were used to verify at equal variance and Dunnett's T3 tests were used as post hoc test to assess the significance of differences between means. Differences were considered significant when $P < 0.05$.

RESULTS

A total of 30 rabbits were used in these experiments. Six rabbits in the HC group and HA group and 5 rabbits in SO group survived to the end of the experiment. At the end of the sixth week after the experiment and after heart failure modeling was completed, 6 rabbits respectively in the HC group and the HA group survived, all of which were listlessness, less active, anorexic, and unresponsive, and exhibited weight loss, tachypnea and a loss of grooming behavior. Moderate and serious aortic valve regurgitations were shown in the echocardiography examinations. Left ventricular end-diastolic diameter(LVIDd), left ventricular internal diameter at systolic phase(LVIDs), end diastolic interventricle septal diameter(IVSd) and left ventricular posterior wall thickness(LVPwd) of animals in HC group and HA group were significantly larger than that in SO group, $P < 0.05$. Left ventricular ejection fraction (LVEF) and left ventricular short axis fractional shortening(LVFS) in HC group and HA group were significantly less than in the SO group, by one-way ANOVA tests, $P < 0.05$(***Table 1***). From the above results, it is apparent that all of the surviving CHF model animals suffered from typical heart failure. In the echocardiography examination at the end of the 10th week after the experiment, it was apparent that LVIDd, LVIDs, IVSd and LVPwd in the HC group were significantly greater than in the SO group, while LVEF and LVFS in the HC group were significantly reduced compared to SO group animals, $P < 0.05$. When data from the HC and HA groups were compared, ventricular structure and cardiac function indices were significantly different between the two groups, by least significant difference values and Dunnett's T3 tests, $P < 0.05$ (***Table 2***). From the above results, it is apparent that the degree of heart failure in the HA group animals is significantly less than that in the HC group animals and the cardiac function is obviously improved.

Serum MMP-2, MMP-9 and TIMP-1 levels in each group of animals were low at the beginning of the experiment and there were no statistically significant differences among the groups, $P > 0.05$(***Tables 3, 4*** and ***5***). Serum MMP-2, MMP-9 and TIMP-1 levels in HA group and HC group were significantly higher than that in SO group at the end of the 6th week after the experiment($P < 0.05$), while there is no statistically significant differences in their levels when the HC and HA mean values were compared, $P > 0.05$(***Tables 3, 4*** and ***5***). However, by the 10th week the serum MMP-2, MMP-9 and TIMP-1 levels in the HA group were significantly lower than in the HC group, by least significant difference values and Dunnett's T3 tests, $P < 0.05$ (***Tables 3, 4 and 5***). These data indicate that continued atorvastain treatment reduced serum MMP-2, MMP9 and TIMP-1 levels in CHF model animals.

DISCUSSION

Ventricular remodeling is the structural basis for the

Table 1 **Comparison of left ventricular structure index and cardiac function index of each group at the end of the 6th week after the experiment** ($\bar{x} \pm s$)

Group	IVSd(cm)	LVIDd(cm)	LVIDs(cm)	LVPWd(cm)	LVEF(%)	LVFS(%)
SO group(n = 5)	0.2586 ± 0.0202	1.3700 ± 0.1241	0.8106 ± 0.0959	0.2530 ± 0.0184	75.22 ± 1.03	40.96 ± 1.24
HC group(n = 6)	0.3142 ± 0.0150*	2.2117 ± 0.0968*	1.1878 ± 0.0875*	0.2922 ± 0.0299*	45.40 ± 2.36*	21.53 ± 1.04*
HA group(n = 6)	0.3060 ± 0.0143*	2.3733 ± 0.0985*	1.0105 ± 0.4025*	0.3218 ± 0.0214*	43.37 ± 2.78*	24.98 ± 4.35*

*compared with SO group, $P < 0.05$.

Table 2 **Comparison of left ventricular structure index and cardiac function index of each group at the end of the 10th week after the experiment** ($\bar{x} \pm s$)

Group	IVSd(cm)	LVIDd(cm)	LVIDs(cm)	LVPWd(cm)	LVEF(%)	LVFS(%)
SO group(n = 5)	0.2588 ± 0.0170	1.3640 ± 0.1270	0.7868 ± 0.1080	0.2552 ± 0.0197	74.54 ± 1.05	41.22 ± 1.37
HC group(n = 6)	0.3233 ± 0.0116*	2.2917 ± 0.0454*	1.2282 ± 0.0932*	0.3025 ± 0.0270*	45.03 ± 2.10*	22.18 ± 1.18*
HA group(n = 6)	0.2868 ± 0.0101**	1.8372 ± 0.1165**	0.9858 ± 0.0172**	0.2886 ± 0.0096**	65.22 ± 2.02**	32.12 ± 1.04**

*-compared with SO group, $P < 0.05$; **-compared with HC group, $P < 0.05$.

Table 3 **Comparison of serum MMP-2 levels at different time of each group in the experiment**

(ng/ml, $\bar{x} \pm s$)

Group	Beginning of experiment	End of 6th week after experiment	End of 10th week after experiment
SO group(n = 5)	36.62 ± 12.84	37.56 ± 11.85	38.17 ± 11.82
HC group(n = 6)	41.40 ± 13.92	1140.84 ± 177.49*	1088.90 ± 181.37*
HA group(n = 6)	33.92 ± 5.76	1068.98 ± 278.79*	391.07 ± 74.07**

*-compared with SO group, $P < 0.05$; **-compared with HC group, by least significant difference values and Dunnett's T3 tests, $P < 0.05$.

Table 4 **Comparison of serum MMP-9 levels at different time of each group in the experiment**

(ng/ml, $\bar{x} \pm s$)

Group	Beginning of experiment	End of 6th week after experiment	End of 10th week after experiment
SO group(n = 5)	2.37 ± 0.66	2.70 ± 0.31	2.71 ± 0.32
HC group(n = 6)	1.93 ± 0.98	101.53 ± 16.61*	100.65 ± 14.58*
HA group(n = 6)	2.49 ± 0.42	91.57 ± 35.08*	38.45 ± 17.12**

*-compared with SO group, $P < 0.05$; **-compared with HC group, by least significant difference values and Dunnett's T3 tests, $P < 0.05$.

Table 5 **Comparison of serum TIMP-1 levels at different time of each group in the experiment**

(ng/ml, $\bar{x} \pm s$)

Group	Beginning of experiment	End of 6th week after experiment	End of 10th week after experiment
SO group(n = 5)	0.82 ± 0.11	0.82 ± 0.11	0.83 ± 0.06
HC group(n = 6)	0.83 ± 0.15	9.81 ± 1.12*	9.00 ± 1.39*
HA group(n = 6)	0.83 ± 0.07	10.35 ± 2.48*	5.06 ± 1.01**

*-compared with SO group, $P < 0.05$; **-compared with HC group, by least significant difference values and Dunnett's T3 tests, $P < 0.05$.

occurrence of chronic heart failure. Degradation of myocardial extracellular matrix(ECM) is the distinct manifestation of ventricular remodeling. Matrix metalloproteinases play an important role in catabolism of ECM and changes of geometrical configuration of the ventricles. Matrix metalloproteinases(MMPs) have been implicated in the process of heart failure and ventricular remodeling[7]. Irregular MMPs expression is an important cause of dilatation and dysfunction of the left ventricle of patients with heart failure[6]. The effect of MMPs on the ECM could be regulated by tissue inhibitors matrix metalloproteinases, TIMP. Both fibroblasts and mast cells in cardiac muscle could be the source of synthesis and secretion of MMP-2, MMP-9 and its specific inhibitor, TIMP-1, into the extracellular space, and this would be reflected in serum concentrations of these substances. The serum MMP-2, MMP-9 and TIMP-1 levels are reported to be proportional to their content in cardiac muscle[8, 9].

Understanding the role of relevant matrix metalloproteinases and their specific inhibitor in ventricular remodeling is an important link in the study of chronic heart failure. Studies have found that along with the changes of cardiac function, TIMP-1 interacts with MMP-2, MMP-3 and MMP-9, which jointly participate in the process of heart failure[10]. Altieri et al[11]. found that TIMP-1 increases along with increases in circulating levels of MMP-2 and MMP-9 in patients with heart failure, and they considered that such changes not only exist in the final stage of heart failure or acute phase of decompensation, but also exist in patients with subclinical heart failure. In the present study, the mechanism of left ventricle chronic failure is further explored by detecting the MMP-2 and MMP-9 in serum of rabbits with experimental CHF and changes of relevant inhibition factor, TIMP-1 level. The results of this study show that serum MMP-2, MMP-9 and TIMP-1 of HC group and HA group at the end of the 6th week and the 10th week were significantly increased compared with the SO group. An increase in serum TIMP-1 level might represent a molecular feedback mechanism in the heart failure reaction and a process of disease development of heart failure that MMP-2 and MMP-9 jointly participated in. Results of this study are consistent with those reported in the relevant literature.

Studies on therapy with statins and their pleitropic mechanisms of action in chronic heart failure have become a hotspot in recent years. But the study of their effective action in heart failure has mainly focused on their impact on left ventricular shape and function. The specific mechanism(s) of action of such drugs in chronic heart failure remains unknown. In the present study the echocardiographic examination at the end of the 10th week of the experiment indicated that LVIDd, LVIDs, IVSd and LVPWd were significantly greater in the HC group than in the SO group or the HA group, while LVEF and LVFS were significantly lower than the values in the SO and HA groups. From the above results it is clear that the degree of heart failure in CHF model animals was improved by the daily administration of atorvastatin, indicating that this agent has therapeutic effectiveness in chronic heart failure. In addition, we

found that serums MMP-2, MMP-9 and TIMP-1 concentrations were significantly elevated in the HC and HA groups, but all three serum values were significantly lower in the HA group than in the HC group. Thus we can postulate that atorvastatin can effectively lower the serum MMP-2, MMP-9 and TIMP-1 levels in the course of heart failure and reduce the ventricular remodeling, and the degree of heart failure, and thereby improve cardiac function.

By analyzing the trends in the changes that occur in the morphological and functional indices used in the experiment, we conclude that the mechanism of action of atorvastatin in heart failure may be related to an intervention in the elevation of MMP-2, MMP-9 and TIMP-1. These proteins might be useful target molecules for the treatment of heart failure. In addition, they may provide a reference index for monitoring the degree of heart failure and assessing the efficacy of drug therapy.

References

[1] Greenberg B.J .Treatment of heart failure: state of the heart and prospectives. *Cardiovasc Pharmacol* 2001;38:S59-S63.

[2] Ueland T, Aukrustl P, Yndestad1 A. Soluble CD40 ligand in acute and chronic heart failure. *Eur Heart J* 2005; 26: 1101-7.

[3] Heart Protection Study Collaborative Group. MRC/BHF heart protection study of cholesterol lowering with simvastatin in 20536 high-risk individuals: a randomized placebo-controlled trial. *Lancet* 2002; 360:7-22.

[4] Joanne MF, Rahman S, Deron G. Statins and mortality among elderly patients hospitalized with heart failure. *Circulation* 2006;113:1086-92.

[5] Mozaffarian D, Nye R, Levy WC. Statin therapy is associated with lower mortality among patients with severe heart failure. *Am J Cardiol* 2004;93:1124-9.

[6] Spinale FG, Coker ML, Bond BR. Myocardial matrix degradation and metalloproteinase activation in the failing heart: a potential therapeutic target. *Cardiovasc Res* 2000; 46: 225-38.

[7] Tyagi SC, Ratajska A, Weber KT. Myocardial matrix metallo proteinase(s): Localization and activation. *Mol Cell Biochem* 1993;126:49-59.

[8] Brower GL, Chancey AL, Thanigaraj S. Cause and effect relationship between myocardial mast cell number and matrix metalloproteinase activity. *Am J Physiol Heart Circ Physiol* 2002;283:H518-25.

[9] Chancey AL, Brower GL, Janicki JS. Cardiac mast cell-mediated activation of gelatinase and alteration of ventricular diastolic function. *Am J Physiol Heart Circ Physiol* 2002;282:H2152-6.

[10] Yang Yongjian,Zhang Xin,Zhu Wenling. Involvement of matrix metalloproteinases in the regulation of myocardial extracellular matrix in patients. *Chin Med J* 2006; 86: 1693-6.

[11] Altieri P, Brunelli C, Gariba S. Metalloproteinases 2 and 9 are increased in plasma of patients with heart failure. *Eur J Clin Invest* 2003;33: 648-56.