Fluvastatin, a 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitor, Attenuates Left Ventricular Remodeling and Failure After Experimental Myocardial Infarction

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Background—Short-term administration of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, has been shown to attenuate ischemia-reperfusion injury. However, the effects of long-term administration of statins on left ventricular (LV) remodeling and failure after myocardial infarction remain unknown.

Methods and Results—Mice were subjected to coronary artery ligation and were treated for 4 weeks with vehicle or fluvastatin (10 mg/kg per day PO). Fluvastatin increased survival (61% versus 86%; \(P < 0.05\)) without affecting the infarct size (52±2% versus 49±3%; \(P = \text{NS}\)). Fluvastatin not only attenuated LV dilatation but also decreased LV end-diastolic pressure and lung weight. Furthermore, it reduced cardiac myocyte hypertrophy and interstitial fibrosis of the noninfarcted LV and also improved LV ejection performance. LV matrix metalloproteinase (MMP)-2 and MMP-13 were increased in myocardial infarction, which was attenuated in fluvastatin-treated mice.

Conclusions—Fluvastatin increased survival in a murine model of postinfarct heart failure, which was associated with the amelioration of LV structural remodeling and contractile failure. Moreover, these effects were associated with the attenuation of increased MMP activity. Thus, long-term treatment with fluvastatin might be beneficial also in patients with heart failure and might improve their long-term survival. (Circulation. 2002;105:868-873.)

Key Words: cholesterol ■ metalloproteinases ■ heart failure ■ myocardial infarction ■ remodeling

Myocardial infarction (MI) frequently produces left ventricular (LV) dilatation associated with myocyte hypertrophy and interstitial fibrosis of the noninfarcted myocardium. These changes in LV geometry, referred to as remodeling, contribute to development of depressed cardiac performance.1 Currently, angiotensin-converting enzyme (ACE) inhibitors have been shown to attenuate LV remodeling and improve the quality of life and decrease the mortality and morbidity of patients with MI and heart failure.2 Although a number of clinical trials have demonstrated the effectiveness of ACE inhibitors in heart failure, they may not sufficiently antagonize disease progression and thereby reduce the risk of major cardiac events in the patients.2,3 Accordingly, it is of critical importance to develop therapeutic strategies that will effectively inhibit the development and progression of LV remodeling and failure after MI. These newer strategies would be adjunctive and/or possibly synergistic with existing therapeutic strategies for treating patients.

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, can lower plasma cholesterol levels and have been associated with reduced morbidity and mortality in patients with coronary artery disease.4,5 It is presumed that statins may cause regression or stabilization of atherosclerotic plaques by lowering serum cholesterol levels and thus exert cardiovascular protective action.6 Recent studies demonstrated that short-term administration of statins attenuated ischemia-reperfusion injury in the brain and heart.7,8 These effects of statins are attributable to their various cellular and subcellular actions independent of cholesterol lowering.

Therefore, the purpose of this study was to determine whether the long-term administration of statins could attenuate the progressive LV chamber dilatation and contractile dysfunction in a murine model of MI.

Methods

Experimental Design

The study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. MI was created in male CD-1 mice, 5 to 8 weeks old and 25 to 35 g in body weight, by ligation of the left coronary artery.9 Our previous studies have shown that most mice have infarction of >40% of the LV and impaired systolic function.9

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Fluvastatin (10 mg/kg per day) was administered daily in the drinking water for 4 weeks. To adjust the daily dose of the drug, the volume of water was recorded every day and the body weight was measured twice a week. Our preliminary studies demonstrated that plasma fluvastatin concentration in the animals treated at this dose was 121±25 ng/mL (mean±SEM, n=10), within the range (50 to 150 ng/mL) in humans achieved after oral administration of its clinical doses (10 to 20 mg).\textsuperscript{10}

**Survival**

During the treatment period, cages were inspected daily for deceased animals. All deceased mice were examined for the presence of pleural effusion and cardiac rupture.

**Echocardiographic and Hemodynamic Measurements**

Echocardiographic studies were performed under light anesthesia with tribromoethanol/amylene hydrate (Avertin; 2.5% wt/vol, 8 μL/g IP) and spontaneous respiration.\textsuperscript{9} After 4 weeks, a 1.4F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and then advanced into the left ventricle to measure LV pressures. One subset of investigators (T.S. and N.S.), who were not informed of the experimental groups, performed in vivo LV function studies.

**Cardiac Histomorphometry**

Mice (8 sham, 9 sham with fluvastatin [FV] treatment [sham+FV], 12 MI, and 12 MI+FV mice) were used for the histomorphometric studies. Infarct size was calculated as total infarct circumference divided by total LV circumference as described previously.\textsuperscript{9} Myocyte cross-sectional area and collagen volume fraction were determined by quantitative morphometry of mid-LV tissue sections.\textsuperscript{9}

**Matrix Metalloproteinase (MMP), ACE, and Endothelial NO Synthase (eNOS)**

Eleven sham, 15 sham+FV, 16 MI, and 12 MI+FV mice were used for the subsequent biochemical studies. The myocardial tissues with MI were carefully dissected into two parts, one consisting of the infarcted left ventricle with the peri-infarct rim (a 0.5- to 1-mm rim of normal-appearing tissue) and the remaining noninfarcted (remote) left ventricle. In all subsequent assays, the comparison was made between noninfarcted LV from MI and control LV from sham.

MMP activity was measured by gelatin zymography.\textsuperscript{9} Within a given experiment, the densitometric values were normalized by using MMP-2 standards concurrently run within the same gel, and the value for each MI or MI+FV was calculated as a ratio of that from sham samples. The gelatinases (MMP-9), the predominant murine form of interstitial collagenases (MMP-13), and the tissue inhibitor of MMP-2 (TIMP-2) were measured by the immunoblotting analysis.\textsuperscript{11} The densitometric signal was normalized to sham values. Tissue ACE activity was measured by the rate of generation of His-Leu from hippuryl-His-Leu substrate and expressed as nmol/mg tissue per hour. eNOS protein levels were measured by Western blot analysis with antibodies against human vascular eNOS.

**Plasma Biochemical Measurement**

Before euthanization, venous blood samples (1 mL) were collected for determination of plasma total cholesterol, blood urea nitrogen, and aspartate aminotransferase.

**Statistical Analysis**

Data are mean±SEM. Comparison of survival was performed with the Kaplan-Meier analysis. For multiple-group comparisons, ANOVA followed by the Bonferroni t test was performed. \( P<0.05 \) was considered statistically significant.

**Results**

**Mortality**

Forty-three sham mice were divided into two groups, as follows: no treatment (sham; \( n=19 \)) or fluvastatin treatment (sham+FV; \( n=24 \)). The survival rate of MI mice at 6 hours after the ligation was 90% (74/82). They were divided into MI (\( n=46 \)) or MI+FV (\( n=28 \)). The survival rate up to 4 weeks was significantly (\( P<0.05 \)) higher in MI+FV than in MI mice (Figure 1). Death was suspected to be attributed to heart failure and/or arrhythmia. Two MI+FV (7%) and 7 MI (15%) mice died from LV rupture (\( P=NS \)). After 4 weeks, total cholesterol was not lowered by fluvastatin, which might be due to the induction of hepatic HMG-CoA reductase in response to fluvastatin in mice and the resultant increase in cholesterol synthesis. Hepatic and renal function were not affected by fluvastatin (Table 1).

**Echocardiography**

Serial echocardiographic measurements were performed at baseline, 1 day, and 28 days after surgery in another group of randomly grouped sham-operated (\( n=15 \)), MI (\( n=15 \)), and MI+FV (\( n=15 \)) mice. Figure 2 demonstrated that LV chamber size increased at day 1 in both MI and MI+FV and the extent of increase was the same between groups, indicating the comparable infarct size. Fluvastatin significantly attenuated LV dilatation and dysfunction caused by MI after 28 days (Figures 2 and 3, and Table 2). In comparison with sham, MI animals showed a significant increase in the thickness of noninfarcted region, which was attenuated by fluvastatin.

**Hemodynamics and Histomorphometry**

There was no significant difference in aortic blood pressure among 4 groups (Table 2). Right ventricular weight/body

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Biochemical Parameters</th>
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<tr>
<td>Total cholesterol, mg/dL</td>
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<tr>
<td>AST, U/L</td>
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<tr>
<td>Blood urea nitrogen, mg/dL</td>
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</tbody>
</table>

Data are mean±SEM. AST indicates aspartate aminotransferase.
weight was increased in the MI mice, and fluvastatin attenuated this increase. LV end-diastolic pressure (EDP) was increased in MI, which was attenuated in MI + FV. Coinciding with an increased LV EDP, lung weight/body weight was increased in the MI group, which was also attenuated by fluvastatin.

Infarct size determined by the histomorphometric analysis of LV sections was comparable between MI and MI + FV groups at 3 days (57±6% versus 54±5%; P=NS) as well as 28 days (49±3% versus 52±2%; P=NS) after operation. Myocyte cross-sectional area was increased in MI, which was significantly attenuated by fluvastatin. These results are concordant with LV wall-thickness data obtained from echocardiography (Table 2). Collagen volume fraction was also increased in MI, which was inhibited by fluvastatin.

MMP Activity and Abundance
MMP-2 zymographic activity was increased in MI compared with sham, which was attenuated in MI + FV mice (Figure 4). MMP-9 levels were not altered in MI, and fluvastatin did not affect them (Figure 5). In contrast, MMP-13 was significantly increased in MI, which was attenuated in MI + FV. TIMP-2 levels tended to increase by fluvastatin treatment in both sham and MI groups compared with no-treatment groups, which, however, did not reach statistical significance.

Myocardial ACE Activity
Compared with sham (22.7±1.6 nmol/mg per hour), MI induced significant increases in myocardial ACE activity.

Fluvastatin did not affect tissue ACE activity (nmol/mg per hour) in the MI group at both 7 days (38.9±2.9 versus 33.1±2.7, n=8 each, P=NS) and 28 days (50.4±2.5 versus 43.6±2.4, n=9 each, P=NS).

eNOS Protein
eNOS protein levels were not altered in MI and fluvastatin did not affect them (Figure 6).

Discussion
The present study demonstrates that fluvastatin can improve the survival in a murine model of postinfarct heart failure. After 4 weeks of treatment, it improved overall LV ejection performance and attenuated remodeling, and it reduced chamber dilatation as well as hypertrophy and fibrosis of the noninfarcted myocardium. These beneficial effects of fluvastatin were independent of cholesterol lowering and were associated with the attenuation of increased MMP levels.

Lefer et al7 have shown that myocardial ischemia-reperfusion injury can be prevented by the statins. However, it has not been shown whether long-term statin treatment affects the postinfarct conditions. Thus, the present study extends the previous observation by demonstrating that statins can inhibit not only ischemia-reperfusion injury but also heart failure. The effects of statins on LV structure may contribute to the increased survival. It was not due to their MI size-sparing effect, because the administration was started 6 hours after ligation. Moreover, based on serial echocardiographic data in randomized groups of mice (Figure 2), it is unlikely that the MI group had larger MI size, but these mice died early, leaving MI size equal between MI and MI + FV in the 28-day survivors. Furthermore, the effects of fluvastatin might not be attributable to its favorable hemodynamic effects because blood pressure and heart rate were not altered.

Several possibilities can explain the beneficial effects of fluvastatin on myocardial structure and function. First, recent studies have demonstrated that activated MMPs play an important role in the development of LV failure12 and that an MMP inhibitor limits the chamber dilatation in a murine model of MI.13 Furthermore, in vitro studies have shown that fluvastatin inhibits the production of MMPs at a concentration as low as 5 μmol/L.14 In the present study, MMP-2 and MMP-13 were increased in the noninfarcted LV, the site of ongoing remodeling, which was significantly attenuated in fluvastatin-treated animals. Taken together, one proposed mechanism of fluvastatin for reverse remodeling might be related to the attenuation of increased MMP levels after MI. In addition, fluvastatin might also influence endothelial cell function, which in turn could modulate the expression of proteolytic enzymes, including MMPs. However, the present study does not provide direct proof of a cause-and-effect relation between MMP inhibition and the attenuation of LV failure after fluvastatin treatment, and further investigation is needed.

Even though the infiltration of inflammatory cells including neutrophils was not prominent within the noninfarcted LV after 28 days, it is shown to be extensive at the infarcted area several days after MI. Therefore, anti-inflammatory effects of fluvastatin can also be involved in its beneficial
effects on LV remodeling. Alternatively, the cardiac renin-angiotensin system is activated after MI. ACE inhibition has been shown to prevent cardiac remodeling and prolong survival in experimental and clinical MI. There is now experimental evidence that the renin-angiotensin system is inhibited by statins in the vasculature and in the myocardium. Statins can directly inhibit angiotensin II–induced cardiac myocyte hypertrophy. Consequently, they may exert the beneficial effects via the inhibition of the tissue renin-angiotensin system. However, this possibility seems to be unlikely because fluvastatin did not affect myocardial ACE activity in our animals. Finally, the upregulation of eNOS might contribute to the effects of fluvastatin. NO has been shown to act as an inhibitor of leukocyte–endothelial cell interaction. In fact, the salutary effects with simvastatin subjected to ischemia-reperfusion have been attributed to the upregulation of NOS. Therefore, statins might increase

TABLE 2. Echocardiographic, Hemodynamic, and Histomorphometric Data

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham+FV</th>
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<td>n</td>
<td>19</td>
<td>24</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>421±9</td>
<td>415±9</td>
<td>410±6</td>
<td>422±7</td>
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<tr>
<td>LV EDD, mm</td>
<td>4.0±0.1</td>
<td>4.0±0.1</td>
<td>5.9±0.1*</td>
<td>4.8±0.1†‡</td>
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<td>LV ESD, mm</td>
<td>2.5±0.1</td>
<td>2.4±0.1</td>
<td>5.2±0.1*</td>
<td>3.9±0.1†‡</td>
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<td>FS, %</td>
<td>38.1±1.6</td>
<td>40.1±1.0</td>
<td>12.4±0.6*</td>
<td>18.6±1.1†‡</td>
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<tr>
<td>Wall thickness</td>
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<tr>
<td>Infarct, mm</td>
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<td>N/A</td>
<td>0.46±0.03</td>
<td>0.47±0.02</td>
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<tr>
<td>Noninfarct, mm</td>
<td>0.75±0.01</td>
<td>0.77±0.02</td>
<td>1.12±0.04*</td>
<td>0.87±0.03†</td>
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<td>Hemodynamic</td>
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<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>72±2</td>
<td>75±1</td>
<td>75±2</td>
<td>74±1</td>
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<tr>
<td>LV EDP, mm Hg</td>
<td>1.9±1.7</td>
<td>0.6±0.2</td>
<td>17.4±1.4†</td>
<td>11.4±1.5†‡</td>
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<tr>
<td>n</td>
<td>8</td>
<td>9</td>
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<tr>
<td>BW, g</td>
<td>35.1±0.6</td>
<td>36.3±0.9</td>
<td>36.8±0.8</td>
<td>36.1±0.6</td>
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<tr>
<td>LV wt/BW, mg/g</td>
<td>2.73±0.08</td>
<td>2.73±0.10</td>
<td>2.84±0.12</td>
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<td>RV wt/BW, mg/g</td>
<td>0.99±0.06</td>
<td>0.96±0.04</td>
<td>1.69±0.07*</td>
<td>1.50±0.09†</td>
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<td>Lung wt/BW, mg/g</td>
<td>5.0±0.1</td>
<td>5.0±0.2</td>
<td>8.2±0.5*</td>
<td>6.9±0.5†</td>
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<tr>
<td>Pleural effusion, %</td>
<td>0</td>
<td>0</td>
<td>68*</td>
<td>33†</td>
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<tr>
<td>Myocyte CSA, μm²</td>
<td>178±7</td>
<td>170±6</td>
<td>375±13*</td>
<td>230±8†</td>
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<tr>
<td>Collagen volume fraction, %</td>
<td>1.2±0.1</td>
<td>1.0±0.1</td>
<td>7.8±0.1*</td>
<td>4.5±0.1†</td>
</tr>
</tbody>
</table>

Data are mean±SEM. n indicates the number of animals studied; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; N/A, not applicable; EDP, end-diastolic pressure; BW, body weight; RV, right ventricular; wt, weight; and CSA, cross-sectional area.

*P<0.01 vs Sham; †P<0.05; ‡P<0.01 vs MI.

Figure 4. A, Representative gelatin zymograms from sham (lane 1), sham+FV (lane 2), MI at day 7 (lane 3), MI+FV at day 7 (lane 4), MI at day 28 (lane 5), and MI+FV at day 28 (lane 6). MMP-2 indicates positive control for purified MMP-2; MWM, molecular weight marker; and *, 68-kDa gelatinase. B, Densitometric analysis of MMP-2 activity in sham+FV (n=7), MI 7 days (n=5), MI+FV 7 days (n=6), MI 28 days (n=8), and MI+FV 28 days (n=6). Data were normalized by purified MMP-2 standards (0.1 ng, Chemicon) concurrently run on the same gel and expressed as the ratio-to-sham values. Values are mean±SEM. S indicates sham. *P<0.01 for difference from sham value. †P<0.05; ‡P<0.01 for difference from corresponding MI value.

Figure 5. A, Representative Western blot analysis comparing MMP-9, MMP-13, and TIMP-2 abundance from sham (lane 1), sham+FV (lane 2), MI (lane 3), and MI+FV (lane 4) groups. B, Densitometric analysis of MMPs and TIMP abundance in sham+FV (n=8), MI (n=8), and MI+FV (n=8) groups. Data are expressed as ratio to sham values concurrently run on the same gel. Values are mean±SEM. *P<0.05; **P<0.01 for difference from sham value. †P<0.05 for difference from MI value.
myocardial blood flow and decrease ischemia. However, the contribution of NO might be minor in our model because fluvastatin was administered after 6 hours of ligation and the resultant infarct size was not affected. In fact, eNOS protein levels remained unchanged by fluvastatin (Figure 6).

Although we should be cautious in extending our results obtained from animal experiments to the clinical setting, the present results imply that statins could be of clinical relevance in patients with heart failure. It has been demonstrated that simvastatin reduces the incidence of heart failure as well as the hospitalization and deaths associated with heart failure in patients with coronary artery disease.22 Even though they might have been due to the prevention of recurrent coronary events, the direct favorable effects of simvastatin on LV remodeling may be also involved. However, the effects of statins on the morbidity and mortality in heart failure patients have not been investigated because previous clinical trials have excluded these patients from the study population.4,5 Therefore, large-scale clinical trials, specifically designed to assess this issue, are warranted. Whether the combination of ACE inhibitors and statins exerts additive or synergistic effects should also be investigated.

The plasma concentrations of fluvastatin in our animals are within the range achieved after oral administration of the clinical doses of fluvastatin.10 Furthermore, these concentrations (0.3 μmol/L) are comparable with those that decrease MMP expression in vitro (0.1 to 10 μmol/L).14 Therefore, clinically relevant doses of fluvastatin can inhibit MMP and attenuate LV remodeling. In addition, we observed the similar effects on LV remodeling also by pravastatin and cerivastatin (data not shown).

There are several limitations in this study. First, as the hemodynamic profile of statins is incompletely described, we could not exclude the contribution of hypothetical favorable hemodynamic effects. Second, the present study could not exclude an additional effect if drugs were initiated at the time of coronary ligation or before ligation. Third, the substrates for MMP-2 include the basement membrane components collagen IV and laminin. Therefore, our finding that the inhibition of MMP is associated with the attenuation of cardiac fibrosis may seem paradoxical, because it may be expected that MMP inhibition prevents collagen degradation and thus promotes collagen accumulation and fibrosis.23 This paradox may be due to the fact that the total collagen content is a function of both synthesis and degradation, and degraded products of matrix proteins may serve as stimuli for further collagen synthesis in the myocardium. However, recent experimental studies have demonstrated that the inhibition of MMP attenuates cardiac fibrosis and failure.24,25 Therefore, even though our findings could not be simply equated with the studies using genetically engineered mice in which the action of MMPs might be potentially different, our results are consistent with them. Nevertheless, further studies are needed to clarify the mechanism in which the inhibition of MMP-2 activity is translated into the inhibition of myocardial collagen deposition. Fourth, even though previous studies have suggested that statins can exert direct inhibitory effects on MMP activation,14 this should be proved in cardiac fibroblasts, predominant cellular sources of MMPs in the heart.

In conclusion, fluvastatin, an HMG-CoA reductase inhibitor, can improve the survival and inhibit the development of cardiac remodeling and failure after MI. These effects were associated with the attenuation of an increase of myocardial MMPs. These drugs might be beneficial in postinfarct patients irrespective of their serum cholesterol levels.

Acknowledgments

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