Pretreatment With Simvastatin Attenuates Myocardial Dysfunction After Ischemia and Chronic Reperfusion

Steven P. Jones, Steven D. Trocha, David J. Lefer

Abstract—We have previously demonstrated that simvastatin attenuates myocardial cell necrosis after acute myocardial ischemia and reperfusion via induction of endothelial cell NO synthase. However, it remains unknown whether the cardioprotective effects of statins can persist after extended periods of reperfusion. Furthermore, it is unknown whether simvastatin therapy can attenuate postischemic cardiac dysfunction. Pretreatment with simvastatin attenuated myocardial injury after 30 minutes of myocardial ischemia and 24 hours of reperfusion. However, the protective effects are not recognized unless simvastatin is given at least 3 hours before myocardial ischemia. Subsequently, we pretreated mice with vehicle or simvastatin and subjected the mice to 30 minutes of myocardial ischemia and 6 months of reperfusion. Myocardial infarct size (percentage of left ventricle) was significantly reduced by 51% in the simvastatin-treated group compared with the vehicle-treated group. Left ventricular diastolic and systolic dilatation was significantly \((P<0.05)\) reduced in simvastatin-treated mice compared with vehicle-treated mice. Additionally, the decrement in fractional shortening after 6 months of reperfusion was minimized in simvastatin-treated mice \((P=\text{NS} \text{ versus baseline})\) compared with vehicle-treated mice \((P<0.05 \text{ versus baseline})\). Left ventricular end-diastolic pressure was significantly \((P<0.01)\) elevated in vehicle-treated mice \((21 \pm 4 \text{ mm Hg})\) but not simvastatin-treated mice \((5 \pm 2 \text{ mm Hg})\) compared with baseline values. These data demonstrate that simvastatin treatment before myocardial ischemia attenuates infarct size and preserves myocardial function after chronic reperfusion in mice. (Arterioscler Thromb Vasc Biol. 2001;21:2059-2064.)

Key Words: inflammation ■ nitric oxide ■ infarct ■ contractile function

Elevated serum cholesterol levels are a known risk factor for the development of cardiovascular disease. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are a class of drugs particularly beneficial in combating this risk factor. Therapeutic doses of statins potently reduce serum cholesterol levels in humans.\(^1\) Furthermore, statins have been shown to decrease coronary heart disease mortality.\(^2\) Statins have also shown to improve endothelial function in humans with elevated serum cholesterol levels.\(^3\) It has become readily apparent through experimental and clinical data that additional benefits of statin therapy may occur independently of cholesterol reduction.\(^4,5\)

Previous experimental studies of statins in ischemia/reperfusion injury have clearly demonstrated cardioprotective\(^6-8\) and neuroprotective\(^9\) effects despite unaltered serum cholesterol levels. The beneficial mechanism potentiated by statins in these studies appeared to be dependent on enhanced release of NO from the coronary/cerebral endothelium. Experimental studies have revealed that acute simvastatin therapy increases the half-life of endothelial NO synthase mRNA and activates the protein kinase Akt pathway, resulting in enhanced NO production. The protective effects of NO have been demonstrated in previous experimental studies in the heart and other organs.\(^10-16\)

Despite evidence supporting acute cardioprotective effects of statins in myocardial ischemia/reperfusion injury, it is unknown whether these effects persist in a chronically reperfused animal model. More specifically, do these effects translate into improvement in ventricular function parameters in vivo? In the present study, we investigated whether simvastatin treatment before myocardial ischemia would confer any sustained cardiovascular benefits by using a murine model of in vivo ischemia and reperfusion. We also established the time frame of pretreatment in which acute cardioprotective effects were evident.

Methods

Mice

Two- to 3-month-old male C57BL/6 mice (Jackson Laboratory, Bar Harbor, Me) were used in all experimental groups. Twenty-six mice were randomized to receive either saline vehicle \((200 \mu\text{L} \text{ IP}, n=15)\) or activated\(^{10}\) simvastatin \((1 \text{ mg/kg IP}, n=11)\) administered 18 hours before myocardial ischemia and chronic reperfusion. Sham-operated mice \((n=5)\) were used to account for possible chronic effects related to the surgical protocol. All mice survived the protocol except for 3 vehicle-treated mice and 2 simvastatin-treated mice. An additional group of 37 mice was used to identify a time course of pretreatment with simvastatin. These mice were reperfused for 24 hours instead of 6 months. None of the functional data in the present study involves these “acutely” reperfused groups of mice. All animal experiments...
Myocardial Ischemia/Reperfusion Protocol
The surgical protocol and infarct size determination were performed similar to methods described previously. Briefly, the mice were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and pentobarbital sodium (50 mg/kg). Subsequent to anesthesia, the mice were orally intubated with polyethylene-90 (PE-90) tubing, connected via loose junction to a rodent ventilator (model 683, Harvard Apparatus) set at a tidal volume of 1.5 mL and a rate of 120 breaths per minute, and supplemented with oxygen. Body temperature was maintained at 37°C by using a rectal thermometer and infrared heating lamp. A median sternotomy was performed, and the proximal left anterior descending coronary artery (LAD) was visualized and ligated with 7-0 silk suture mounted on a tapered needle (BV-1, Ethicon). Ischemia was confirmed by the appearance of hypokinesis and pallor distal to the occlusion. After 30 minutes of LAD occlusion, the ligature was removed, and reperfusion was visually confirmed. The chest wall was closed, and the mice were given butorphanol tartrate (0.1 mg/kg) and allowed to recover in a temperature-controlled area.

Evaluation of Arterial and LV Hemodynamics
To assess the closed-chest hemodynamic status of both groups of mice after 6 months of reperfusion, a 1.4F Millar (SPR-671) pressure transduction catheter was inserted similar to methods described previously. Mice were anesthetized with ketamine (50 mg/kg IP) and pentobarbital (50 mg/kg IP) and supplemented with oxygen via a nasal cone. The right common carotid artery was isolated, and the catheter was inserted and advanced to the aorta. Data were recorded for ~10 seconds. Offline assessment of these data yielded systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and heart rate (HR). The catheter was then advanced through the aortic valve into the left ventricle (LV). Data were recorded for ~10 seconds. Subsequent offline evaluation provided LV systolic pressure, LV end-diastolic pressure (LVEDP), LV developed pressure, and the first derivative of the LV pressure curve (minimum and maximum dp/dt). Data for each animal were calculated from at least 10 seconds of chart recording (arithmetic mean of at least 50 cardiac cycles).

Echocardiographic Assessment of the LV
In vivo transthoracic echocardiography of the LV with use of a 15-MHz linear array transducer (15L8) interfaced with a Sequoia C256 (Acuson) was performed as described previously. Ventricular parameters were measured by using the leading-edge technique. M-mode (sweep speed 200 mm/s) echocardiograms were captured from parasternal, short-axis, and long-axis 2D views of the LV at the midpapillary level. LV end-diastolic diameters (LVEDDs), LV end-systolic diameters (LVESDs), aortic diameter (AoD), aortic velocity time integral (AoVTI), and HR were measured before ischemia and at 6 months of reperfusion. For measurement of the AoVTI, angle correction of the Doppler signal was incorporated to account for the difference between the ultrasound beam and aortic flow (~90°). LV percent fractional shortening (LV%FS) was calculated according to the following equation: LV%FS=[(LVEDD−LVESD)/LVEDD]×100. Stroke volume was calculated from the product of the aortic cross-sectional area, ie, π×(AoD/2)², and the AoVTI. Cardiac output was calculated from the product of the stroke volume and HR. The cardiac output values were corrected for the weights of the animals (in microliters per minute per gram). Anterior and posterior wall dimensions were also assessed in diastole and systole in all groups of mice. All data were calculated from 10 independent cardiac cycles per experiment.

Statistical Analysis
All data were analyzed by a Student unpaired t test with the use of StatView 4.5 (Abacus Concepts). Values are reported as mean±SEM, with significance set at P<0.05.

Results

Myocardial Infarct Size
To ascertain the onset of the protective effects of simvastatin, we performed a time course of simvastatin pretreatment in which a separate group of mice was given vehicle or 1 mg/kg simvastatin before 30 minutes of myocardial ischemia (Figure 1). These data demonstrate that pretreatment with simvastatin as late as 3 hours before the onset of myocardial ischemia exerted protective effects. However, pretreatment with simvastatin 1 hour before myocardial ischemia did not confer any demonstrable cardioprotective effects.

In the chronically reperfused group of mice, surviving mice were euthanized, and the amount of necrosis per LV was determined after 6 months. As shown in Figure 2, it was clear that hearts from mice that received simvastatin (3.8±0.8% infarct per LV, n=7) before myocardial ischemia maintained less (P<0.05) injury than did hearts from vehicle-treated mice (7.8±0.9% infarct per LV, n=11). In addition, LV weights were not significantly different between the vehicle- and simvastatin-treated groups when expressed as raw values or when corrected for body weight (data not shown).

Arterial and LV Hemodynamics
Insertion of a 1.4F pressure catheter through the carotid artery into the aorta and LV yielded in vivo hemodynamics in closed-chest mice. None of the arterial hemodynamic param-
Echocardiographic Assessment of LV Function and Dimensions

Using transthoracic echocardiography, we assessed closed-chest in vivo ventricular function and dimensions before ischemia and after chronic reperfusion in sham-operated mice and in mice given vehicle or simvastatin. Table 2 contains ventricular dimensions of systole and diastole in all groups of mice. Although vehicle- and simvastatin-treated mice experienced significant ventricular dilatation at 6 months after myocardial ischemia, the percent change in diastolic and systolic diameters from baseline was significantly greater in the vehicle-treated group compared with the simvastatin-treated group (Table 2). In addition, the percent fractional shortening in simvastatin-treated hearts was maintained near baseline levels, whereas the vehicle-treated hearts suffered a small but significant reduction in fractional shortening (Table 2).

Regional wall dimensions and function were also assessed in sham-operated, vehicle-treated, and simvastatin-treated mice after 6 months of reperfusion (Table 3). Anterior wall thickening was significantly depressed in vehicle- and simvastatin-treated mice after 6 months of reperfusion compared with sham-operated mice. The thickness of the posterior wall in diastole and systole was significantly greater in the vehicle-treated mice compared with the sham-operated mice. Furthermore, the ratio of the anterior wall thickness to posterior wall thickness was significantly diminished in vehicle-treated mice compared with sham-operated mice.

Discussion

The present study demonstrates that mice subjected to coronary artery occlusion followed by chronic reperfusion have significant long-standing cardiac dysfunction. More important, these data provide evidence that a single dose of simvastatin can at least partially preserve normal cardiac function in the chronically reperfused mouse myocardium. It is important to consider the effects of an agent after more prolonged periods of reperfusion to ascertain whether the acute cytoprotective effects would be associated with any improvement in myocardial function. This point is particularly important because previous studies have clearly demonstrated that some acutely cardioprotective agents do not confer sustained cardiac benefits.24–26 Therefore, the present study addresses this vital issue as it relates to the cardioprotective effects of simvastatin.

Ever since the first demonstration of the protective effects of statins in a model of cerebral ischemia/reperfusion injury,9 we have documented similar findings in the myocardium.6–8 These studies were largely driven by in vitro evidence demonstrating an important effect of HMG-CoA reductase inhibitors on endothelial NO synthase.27–30 In addition to the attenuation of ischemia/reperfusion injury in the brain9 and heart,6–8 statins have also been shown to promote angiogenesis in ischemic limbs of rabbits,32 attenuate leukocyte–endothelial cell interactions,7,8,33 and retard the progression of

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<th>Table 1. Body Weight and General Hemodynamics</th>
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<td>Statin</td>
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Values are mean±SEM. Body weight, cardiac output, HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP) were measured in closed-chest mice before myocardial ischemia (baseline) and after 6 months of reperfusion (after myocardial ischemia [MI]). Although body weight significantly increased ($P<0.05$ vs baseline) over the 6-month period in all groups of mice, none of the hemodynamic parameters were significantly different between groups or compared with baseline (n=5 per group).

Figure 3. LVEDP before myocardial ischemia (baseline) and after 6 months of reperfusion (6 months after myocardial ischemia [MI]) in wild-type mice treated with vehicle or simvastatin or with sham operation. LVEDP was significantly elevated (**$P<0.01$ vs baseline) in the vehicle-treated mice. However, simvastatin treatment abolished this rise in LVEDP.

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1. Jones et al Simvastatin Preserves Myocardial Function

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The protective effects of statins in myocardial ischemia/reperfusion models have been demonstrated in normocholesterolemic rats, normocholesterolemic mice, hypercholesterolemic mice, and diabetic mice. The protective effects in all of these studies are largely mediated by induction of endothelial cell–derived NO, which is an essential cardioprotective and anti-inflammatory factor.

Despite consistent demonstrations of a reduction in myocardial reperfusion injury with statin treatment, the functional consequences of this protection remained unknown until now. Although the preservation of function in the statin group is not entirely surprising because statin treatment does not completely block myocardial necrosis in the present study or completely abolish ventricular dilatation or the diminution of function after 6 months of reperfusion. This finding is not entirely surprising because statin treatment does not completely block myocardial necrosis in the present study or other studies of acute myocardial ischemia/reperfusion. It is critical to indicate important differences in the method of reporting the 2 groups of infarct sizes. In Figure 1, we report the myocardial infarct size with respect to the area at risk, whereas in Figure 2, it is reported with respect to the LV. The expression of infarct size with respect to the area at risk after 6 months of reperfusion was not possible because of the inability to locate the exact position of the previous ligation. The religation is necessary to counterstain the nonischemic zone and delineate it from the area at risk. If data from the acutely reperfused group (Figure 1) were expressed with respect to the LV, the values would still be significantly greater than the values for the chronically reperfused group (Figure 2). However, this difference could likely be due to scar formation and the remodeling process of the posts ischemic myocardium.

Although simvastatin did not completely abolish the deleterious functional consequences of myocardial ischemia/reperfusion in the present study, several important parameters of cardiovascular function were protected. It is clear that the extent of myocardial infarction was significantly attenuated in the simvastatin group compared with the vehicle-treated group. However, one of the most striking functional observations is the elevated LVEDP in the vehicle group that was not observed in the statin group. The augmented LVEDP in the vehicle group likely results from alterations in myocardial compliance that are due to the larger area of myocardial infarction. Ultimately, when the finding of elevated LVEDP is combined with a normal cardiac output, it appears that mice receiving vehicle exhibit a compensated form of heart failure. Another possibly related explanation of the elevated LVEDP could involve alterations in regional wall function. The data in Table 3 may indicate that there is compensatory hypertrophy of the posterior (noninfarcted) wall. Furthermore, the ratio of the anterior wall to the posterior wall is significantly lower in the vehicle-treated mice compared with the sham-treated mice.

**TABLE 3. LV Regional Wall Dimensions**

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<tr>
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<th>Diastole</th>
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<tr>
<td></td>
<td>Anterior, mm</td>
<td>Posterior, mm</td>
<td>Anterior, mm</td>
<td>Posterior, mm</td>
<td>Anterior Thickening, %</td>
<td>Posterior Thickening, %</td>
<td>AW/PW Ratio</td>
</tr>
<tr>
<td>Sham</td>
<td>0.78±0.06</td>
<td>1.08±0.14</td>
<td>1.20±0.03</td>
<td>1.34±0.12</td>
<td>51.6±7.9</td>
<td>26.0±6.3</td>
<td>0.75±0.07</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.89±0.02</td>
<td>1.34±0.05*</td>
<td>1.08±0.06</td>
<td>1.60±0.06*</td>
<td>33.0±4.6*</td>
<td>16.4±1.7</td>
<td>0.60±0.03*</td>
</tr>
<tr>
<td>Statin</td>
<td>0.83±0.04</td>
<td>1.18±0.08</td>
<td>1.21±0.04</td>
<td>1.41±0.09</td>
<td>37.1±2.5*</td>
<td>21.2±1.8</td>
<td>0.78±0.04</td>
</tr>
</tbody>
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Values are mean±SEM. LV anterior and posterior wall thickness in diastole and systole of sham-operated, vehicle-treated, and simvastatin-treated mice after 30 minutes of LAD ischemia and 6 months of reperfusion. Percent thickening of the anterior wall, percent thickening of the posterior wall, and the anterior to posterior wall (AW/PW) ratio were also measured in all groups of mice by use of transthoracic echocardiography (n=5 per group). The posterior wall was significantly thicker (P<0.05 vs sham) in the vehicle-treated group in diastole and systole. The vehicle- and statin-treated groups experienced a significant reduction in the percent thickening of the anterior wall (P<0.05 vs sham). The AW/PW ratio was significantly lower in the vehicle-treated group compared with the sham-operated group (P<0.05 vs sham).
operated (and simvastatin-treated) mice. These findings likely result from the infarct-reducing effect of simvastatin. Although simvastatin may beneficially affect the remodeling process in this model (independent of infarct size reduction), this is probably unlikely because of the transient administration of simvastatin that we used. However, this important question cannot be answered in the present study.

Preservation of normal LVEDP was accompanied by a less impressive effect on ventricular dimensions. Both groups of mice underwent a significant degree of ventricular dilatation after the infarct. However, the change in LV diameter from baseline was more pronounced in the vehicle-treated group in diastole and in systole. Although the vehicle-treated group experienced a significant decrement in fractional shortening, statin therapy virtually abolished this defect. The attenuated dilatation and preservation of fractional shortening in the statin group likely resulted from the smaller area of necrosis after the infarct. In any event, simvastatin therapy before myocardial ischemia exerts significant and persistent cardiovascular protective effects.

A major limitation of the present study is the use of otherwise healthy animals to investigate the pathophysiology of acute myocardial infarction. Most cardiac patients suffer from a variety of risk factors (eg, hypertension, diabetes, and hypercholesterolemia) that significantly reduce endothelial cell NO synthase function. It may not be possible to realize a significant benefit in the setting of myocardial ischemia/reperfusion in such individuals. In addition, we pretreated mice with simvastatin before the onset of myocardial ischemia, which is not usually possible in humans. Furthermore, we also found that the protective effect of simvastatin was not apparent if the mice were treated as late as 1 hour before myocardial ischemia. However, our results do provide insights into the mechanism responsible for the significant reduction in cardiac disease events in statin-treated patients.

In summary, the present study demonstrates that simvastatin treatment before myocardial ischemia attenuates ventricular dilatation, preserves ventricular function, and abrogates the elevation in end-diastolic pressure after long-term reperfusion. Future studies should evaluate the effect of chronic statin therapy before and/or after myocardial ischemia to ascertain any added benefits or possible deleterious effects. The present and future findings may have profound implications for the future treatment of myocardial infarction and preservation of function after ischemia.

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