Improvement of Left Ventricular Remodeling and Function by Hydroxymethylglutaryl Coenzyme A Reductase Inhibition With Cerivastatin in Rats With Heart Failure After Myocardial Infarction

Johann Bauersachs, MD; Paolo Galuppo, PhD; Daniela Fraccarollo, PhD; Michael Christ, MD; Georg Ertl, MD

Background—Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) attenuate angiotensin II-induced cellular signaling. Because angiotensin II is involved in left ventricular (LV) remodeling after myocardial infarction (MI), we examined the effects of statin treatment in an experimental model of chronic heart failure after MI.

Methods and Results—Rats with extensive MI were treated with placebo or cerivastatin (0.3 mg/kg per day) as a dietary supplement or via gavage for 11 weeks starting on the 7th postoperative day. Infarct size and cholesterol levels were similar among all groups. LV cavity area, an index of LV dilatation, was reduced in MI rats on cerivastatin compared with placebo. LV end-diastolic pressure was increased in MI rats on placebo (24.1±4.1 mm Hg versus sham: 5.1±0.3 mm Hg; P<0.01), and it was significantly reduced by cerivastatin treatment (13.7±2.7 mm Hg; P<0.05 versus placebo). Cerivastatin partially normalized LV dP/dt max and dP/dt min, indices of LV systolic and diastolic function, which were significantly reduced in MI rats on placebo. Improvement of LV function by cerivastatin was accompanied by a reduced expression of collagen type I and β-myosin heavy chain. LV endothelial nitric oxide synthase was increased, whereas the nitrotyrosine protein level was decreased in MI rats by cerivastatin treatment.

Conclusions—Cerivastatin improved LV remodeling and function in rats with heart failure. This effect was associated with an attenuated LV expression of fetal myosin heavy chain isoenzymes and collagen I. Statin treatment may retard the progression of chronic heart failure.

Key Words: myocardial infarction ■ heart failure ■ myocardium ■ remodeling ■ nitric oxide synthase

A

ute neurohumoral activation after myocardial infarc-

ion (MI) helps maintain cardiac output and peripheral perfusion in the acute phase of MI. However, chronic stimulation of neurohumoral systems, such as angiotensin II and endothelin-1, results in increased cardiac load and left ventricular (LV) hypertrophy, which eventually leads to ventricular enlargement and progression of heart failure.1,2 The detrimental effects of neurohumoral activation on ventricu-

lar remodeling seem to depend on the increased generation of reactive oxygen species such as superoxide anions (O$_2^-$).3–5 Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) attenuate O$_2^-$ formation and simultaneously increase the expression of endothelial nitric oxide synthase (eNOS),6–8 resulting in a beneficial shift in the balance between NO and O$_2^-$ that may improve LV remodeling after MI.9 Interestingly, statins attenuate angiotensin II–induced myocyte hypertrophy10 in cultured neonatal rat cardiomyocytes in a manner that is probably mediated by the attenuation of radical generation. Therefore, we examined whether long-term treatment with cerivastatin improves LV remodeling and function in rats with extensive MI.

Methods

Study Protocol, MI, and Hemodynamic Measurements

Left coronary artery ligations were performed in adult male Wistar rats (250 to 300 g).11 On the seventh postoperative day, rats were randomly allocated to treatment with placebo or cerivastatin (0.3 mg/kg per day), which was given as either a dietary supplement or via gavage. Hemodynamic studies were performed 12 weeks after MI under barbiturate anesthesia and controlled respiration.11

Sample Collection, Determination of Infarct Size, and Ventricular Remodeling

The heart was excised and dissected into the right and left ventricles, including the septum. The LV was cut into 3 transverse sections: apex, middle ring (~3 mm), and base. From the middle ring, 5-μm
sections were cut at 100-μm intervals and stained with picrosirius red. The boundary lengths of the infarcted and noninfarcted endocardial and epicardial surfaces were traced with a planimeter digital image analyser. Infarct size (fraction of the infarcted LV) was calculated as the average of all slices and expressed as the percentage of length of circumference, and only rats with extensive infarcts (>40%) were included in the study. LV cavity area (area enclosed by LV endocardial circumference) was taken as an index of LV dilatation.

**Myosin Heavy Chain Isoenzyme and Collagen I Expression**

Total RNA was isolated from surviving LV myocardium (septum) using TRIzol reagent. α- and β-myosin heavy chain (MHC) mRNA was amplified by polymerase chain reaction after reverse transcription (SuperScript, Life Technologies, Germany; Table 1). Fragments of the amplification product were separated on 8% polyacrylamide gels after enzymatic digestion with Tru9I (lengths: 310 bp for α- and 257+53 bp for β-MHC), and the ratio of β- to α-MHC mRNA was quantified. mRNA expression of collagen α1(I) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was determined by competitive polymerase chain reaction using internal standards. After separation on a 2% agarose gel, amplification products were visibly polymerase chain reaction.

**Western Blot Analysis**

Crude protein extracts (20 μg) were subjected to a 7.5% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. eNOS and nitrotyrosine protein levels were detected using specific antibodies (Transduction Laboratories and Upstate Biotechnology) and were visualized by enhanced chemiluminescence.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer Sequences</th>
<th>Genebank No.</th>
<th>PCR Product Size, bp</th>
<th>Temperature, °C</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>α/β-MHC</td>
<td>GCAGACCATCAGGAGAAGCCT-se</td>
<td>X15938/9</td>
<td>310</td>
<td>63</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>GTTGCGCTTGTTCTCTGCCG-as</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>collagen α1(I)</td>
<td>TCAGGTAAGGATGGAAGCCT-se</td>
<td>Z78279</td>
<td>462</td>
<td>58</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>CACAACGCTGCTGTAAGCTGA-as</td>
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<tr>
<td>GAPDH</td>
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<td>M17701</td>
<td>408</td>
<td>61</td>
<td>26</td>
</tr>
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</table>

| PCR indicates polymerase chain reaction. |

**Materials**

All biochemicals were obtained from Sigma. Cerivastatin was provided by Bayer AG (Wuppertal, Germany).

**Statistics**

Statistical analysis was performed by 2-factor ANOVA followed by a Newman-Keuls test or by the 2-tailed Student’s t test, where appropriate. Values are expressed as mean±SEM, and *P<0.05 was considered statistically significant.

**Results**

Global parameters are shown in Table 2. Infarct size, body weight, and plasma cholesterol levels did not differ among MI rats on placebo or cerivastatin. LV cavity area, which was increased in MI rats, was reduced by cerivastatin. Mean arterial pressure and LV systolic pressure were significantly lower in placebo-treated MI rats (Table 2), and LV end-diastolic pressure and right atrial pressure were substantially elevated compared with sham-operated animals (Figure 1). In MI rats on cerivastatin treatment, LV systolic pressure and mean arterial pressure were significantly increased, whereas LV end-diastolic pressure and right atrial pressure were reduced (*P<0.05 versus placebo). Cerivastatin partially normalized LV dp/dt max, an index of myocardial contractility, and dp/dt min, an index of diastolic relaxation, which were both significantly reduced in MI rats on placebo (Figure 1). Reduction of systolic and diastolic function was associated with an increased ratio of LV β-MHC to α-MHC mRNA and a marked increase in LV collagen I mRNA expression in MI rats on placebo or cerivastatin.

**TABLE 1. RNA Amplification Information**

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**TABLE 2. Global Parameters of Sham-Operated Rats and Rats With Heart Failure 12 Weeks After MI**

<table>
<thead>
<tr>
<th></th>
<th>Plac Sham (n=11)</th>
<th>Plac Infarct (n=8)</th>
<th>Cer-D Infarct (n=7)</th>
<th>Cer Gav Infarct (n=5)</th>
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<tr>
<td>Infarct size, %</td>
<td>...</td>
<td>49.6±2.5</td>
<td>49.2±1.4</td>
<td>52.6±0.5</td>
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<td>LVCA, mm²</td>
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<td>50.2±4.1</td>
<td>45.8±2.3</td>
<td>40.9±3.6†</td>
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<tr>
<td>LVSP, mm Hg</td>
<td>148±3</td>
<td>116±4</td>
<td>131±4</td>
<td>133±4†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>125±4</td>
<td>95±4</td>
<td>111±4</td>
<td>113±4†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>502±6</td>
<td>506±19</td>
<td>503±13</td>
<td>528±14</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td>88±8</td>
<td>74±9</td>
<td>98±7</td>
<td>102±11</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Rats were treated with either placebo (Plac) or with cerivastatin (0.3 mg/kg per day starting on the seventh postoperative day) given as a dietary supplement (Cer-D) or via gavage (Cer Gav). MAP indicates mean arterial pressure; LVSP, left ventricular systolic pressure; and LVCA, left ventricular cavity area.

*P<0.001 vs Plac Sham; †P<0.05 vs Plac Infarct.
rats compared with sham-operated animals. Both parameters were beneficially modulated by cerivastatin treatment (Figure 2). The reduction in collagen expression significantly correlated with the improvement in dP/dt_{min} (r=0.72, P<0.01) and dP/dt_{max} (r=0.75, P<0.01).

LV eNOS protein was significantly increased in rats with chronic MI (3.6±0.1 versus 2.7±0.2 arbitrary units [aU] in sham), and it was further enhanced in both cerivastatin-treated groups. LV nitrotyrosine protein level as a marker for peroxynitrite formation was enhanced in MI rats (2.9±0.3 versus 1.8±0.4 aU in sham) and attenuated by cerivastatin (2.0±0.4 versus 1.8±0.5 aU in sham, n=3; P<0.05; Figure 2C).

Discussion
The major novel result of this study was that cerivastatin improves LV systolic and diastolic function in rats with chronic heart failure after experimental MI. This effect was associated with an attenuated LV expression of fetal genes such as β-MHC, a marked reduction of collagen I gene expression, an increase in LV eNOS expression, and a decrease in tyrosine nitration.

Improvement of LV function by cerivastatin may be explained by the beneficial effects of statins on afterload, such as a direct reduction of systemic vascular resistance. However, a cardioprotective effect of cerivastatin secondary to afterload lowering is unlikely because mean arterial pressure increased during treatment. Thus, cerivastatin seems to modulate cardiac function directly, as indicated by the improvement of dP/dt_{max} and the attenuation of fetal β-MHC expression. In addition, we showed that statin treatment leads to a substantial reduction of LV collagen I expression, which correlated with the improvement in LV systolic and diastolic function. Myocardial fibrosis is a major feature of LV remodeling after MI, which is mainly driven by angiotensin II.1,2 Statins have been shown to prevent angiotensin II–induced hypertrophy in cultured neonatal rat cardiac myocytes, probably by attenuating angiotensin II–stimulated p21 ras activity.10 This effect could be reversed by mevalonic acid, an immediate precursor of isoprenoids, suggesting that statins reduce angiotensin II–dependent hypertrophy by blocking the isoprenylation of small (21 kDa) guanine nucleotide-binding proteins (G-proteins). The critical steps for...
angiotensin II–induced effects on myocardial remodeling seem to depend on the generation of reactive oxygen species. Indeed, statins have been shown to reduce the formation of \( \text{O}_2^\cdot \) by preventing the isoprenylation of p21 Rac, which is critical for the assembly of NADPH oxidase.7 Furthermore, statins upregulate eNOS expression by inhibiting geranylgeranylation of Rho GTPase, another small G-protein.6,8

LV eNOS expression was markedly increased and protein tyrosine nitration was reduced by cerivastatin in our study, suggesting an interaction of cerivastatin treatment with the above-discussed intracellular pathways. Thus, the beneficial modulation of LV remodeling by cerivastatin may be mediated by an improved NO/O\( \text{O}_2^\cdot \) balance. This hypothesis is supported by the observed decrease of protein tyrosine nitration, indicating reduced peroxynitrite formation. Reduced NO bioavailability contributes to the deterioration of LV function after MI, whereas an increase of NO bioavailability combined with reduced O\( \text{O}_2^\cdot \) formation may synergistically improve LV remodeling and function.5,9 We interpret the slight increase in eNOS expression in MI rats on placebo as a failed counter-regulatory mechanism in response to the increase in O\( \text{O}_2^\cdot \) formation, which has a decisive role for LV remodeling. This response was presumably not sufficient to counteract the marked increase in radical generation.

Furthermore, the beneficial effects of cerivastatin on LV remodeling after MI may be mediated by an attenuation of endothelin-1 synthesis, which has been demonstrated previously in vascular endothelial cells. Thus, cerivastatin may alleviate the deleterious consequences of an increased expression of endothelin-1 after coronary ligation.1,2,11

In conclusion, we show for the first time that cerivastatin improves LV remodeling and function in rats with heart failure, which was associated with a marked reduction of collagen expression and an attenuated expression of fetal MHC isoenzymes. The reduction of overall mortality by statins in the secondary prevention of coronary heart disease presumably depends on plaque stabilization; however, our data indicate that statins may also retard the progression of heart failure, presumably by a beneficial modulation of cellular responses to neurohormonal activation after large MI.

Acknowledgments
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References
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