Mechanisms Involved in Cardioprotective Effects of Pravastatin Administered during Reoxygenation in Human Myocardium In Vitro

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ABSTRACT

Background: The authors investigated the effect of pravastatin during reoxygenation after myocardial hypoxia and examined the involvement of nitric oxide synthase, mitochondrial permeability transition pore, and expression of markers of apoptosis in human myocardium in vitro.

Methods: Human atrial trabeculae were exposed to hypoxia for 30 min and reoxygenation for 60 min (control group; n = 10). Pravastatin (5, 10, 50, 75 μM; n = 6 in each group) was administered throughout the reoxygenation. In separate groups (n = 6 in each group), pravastatin 50 μM was administered in the presence of 200 μM L-NG-nitroarginine methyl ester, a nitric oxide synthase inhibitor, and 50 μM atracyloside, the mitochondrial permeability transition pore opener. The primary endpoint was the developed force of contraction at the end of reoxygenation, expressed as a percentage of baseline (mean ± SD). Protein expression of BAD, phospho-BAD, caspase 3, Pim-1 kinase, and Bcl-2, and preserving the myocardium against the mitochondrial permeability transition pore opening, and antiapoptotic effects.

Results: Pravastatin administered at the reoxygenation period protects human myocardium against hypoxia or reoxygenation injury via activation of nitric oxide synthase, inhibition of mitochondrial permeability transition pore opening, and antiapoptotic effects.

Conclusions: Pravastatin, administered at reoxygenation, protected the human myocardium by preventing the mitochondrial permeability transition pore opening, phosphorylating BAD, activating the expression of Pim-1 kinase and Bcl-2, and preserving the myocardium against the caspase 3 activation.

What We Already Know about This Topic

• Previous studies have demonstrated statins are protective in patients undergoing major vascular or cardiac surgery and reduce the risk of perioperative myocardial ischemia. However, few studies have focused on the direct effect of statins during reperfusion.

• This study determined the cardioprotective effect of statins on the in vitro ischemic human myocardium when administered at the time of reoxygenation.

What This Article Tells Us That Is New

• Pravastatin administered at the reoxygenation period protects human myocardium against hypoxia or reoxygenation injury via activation of nitric oxide synthase, inhibition of mitochondrial permeability transition pore opening, and antiapoptotic effects.

PERIOPERATIVE myocardial ischemia is a major adverse event that dramatically increases postoperative morbidity and mortality. The restoration of oxygenated...
blood to ischemic myocardium is mandatory to prevent extensive myocardial infarction and preserve cardiac function. However, myocyte cell death is temporarily enhanced by reperfusion, a phenomenon referred to as “reperfusion injury.” Increasing research has focused on limiting reperfusion-induced injury, which could result in increased salvage of ischemic myocardium and possibly improved mortality and morbidity.

Statins have been reported to have pleiotropic effects beyond their cholesterol-lowering effects. Statins may be considered a global perioperative protective therapy for patients undergoing major vascular surgery and have been shown to reduce the risk of perioperative myocardial ischemia. Few studies have focused on the direct effect of statins during reperfusion. Furthermore, mechanisms involved in statin-induced cardioprotection remain incompletely studied. It has been suggested that bioavailability of nitric oxide mediates the cardioprotective effect of pravastatin by inhibiting vascular inflammation and cardiac cell death. Evidence shows that the key signaling pathways of cardioprotection converge on the mitochondria. The mitochondrial pore transition permeability (mPTP) seems to be an end effector of cardioprotection; nevertheless, its role in statin-induced cardioprotection has never been examined. Finally, apoptotic cell death during the reperfusion phase has been suggested to have a role in lethal reperfusion-induced injury. Many proteins have been reported to be linked to the regulation of apoptosis under physiologic and pathologic conditions. Among them, the antiapoptotic protein Bcl-2 and Pim-1 kinase have been implicated in myocardial postconditioning.

Thus, the aim of the current study was to investigate the cardioprotective effect of statin administered at reoxygenation in isolated human myocardium in vitro. The role of nitric oxide and the mPTP opening was examined. Finally, the possible antiapoptotic effects of statin were evaluated through the expression of caspase 3, BAD, phospho-BAD, Bcl-2, and Pim-1 kinase.

Materials and Methods

After approval of the local medical ethics committee (Comité de Protection des Personnes Nord Ouest III, Caen, France) and written informed consent were received, right atrial appendages were obtained during cannulation for cardiopulmonary bypass from patients scheduled for coronary artery bypass surgery and aortic valve replacement. All patients received total intravenous anesthesia with propofol, remifentanil, and pancuronium. Patients with chronic atrial arrhythmia and diabetes mellitus treated with insulin or oral hypoglycemic agents were excluded from the study because these diseases have been shown to modify the structure and function of the myocardium. In particular, diabetes and hyperglycemia may interfere with signaling pathways involved in cardioprotection.

Human Atrial Trabeculae Model of Hypoxia or Reoxygenation Injury

Experimental Conditions. Right atrial trabeculae (one per appendage) were dissected and suspended vertically between an isometric force transducer (MLT0202; ADInstruments, Sydney, Australia) and a stationary stainless clip in a 200-ml jacketed reservoir filled with daily prepared Tyrode’s modified solution containing 120 mM NaCl, 3.5 mM KCl, 1.1 mM MgCl2, 1.8 mM NaH2PO4, 25.7 mM NaHCO3, 2.0 mM CaCl2, and 5.5 mM glucose. The jacketed reservoir was maintained at 34°C by a thermostatic water circulator (Polystat micropros; Bioblock, Illkirch, France). The bathing solution was insufflated with carbogen (95% O2 5% CO2), resulting in a pH of 7.40 and a partial pressure of oxygen of 600 mmHg. Isolated muscles were field-stimulated at 1 Hz by two platinum electrodes with rectangular wave pulses of 5-ms duration 20% above threshold (CMS 95107; Bionic Instrument, Paris, France).

Trabeculae were equilibrated for 60–90 min to allow stabilization of their optimal mechanical performance at the apex of the length active isometric tension curve (Lmax). The force developed was measured continuously, digitized at a sampling frequency of 400 Hz, and stored in a computer (PowerLab; ADInstruments). At the end of the experiment, the muscle cross-sectional area was calculated from its weight and length assuming a cylindrical shape and density of 1. To avoid core hypoxia, trabeculae included in the study must have a cross-sectional area less than 1.0 mm2, a force of contraction normalized per cross-sectional area (FoC) greater than 5.0 mN/mm2, and a ratio of resting force or total force less than 0.50; otherwise they were excluded a posteriori.

Experimental Protocol. At the end of the stabilization period, the trabeculae were randomly assigned (sealed envelopes) to one of the experimental groups. In all groups, hypoxia or reoxygenation was performed by replacing 95% O2 5% CO2 with 95% N2 5% CO2 in the buffer for 30 min, followed by a 60-min oxygenated recovery period. In the control group (control, n = 10) trabeculae were exposed to hypoxia and reoxygenation only (fig. 1). In the statin treatment groups: pravastatin (Sigma Aldrich, Saint Quentin Fallavier, France) was administered at 5, 10, 50, and 100 μM L-NG-nitroarginine methyl ester (L-NAME) (Sigma Aldrich), a nonselective nitric oxide synthase inhibitor (pravastatin + L-NAME; n = 6), and 50 μM L-NAME (Sigma Aldrich), the mPTP opener (pravastatin + atacryloside; n = 6) (fig. 1). In additional groups, muscles were exposed to 200 μM L-NAME (Sigma Aldrich; n = 6) and 50 μM atacryloside (atacryloside; n = 6) 10 min before and during reoxygenation (fig. 1). These concentrations of...
L-NAME and atractyloside have been demonstrated to abolish cardioprotection in human myocardium in vitro.\textsuperscript{13}

**Western Blot Analysis.** The right atrial appendage was pinned in a chamber (25 ml) containing Tyrode's modified solution, oxygenated with 95% O\textsubscript{2} 5% CO\textsubscript{2}, and maintained at 34° ± 0.5°C (Polystat micropros). The preparation was stimulated at a frequency of 1 Hz.

In four separate groups, after a 90-min equilibration period, hypoxia was performed by replacing 95% O\textsubscript{2} 5% CO\textsubscript{2} with 95% N\textsubscript{2} 5% CO\textsubscript{2} in the buffer for 30 min, followed by a 15-min oxygenated recovery period alone or in the presence of 50 μM pravastatin (control, 15 min reoxygenation; pravastatin, 15 min reoxygenation; n = 6 in each group), and a 60-min oxygenated recovery period alone or in the presence of 50 μM pravastatin (control, 60 min reoxygenation; pravastatin, 60 min reoxygenation; n = 6 in each group; fig. 1).

Atrial samples were frozen in liquid nitrogen and stored at −80°C before protein extraction and Western blot analysis. Frozen tissue samples were extracted into extraction buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin-pepstatin A-protinin and homogenized with a polytron. Homogenates were centrifuged at 10,000 g for 15 min, the supernatant was decanted, and protein concentration was determined using the BCA protein assay (Bradford colorimetric method; Bio-Rad, Marnes-la-Coquette, France). Extracted protein samples were reduced with 100 mM threo-1,4-dimercapto-2,3-butanediol and denatured at 95°C for 3 min. Denatured proteins (30 μg/lane) from human atrial tissues were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred on nitrocellulose. Membranes were blocked for 1 h in TRIS-buffered saline Tween buffer (0.02 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.05% Tween 20) containing 5% nonfat dry milk at room temperature.

The membranes were incubated with a rabbit polyclonal antibody recognizing total caspase 3, a rabbit polyclonal an-

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**Fig. 1.** Schematic diagram depicting the experimental protocol. Contracting muscle experimental protocols. In the pravastatin plus inhibitor groups and inhibitor groups, 200 μM L-NG-nitroarginine methyl ester (L-NAME) was administered and 50 μM atractyloside was administered (A). Western blot experimental protocols (B). Atract = atractyloside; Prava = pravastatin.
tibody recognizing phospho-BAD (Ser 112), a rabbit polyclonal antibody recognizing BAD total, a rabbit polyclonal antibody recognizing Bcl-2, and a rabbit polyclonal antibody recognizing Pim-1 (1/1,000 dilution each; Cell Signaling Technology, Ozyme, Saint Quentin Yvelines, France) for 1 h at 4°C. After washing in TRIS-buffered saline Tween, the blots were incubated with a secondary antibody (goat antirabbit, 1/1,000 dilution) coupled to peroxidase (Santa Cruz Technology, Le Perray en Yvelines, France) for 1 h at room temperature. The blots were washed again in TRIS buffered saline Tween, and the bands were detected using chemiluminescence reagent (Pierce Perbio Science, Brebieres, France) before exposure to photography film. The Western blots of each group were stripped and probed again with an antibody against glyceraldehyde 3-phosphate dehydrogenase (1/1,000 dilution; Santa Cruz Technology) to ensure equivalent loading.

The developed films were scanned, and the band densities were quantified using National Institutes of Health Image J software (Research Service Branch, National Institutes of Mental Health, Bethesda, MD).

Statistical Analysis
The primary endpoint of the study was the recovery of FoC at 60 min of reoxygenation (FoC60, expressed as percent of baseline).

Data are expressed as mean ± SD. Baseline values of main mechanical parameters, age, preoperative left ventricular ejection fraction, and FoC were compared by univariate analysis of variance with group factor as the independent variable. Statistical analysis has been performed with Statview software (version 5.0; Delsasoft, Meylan, France). All P values were two-tailed, and a P value of <0.05 was considered significant. If the P value was <0.05, a Bonferroni post hoc analysis was performed. Within-group data were analyzed over time using a two-way analysis of variance for repeated measures and Bonferroni post hoc analysis with group factor and time (baseline, hypoxia 5, 10, 20, 30 min, and reoxygenation 5, 10, 20, 30, 40, 50, and 60 min) as independent variables.

In Western blotting experiments, band densities for protein of interest were normalized to that of the band for glyceraldehyde 3-phosphate dehydrogenase in the same sample. The ratio of phosphorylated-BAD/BAD total and the concentrations of caspase 3, Bcl-2, and Pim-1 kinase were compared by univariate analysis of variance with group factor as the independent variable. If the P value was <0.05, a Bonferroni post hoc analysis was performed. Western blot results are expressed as mean ± SD.

Results
There were no statistical differences between groups for the main patient characteristics and left ventricular ejection fraction (table 1). Fifty-eight human right atrial trabeculae and 24 right atrial appendages were studied. There were no significant differences between the groups in terms of the trabecular length at the apex of the length or active isometric tension curve, cross-sectional area, ratio of resting-to-total force (table 2).

Effect of Pravastatin Administration during Reoxygenation Period
The administration of 5 μM pravastatin did not modify the FoC60 compared with the control group (49 ± 10% of baseline vs. 49 ± 11% of baseline in control group; P = 0.94). The administration of 10 (FoC60: 77 ± 5% of baseline), 50 (FoC60: 86 ± 6% of baseline), and 75 μM pravastatin (FoC60: 88 ± 13% of baseline) induced a significant increase of FoC60 compared with control group (P < 0.001) (fig. 2).

Effect of L-NAME and Atractyloside Pretreatment on Pravastatin-induced Cardioprotection
Pretreatment with L-NAME (FoC60,5 11% of baseline; FoC60,7 11% of baseline; FoC60,5 0.94) modify the FoC60 compared with control group (fig. 3).

Effect of Pravastatin on the Phosphorylation of BAD
No significant difference among groups was found in the expression of total BAD.

Tissue samples obtained at 15 and 60 min of reoxygenation and exposed to 50 μM pravastatin showed a significant increase in the ratio phospho-BAD (Ser 112)/BAD total (table 2).

Effect of Pravastatin on Caspase 3 Expression
At 60 min of reoxygenation, in the control group, caspase 3 expression was significantly decreased compared with that observed at 15 min reoxygenation (±71% in pravastatin 15-min reoxygenation vs. control 15-min reoxygenation and ±87% in pravastatin 60-min reoxygenation vs. control 60-min reoxygenation; P < 0.01) (fig. 4A).

Effect of Pravastatin on Bcl-2 Expression
At 60 min of reoxygenation, in the control group, Bcl-2 expression was significantly decreased compared with that observed at...
Table 1. Patient Demographic Data, Preoperative Drug Treatments, and Preoperative Left Ventricular Ejection Fraction

<table>
<thead>
<tr>
<th>Groups and Heart Disease</th>
<th>Age (yr)</th>
<th>Preoperative Drug Treatments</th>
<th>LVEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control AVR (n = 6); CABG (n = 4)</td>
<td>67 ± 12</td>
<td>ACE (3), bAB (2), BZD (3), CA (2), COR (0), STA (6), NT (1)</td>
<td>63 ± 15</td>
</tr>
<tr>
<td>Pravastatin 5 μM AVR (n = 3); CABG (n = 3)</td>
<td>74 ± 5</td>
<td>ACE (1), bAB (2), BZD (0), CA (2), COR (0), STA (4), NT (2)</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Pravastatin 10 μM AVR (n = 2); CABG (n = 4)</td>
<td>65 ± 13</td>
<td>ACE (3), bAB (3), BZD (2), CA (1), COR (2), STA (5), NT (1)</td>
<td>66 ± 13</td>
</tr>
<tr>
<td>Pravastatin 50 μM AVR (n = 2); CABG (n = 4)</td>
<td>66 ± 20</td>
<td>ACE (5), bAB (4), BZD (1), CA (2), COR (1), STA (4), NT (0)</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>Pravastatin 75 μM AVR (n = 4); CABG (n = 2)</td>
<td>69 ± 8</td>
<td>ACE (3), bAB (2), BZD (1), CA (0), COR (0), STA (5), NT (1)</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Prava + L-NAME AVR (n = 3); CABG (n = 3)</td>
<td>70 ± 7</td>
<td>ACE (4), bAB (3), BZD (1), CA (2), COR (3), STA (5), NT (1)</td>
<td>55 ± 16</td>
</tr>
<tr>
<td>Prava + Atract AVR (n = 3); CABG (n = 3)</td>
<td>61 ± 11</td>
<td>ACE (2), bAB (2), BZD (2), CA (0), COR (0), STA (3), NT (1)</td>
<td>73 ± 9</td>
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<tr>
<td>L-NAME AVR (n = 3); CABG (n = 3)</td>
<td>67 ± 8</td>
<td>ACE (3), bAB (2), BZD (1), CA (4), COR (0), STA (5), NT (1)</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>Atract AVR (n = 5); CABG (n = 1)</td>
<td>66 ± 10</td>
<td>ACE (1), bAB (1), BZD (0), CA (4), COR (2), STA (4), NT (0)</td>
<td>67 ± 12</td>
</tr>
<tr>
<td>Western blot: contol 15-min reox AVR (n = 2); CABG (n = 4)</td>
<td>63 ± 15</td>
<td>ACE (3), bAB (5), BZD (2), CA (0), COR (0), STA (5), NT (0)</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>Western blot: prava 15-min reox AVR (n = 2); CABG (n = 4)</td>
<td>64 ± 10</td>
<td>ACE (2), bAB (4), BZD (2), CA (1), COR (0), STA (4), NT (1)</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Western blot: control 60-min reox AVR (n = 3); CABG (n = 3)</td>
<td>72 ± 11</td>
<td>ACE (4), bAB (5), BZD (2), CA (2), COR (0), STA (4), NT (0)</td>
<td>70 ± 11</td>
</tr>
<tr>
<td>Western blot: prava 60-min reox AVR (n = 3); CABG (n = 3)</td>
<td>73 ± 7</td>
<td>ACE (2), bAB (4), BZD (0), CA (0), COR (0), STA (5), NT (1)</td>
<td>59 ± 10</td>
</tr>
</tbody>
</table>

Age and left ventricular ejection fraction are expressed as mean ± SD. The numbers in parentheses after heart disease and drug abbreviations indicate the number of patients.

ACE = angiotensin-converting enzyme inhibitors; Atract = atracyloside; AVR = aortic valve replacement; BZD = β-adrenergic blocking drugs; BZD = benzodiazepine; CA = calcium channel antagonists; CABG = coronary artery bypass graft; COR = amiodarone; L-NAME = L-NG-nitroarginine methyl ester; LVEF = preoperative left ventricular ejection fraction; NT = nitroglycerin; Prava = pravastatin; reox = reoxygenation; STA = statins.

At 15 min of reoxygenation (−32%) in control 60-min reoxygenation vs. control 15-min reoxygenation; P < 0.01).

At 60 min of reoxygenation, the concentration of Bcl-2 was enhanced in the presence of 50 μM pravastatin compared with the respective control group (+53% in pravastatin 60-min reoxygenation vs. control 60-min reoxygenation; P < 0.01), suggesting that 50 μM pravastatin abolished the decrease of Bcl-2 expression observed in the control group (fig. 4C).

Effect of Pravastatin on Pim-1 Expression
At 15 min of reoxygenation, as well as at 60 min of reoxygenation, the Pim-1 kinase expression was enhanced in the

Table 2. Control Values of Main Mechanical Parameters of Human Right Atrial Trabeculae

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>L_max (mm)</th>
<th>CSA (mm²)</th>
<th>FoC (mN/mm²)</th>
<th>RF/TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>6.7 ± 1.2</td>
<td>0.54 ± 0.19</td>
<td>21 ± 12</td>
<td>0.35 ± 0.12</td>
</tr>
<tr>
<td>Pravastatin 5 μM (n = 6)</td>
<td>5.0 ± 0.6</td>
<td>0.41 ± 0.15</td>
<td>28 ± 14</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>Pravastatin 10 μM (n = 6)</td>
<td>6.3 ± 1.7</td>
<td>0.52 ± 0.15</td>
<td>20 ± 14</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>Pravastatin 50 μM (n = 6)</td>
<td>6.8 ± 1.2</td>
<td>0.42 ± 0.19</td>
<td>29 ± 18</td>
<td>0.34 ± 0.11</td>
</tr>
<tr>
<td>Pravastatin 75 μM (n = 6)</td>
<td>5.8 ± 0.8</td>
<td>0.42 ± 0.07</td>
<td>19 ± 5</td>
<td>0.37 ± 0.12</td>
</tr>
<tr>
<td>Prava + L-NAME (n = 6)</td>
<td>5.8 ± 1.2</td>
<td>0.48 ± 0.20</td>
<td>20 ± 10</td>
<td>0.39 ± 0.10</td>
</tr>
<tr>
<td>Prava + Atract (n = 6)</td>
<td>5.4 ± 1.5</td>
<td>0.43 ± 0.19</td>
<td>25 ± 5</td>
<td>0.29 ± 0.09</td>
</tr>
<tr>
<td>L-NAME (n = 6)</td>
<td>5.6 ± 1.6</td>
<td>0.58 ± 0.13</td>
<td>16 ± 7</td>
<td>0.36 ± 0.13</td>
</tr>
<tr>
<td>Atract (n = 6)</td>
<td>6.0 ± 1.6</td>
<td>0.59 ± 0.15</td>
<td>20 ± 8</td>
<td>0.28 ± 0.05</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

Atract = atracyloside; CSA = cross-sectional area; FoC = isometric force of contraction normalized per cross-sectional area; L_max = maximal length at the apex of the length-active force curve; L-NAME = L-NG-nitroarginine methyl ester; Prava = pravastatin; RF/TF = ratio of resting force to total force.

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Discussion

This study showed that pravastatin administered during reoxygenation enhanced the recovery of FoC of isolated human myocardium exposed to hypoxia reoxygenation in vitro. The cardioprotection elicited by pravastatin involved nitric oxide production and prevented the mPTP opening. Moreover, pravastatin preserved the myocardium against the caspase 3 activation, increased BAD phosphorylation, and activated the Bcl-2 and Pim-1 kinase expression during the reoxygenation period.

Clinical trials with pravastatin have shown reduction in myocardial infarct size beyond those predicted by reductions in low-density lipoprotein cholesterol. Similar benefits were observed in patients without hypercholesterolemia after myocardial infarction. Importantly, a double-blind, placebo-controlled study showed that perioperative treatment with statins improved postoperative cardiac outcome in patients undergoing vascular surgery. It has been shown that pretreatment with pravastatin results in a significant gain in tolerance to ischemia during angioplasty. In contrast, data from experimental models of myocardial ischemia reperfusion are contradictory. In rabbits undergoing myocardial infarction through reversible coronary artery occlusion, preschismic but not prereperfusion treatment with pravastatin reduced the myocardial infarct size. However, it is well recognized that the cardioprotection window during reperfusion starts within seconds of the reperfusion and lasts a few minutes. Thus, it has been reported that pravastatin administered at the beginning of the reoxygenation period conferred cardioprotection in neonatal rat cardiomyocytes and isolated human cardiomyocytes obtained from children undergoing elective surgery for congenital heart disease. However, because important functional and structural differences exist between young and old myocardium, these results cannot be extrapolated to adult or senescent myocardium.

Thus, age-related differences have been suggested in the cardioprotective effects of volatile anesthetics on isolated human myocardocytes. In addition, experiments using isolated cardiomyocytes may have some limits, including the lack of functional gap junctions, fibroblasts, and endothelial cells, and the impossibility of studying myocardial contractility, which is a major issue in ischemia reperfusion injury. The current study shows that pravastatin administered during reoxygenation enhanced the recovery of FoC of isolated human myocardium exposed to hypoxia reoxygenation in vitro. Furthermore, a concentration-dependent cardioprotective effect was suggested because 5 μM pravastatin did not modify the recovery of FoC compared with that of the control group. In contrast, 10, 50, and 75 μM pravastatin enhanced the recovery of FoC at the end of the reoxygenation period. In accordance, Sanada et al. showed that pravastatin dose-dependently reduced the infarct size. Nevertheless, because of a statistical type 2 error, the current study cannot definitely conclude on the lack of difference between the effect measured at 10 μM and that measured at 50 and 75 μM.
The mechanisms involved in statin-induced cardioprotection at reperfusion remain incompletely understood. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production.

**Fig. 4.** Western blotting and densitometry results. Western blot analysis showing concentrations of total BAD, phosphorylated BAD (Ser 112) (A), caspase 3 (B), Bcl-2 (C), and Pim-1 kinase (D) after 15 min of reoxygenation alone (control 15-min reoxygenation, n = 6) or in the presence of 50 μM pravastatin (Prava 15-min reoxygenation, n = 6) and after 60 min of reoxygenation alone (control 60-min reoxygenation, n = 6) or in the presence of pravastatin 50 μM (Prava 60-min reoxygenation, n = 6). Equal loading was confirmed by Western blot with an antiglyceraldehyde 3-phosphate dehydrogenase antibody. The relative total BAD, phospho-BAD, caspase 3, Bcl-2, and Pim-1 kinase protein concentrations were calculated by averaging the results obtained from six independent experiments for each time. Data are presented as the mean ± SD. *P < 0.01 versus control 15-min reoxygenation group. #P < 0.01 versus control 60-min reoxygenation group. §P < 0.05 versus control 15-min reoxygenation group. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; Prava = pravastatin; reox = reoxygenation.

The mechanisms involved in statin-induced cardioprotection at reperfusion remain incompletely understood. It has been shown that the cardioprotective effect of pravastatin was dependent on nitric oxide production. The current results show that the cardioprotective effect of pravastatin was abolished in the presence of L-NAME, suggesting that nitric oxide production was, at least in part, involved. A growing body of evidence suggests that mitochondrion is a major component of cellular cardioprotective pathways. Although the role of mitochondrial adenosine triphosphate-sensitive potassium channels in statin-induced cardioprotection has been suggested, the involvement of mPTP has never been investigated. The key role of mPTP in cardioprotection was established by Argaud et al. in rabbits in vivo, showing that administration of mPTP inhibitors at the time of the reperfusion significantly decreased infarct size.
attracyloside, an mPTP opener, indirectly suggesting that pravastatin would prevent the opening of the mPTP during the reoxygenation period. These data provide additional insight into the mechanisms of the cardioprotective effects of pravastatin, but additional studies using isolated mitochondria are required to confirm our results.

Because mitochondrion has also been shown to play a critical role in apoptosis, we investigated whether the inhibition of apoptosis could contribute to the pravastatin-induced cardioprotection. The current Western blot findings show that the expression of total caspase 3 was significantly decreased at 60 min of reoxygenation, which may be related to reoxygenation-induced caspase 3 cleavage. Pravastatin prevented the decrease in total caspase 3 expression, suggesting that it could have decreased the activation of caspase 3 involved in apoptosis. Bergmann et al. have shown that the reduction of the cardiomyocyte apoptosis in the presence of pravastatin was associated with a decrease of caspase-3 activation.20 In addition, the current study showed that the expression of phosphorylated BAD was increased by pravastatin administered during the reoxygenation period. It has been shown that the prosurvival kinase signaling cascades, PI3K/Akt and MEK/ERK1/2, provided protection against apoptosis.21 The phosphorylation of BAD has also been shown to be involved in the proapoptotic effect of Bcl-2.22 Furthermore, the importance of Pim-1 kinase in cardioprotection has been highlighted in Pim-1 KO mice and Pim-1– overexpressing transgenic hearts.32 The current Western blot findings show that pravastatin administration during the reoxygenation period abolished the decrease in Bcl-2 expression in isolated mitochondria.30 The Pim-1 kinase may act, in part, as an upstream regulator of Bcl-2 expression.31 Furthermore, the importance of Pim-1 kinase in cardioprotective pathways has been highlighted in Pim-1 KO mice and Pim-1– overexpressing transgenic hearts.32 Cardiac-specific overexpression of Pim-1 resulted in higher concentrations of Bcl-2 and preserved the mitochondrial integrity.33 The current Western blot results show that pravastatin significantly enhanced Pim-1 kinase expression during the reoxygenation period. Finally, the current results strongly suggest that pravastatin administered at the reoxygenation period enhanced the expression of Pim-1 kinase, phosphorylated BAD, and Bcl-2 expression, which altogether may exert an antiapoptotic effect. Additional studies using specific experimental methods are needed to confirm the antiapoptotic effect suggested.

Several limitations must be considered in the interpretation of the current results. First, the concentrations of pravastatin used in the current experimental study are higher than plasma concentrations measured in patients treated long term.34 Second, the effects of anesthetic drugs,35.36 physiologic conditions, including other disease, or medical treatments received by the patients before obtaining the atrial appendages cannot be ruled out, and because of the small sample size and the number of groups, differences in demographic data cannot be excluded. However, it is of interest to examine the effect of cardioprotective strategies in conditions closely resembling the clinical situations. Third, age has been shown to impair the effects of certain cardioprotective strategies (see Boengler et al.).37 Fourth, our experiments are performed under moderate hypothermia (34°C) to ensure stability of trabeculae over time. It has been shown that hypothermia may decrease the mitochondrial adenosine triphosphate-sensitive potassium channel sensitivity.38 However, during surgical procedures moderate hypothermia may occur. Fifth, the specificity of the antagonist used in the current study must be discussed. Although L-NAME is a widely used nonspecific inhibitor of nitric oxide synthases, it has been shown that L-NAME can have inhibitory effects on iron-containing systems (largely present in mitochondria) and may exert antioxidant actions. Our data strongly suggest a key role for mPTP inhibition in pravastatin-induced cardioprotection. Nevertheless, it has been reported that attracyloside not only opens mPTP but also inhibits adenosine diphasphate transport by inhibition of adenine nucleotide translocase,39 therefore limiting oxidative phosphorylation. Thus, it would be difficult to differentiate the effects of attracyloside on mPTP opening versus loss of energy production as causative factors in attenuated protection. Our results also require qualification because the actions of pravastatin with or without attracyloside pretreatment on mPTP channel activity in isolated mitochondria were not investigated. Sixth, we studied isolated contracting human atrial trabeculae but not myocardial ventricular infarct size (for ethical reasons). In addition, as described in myocardial preconditioning, the beneficial effects of postconditioning have been described on reperfusion-induced arrhythmias30 and myocardial contractility.41

In conclusion, in isolated human myocardium, pravastatin conferred cardioprotection when administered at reoxygenation. The mechanisms involved in pravastatin-induced cardioprotection include nitric oxide production and inhibition of mPTP opening. Furthermore, antiapoptotic effects of pravastatin are suggested by an increase in BAD phosphorylation, Pim-1 kinase, and Bcl-2 expression and a decrease in total caspase 3 expression.

References

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ANESTHESIOLOGY REFLECTIONS

Egidius’ 1484 De Pulsibus: The First Printed Book on the Pulse

From Cos’ Hippocrates, Pergamon’s Galen, Baghdad’s al-Rhazi, and Kairouan’s Israeli, medical wisdom was passed in Salerno, Italy to Egidius Corboliensis (c.1140–c.1224), who had been born in France as “Pierre-Gilles de Corbeil.” Continuing as a Salernitan medical schoolmaster, Egidius composed 380 lines of Latin verse, his De Pulsibus, as a handwritten mnemonic for students learning Galenic pulsology. Not actually printed until 1484, this Paduan imprinting made extensive use of abbreviations, contractions, and symbols to preserve either verse (dactylic hexameter) or vellum (or, in this case, rag paper). Wasted pages could also be avoided in 1484 by forsaking the then recent invention of the title page for the classic colophon (“summit” or “culmination”) of authorship wedged onto the bottom of the final page (above). In purple beneath the colophon is the stamp of the library which deaccessioned this book and unwittingly provided provenance for the Wood Library-Museum’s copy of the “world’s first printed book on the pulse.” (Copyright © the American Society of Anesthesiologists, Inc.)

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