Simvastatin Reduces Myocardial Injury Undergoing Noncoronary Artery Cardiac Surgery

A Randomized Controlled Trial

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**Objective**—Myocardial injury during cardiac surgery is a major cause of perioperative morbidity and mortality. We determined whether perioperative statin therapy is cardioprotective in patients undergoing noncoronary artery cardiac surgery and the potential mechanisms.

**Methods and Results**—One hundred fifty-one patients undergoing noncoronary artery cardiac surgery were randomly assigned to either a statin group (n=77) or a control group (n=74). Simvastatin (20 mg) was administered preoperatively and postoperatively. Plasma were analyzed for troponin T, isoenzyme of creatine kinase, C-reaction protein, interleukin-6, interleukin-8, creatinine, and blood urea nitrogen. Cardiac echocardiography was performed. Endothelial nitric oxide synthase (eNOS), Akt, p38, heat shock protein 90, caveolin-1, and nitric oxide (NO) in the heart were detected. Simvastatin significantly reduced plasma troponin T, isoenzyme of creatine kinase, C-reaction protein, blood urea nitrogen, creatinine, interleukin-6, interleukin-8, and the requirement of inotropic postoperatively. Simvastatin increased NO production, the expression of eNOS and phosphorylation at serine1177, phosphorylation of Akt, expression of heat shock protein 90, heat shock protein 90 association with eNOS and decreased eNOS phosphorylation at threonine 495, phosphorylation of p38, and expression of caveolin-1. Simvastatin also improved cardiac function postoperatively.

**Conclusion**—Perioperative statin therapy can improve cardiac function and renal function by reducing myocardial injury and inflammatory response through activating Akt-eNOS and attenuating p38 signaling pathways in patients undergoing noncoronary artery cardiac surgery. *(Arterioscler Thromb Vasc Biol. 2012;32:2304-2313.)*

**Clinical Trial Registration**—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01178710.

**Key Words:** cardiopulmonary bypass ■ cardiac surgery ■ myocardial injury ■ statin ■ endothelial nitric oxide synthase

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Despite the advances in cardiopulmonary bypass (CPB) technology and myocardial protection techniques, morbidity and mortality remain high in patients with poor preoperative cardiac function, long surgical times, complicated surgical procedures, or incomplete correction of the anatomic malformation. Myocardial injury during cardiac surgery is the primary contributor to these high mortality and morbidity rates. It is, therefore, necessary to search for approaches to reduce myocardial injury during cardiac surgery.

Statins are well known for their ability to decrease hyperlipidemia and are used in the prevention and treatment of coronary artery disease.1,2 In addition to lowering hyperlipidemia, statins also exert other pleiotropic effects, such as improving endothelial dysfunction, increasing nitric oxide (NO) bioavailability, enhancing antioxidant effects and strengthening anti-inflammatory properties.3 These properties of statins suggest that these drugs have the potential ability to attenuate myocardial injury in patients undergoing cardiac surgery with CPB.

Some retrospective studies have shown that perioperative statin therapy reduced myocardial injury, morbidity, and mortality in patients undergoing both coronary artery bypass grafting and valvular heart surgery.4,5 Statin decreased mortality in patients undergoing cardiac surgery even in the face of normal cholesterol levels, suggesting that the cardiac protective effect of statins may be independent of their ability to reduce lipid levels.6 In contrast, another large retrospective study reported that preoperative statin use was not associated with a reduction of in-hospital mortality or major morbidity after cardiac surgery.7

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.orglookup/suppl/doi:10.1161/ATVBHA.112.252098/-/DC1. Correspondence to Jing-Song Ou, MD, PhD, FCCP, Division of Cardiac Surgery, The First Affiliated Hospital, Sun Yat-sen University, 58 Zhong Shan Er Road, Guangzhou, 510080, P.R. China (e-mail oujs@mail.sysu.edu.cn or oujs2000@yahoo.com) and Zhi-Ping Wang, MD, PhD (email: wangzhi21345@yahoo.com.cn).

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Some small, prospective, randomized studies demonstrated that statins reduced myocardial injury; however, all of those studies were performed in patients undergoing coronary artery bypass grafting. Because patients with coronary artery disease may also benefit from statin's ability to lower hyperlipidemia, it is difficult to confirm that statins really reduce myocardial injury in patients undergoing cardiac surgery with CPB. In addition, although several reports showed that statins decreased the systemic inflammatory response during CPB, reported that acute preoperative statin therapy failed to limit the inflammatory response to CPB in a small randomized trial. Those results prompted the study’s authors to indicate that whether statins protect myocardium during cardiac surgery with CPB remains unclear. The purpose of this single-blinded, randomized, controlled trial was to determine whether statins had cardioprotective effects in patients undergoing cardiac surgery with CPB independent of their ability to reduce lipid levels.

**Patients and Methods**

**Patients, Randomization, and Masking**

Patients referred for elective noncoronary artery cardiac surgery between September 2010 and June 2011 were recruited. Patients with coronary artery disease, contraindications to statins treatment, and women who were gestating or lactating were excluded. Patients <10 years of age were also excluded because it is unknown whether statins are safe for these young patients. Patients with noncyanotic congenital heart disease without pulmonary hypertension were also excluded because these patients can be safely treated with current CPB and myocardial protective techniques. One hundred fifty-one patients were eligible and were randomly assigned to either the statin group (n=77) or control group (n=74) by a random number produced by a computer. Simvastatin (20 mg) was administered every day for 5 to 7 days preoperatively, but not on the day of surgery in the statin group. Then simvastatin was readministered on the second day postoperatively. The control group was administered all of the same routine medications as the statin group, such as digoxin, furosemide, but without statin therapy. The patients, surgeons, anesthesiologists, perfusionists, ultrasound physicians, the individuals collecting the samples, and people performing data analysis were all blinded. This randomized controlled trial was approved by The First Affiliated Hospital, Sun Yat-sen University Ethics Review Board. Informed consent was obtained from all patients enrolled in this trial and the procedures followed were in accordance with institutional guidelines. The trial profile has been summarized in Figure 1.

**Cardiac Function Measurement**

Cardiac echocardiography was performed in patients preoperatively and again 7 days and 1 month postoperatively.

**Nitric Oxide Measurement**

Frozen right atrial appendages from −80°C were pulverized and homogenized for NO measurement. NO was determined spectrophotometrically by measuring total nitrate plus nitrite (NO−3 + NO−2) with a NO detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions using Griess reagent at an absorbance of 550 nm, as previously described. Results were expressed as μmol/g protein.

**Western Analysis**

Western analysis and protein association in the heart (right atrial appendage) were performed using standard protocol, as previously described. Anti-eNOS and anti–heat shock protein 90 (Hsp90)
were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Ser1177-phospho-eNOS (P-eNOS S1177), anti-Thr495-phospho-eNOS (T495), anti-AKT, anti-Thr308-phospho-AKT (P-Akt), and Anti-P38, and phosphorylation of P38 and anti-caveolin-1 were from Cell Signaling Technology (Danvers, MA). Anti-GAPDH was from Proteintech Group (Chicago, IL). eNOS was immunoprecipitated with H-32 antibody (Enzo Life Sciences, Farmingdale, NY) or with anti-eNOS (BD Biosciences, San Jose, CA). Proteins were visualized using a Western blotting luminol reagent (Santa Cruz Biotechnology).

Statistical Methods
Data were presented as means, SD, and IQR. Comparisons between treatment groups were made with the unpaired Student t test or repeated measures ANOVA where appropriate. Categorical data were expressed as frequency and percentage and compared with the Fisher exact test where appropriate. The total area under the curve was calculated using the trapezoidal method for each patient and compared using the t test. Estimations were presented with 95% CIs. A value of *P*≤0.05 was regarded as significant. All statistical analyses was performed using SPSS for windows, version 17.0 (Chicago, IL) and MedCalc (version 11.0.0.0, Frank Schoonjans, Mariakerke, Belgium).

See the online-only Data Supplement for additional details in the Methods section.

Results

General Preoperative Comparison
One hundred fifty-one patients were randomly assigned to either the statin group (n=77) or control group (n=74). In total, 132 patients (68 in the statin group and 64 in the control group) completed the study. Baseline characteristics have been summarized in Table 1.

Operative Characteristics and Postoperative Outcomes
There were no significant differences in the operative characteristics between the 2 groups (Table 1). Preoperative atrial fibrillation was identified in 15 patients in the statin group and 13 patients in the control group. There were no differences in cholesterol levels between the 2 groups before administration of simvastatin. There was a tendency for reduction of total cholesterol and low-density lipoprotein levels in 1 week postoperatively. Dyslipidemia (high levels of total cholesterol, low-density lipoprotein, or triglyceride) was diagnosed in 8 patients in the statin group and 7 patients in the control group. Diabetes mellitus was diagnosed in 4 patients in the statin group and 2 patients in the control group. General preoperative comparison of simvastatin. There was a tendency for reduction of cholesterol levels between the 2 groups before administration of simvastatin. There was a tendency for reduction of total cholesterol and low-density lipoprotein levels in 1 week postoperatively. Dyslipidemia (high levels of total cholesterol, low-density lipoprotein, or triglyceride) was diagnosed in 8 patients in the statin group and 7 patients in the control group. Diabetes mellitus was diagnosed in 4 patients in the statin group and 2 patients in the control group. Preoperative atrial fibrillation was identified in 15 patients in the statin group and 13 patients in the control group. Postoperative development of atrial fibrillation occurred in 1 patient in the control group. One case of preoperative atrial fibrillation disappeared postsurgically in the statin group.

Plasma TnT
Preoperatively, plasma TnT concentrations were <0.01 μg/L in both the groups and increased postoperatively—patients administered perioperative simvastatin therapy released less TnT than the patients who did not receive perioperative statins. Specifically, the mean TnT level was 0.88 μg/L (SD, 0.56; IQR, 0.49–1.12) versus 1.35 μg/L (SD, 1.56; IQR, 0.56–1.48) (*P*=0.020) at 6 hours, 0.81 μg/L (SD, 0.55; IQR, 0.40–1.12) versus 1.22 μg/L (SD, 1.47; IQR, 0.53–1.32) (*P*=0.037) at 12 hours, 0.59 μg/L (SD, 0.44; IQR, 0.29–0.76) versus 0.91 μg/L (SD, 1.08; IQR, 0.38–0.99) (*P*=0.031) at 24 hours, and 0.36 μg/L (SD, 0.29; IQR, 0.15–0.42) versus 0.61 μg/L (SD, 0.74; IQR, 0.29–0.69) (*P*=0.013) at 72 hours in the statin and control groups, respectively (Figure 2A). The total TnT released 72 hours postoperatively was reduced by 36.7%, from 60.33 μg/L (SD, 71.12) in the control group to 38.16 μg/L (SD, 26.58) in the statin group (mean difference was 22.17 [SD, 7.28]; 95% CI, 3.89–40.45; *P*=0.018). After excluding the 15 patients with dyslipidemia, similar results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statin Group</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD), y</td>
<td>45.5 (14.5)</td>
<td>41.5 (18.7)</td>
<td>0.17</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>30 (44.1)</td>
<td>35 (54.7)</td>
<td>0.3</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>38 (55.9)</td>
<td>29 (45.3)</td>
<td></td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>57.6 (20.7)</td>
<td>53.6 (12.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Cardiac function NYHA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, n (%)</td>
<td>8 (11.8)</td>
<td>7 (10.9)</td>
<td>0.94</td>
</tr>
<tr>
<td>II, n (%)</td>
<td>11 (16.2)</td>
<td>12 (18.8)</td>
<td></td>
</tr>
<tr>
<td>III, n (%)</td>
<td>48 (70.6)</td>
<td>43 (67.2)</td>
<td></td>
</tr>
<tr>
<td>IV, n (%)</td>
<td>1 (1.5)</td>
<td>2 (3.1)</td>
<td></td>
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<tr>
<td>Chronic AF, n (%)</td>
<td>15 (22.1)</td>
<td>13 (20.3)</td>
<td>0.83</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>4 (5.9)</td>
<td>2 (3.1)</td>
<td>0.68</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>8 (11.8)</td>
<td>7 (10.9)</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>9 (13.2)</td>
<td>6 (9.4)</td>
<td>0.58</td>
</tr>
<tr>
<td>Ejection fraction (SD), %</td>
<td>62.4 (10.9)</td>
<td>64.3 (8.4)</td>
<td>0.23</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (SD), mm/L</td>
<td>22.5 (19.4)</td>
<td>19.4 (17.1)</td>
<td>0.32</td>
</tr>
<tr>
<td>Lactic acid salt dehydrogenase(SD), U/L</td>
<td>215.9 (75.2)</td>
<td>233.3 (103.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Total operative time (SD), min</td>
<td>257.2 (69.6)</td>
<td>266.9 (100.25)</td>
<td>0.41</td>
</tr>
<tr>
<td>Total cardiopulmonary bypass time (SD), min</td>
<td>109.7 (41.3)</td>
<td>119.8 (53.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Aortic cross-clamp time (SD), min</td>
<td>64.8 (34.1)</td>
<td>72.7 (32.4)</td>
<td>0.18</td>
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<tr>
<td>Postoperative AF, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Usage of intraaortic balloon assistance, %</td>
<td>1 (1.5)</td>
<td>1 (1.6)</td>
<td>1</td>
</tr>
<tr>
<td>Postsurgery cardiac tamponade, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Postsurgery AF disappear, n (%)</td>
<td>1 (1.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Before administration of simvastatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.63±1.19</td>
<td>4.54±0.97</td>
<td>0.7</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.38±0.59</td>
<td>1.23±0.57</td>
<td>0.76</td>
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<tr>
<td>Low-density lipoprotein, mmol/L</td>
<td>2.95±1.03</td>
<td>2.88±0.93</td>
<td>0.72</td>
</tr>
<tr>
<td>High-density lipoprotein, mmol/L</td>
<td>1.15±0.35</td>
<td>1.18±0.28</td>
<td>0.62</td>
</tr>
<tr>
<td>1 week postoperation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>3.86±0.97</td>
<td>4.16±0.90</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.23±0.59</td>
<td>1.18±0.53</td>
<td>0.68</td>
</tr>
<tr>
<td>Low-density lipoprotein, mmol/L</td>
<td>2.41±0.85</td>
<td>2.65±0.77</td>
<td>0.13</td>
</tr>
<tr>
<td>High-density lipoprotein, mmol/L</td>
<td>1.10±0.40</td>
<td>1.06±0.32</td>
<td>0.54</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; NYHA, New York Heart Association.
as those described above were obtained, indicating that statins reduced myocardial injury during CPB independent of their ability to reduce lipid levels (data not shown).

Plasma CKMB
Preoperative plasma CKMB concentrations were not significantly different between the 2 groups (P>0.05). Postoperatively, a significant reduction in plasma CKMB concentrations was identified in patients treated with simvastatin compared with the control group (Figure 2B). The total CKMB released 72 hours postoperatively was reduced by 27.6%, from 1618.68 μg/L (SD, 1232.15) in the control group to 1171.55 μg/L (SD, 776.86) in the statin group. The mean difference was 447.12 μg/L (SD, 31.99; 95% CI, 94.61–799.64; P=0.013).

Plasma CRP
There were no significant differences in preoperative plasma concentrations of CRP between both the groups. In contrast, less amounts of CRP release were observed in the statin group in comparison with the control group postoperatively (Figure 2C). After excluding the patients with dyslipidemia, the same significant differences were obtained (data not shown).

Blood Urea Nitrogen, Serum Creatinine, Plasma IL-6, and IL-8
There were no significant differences between the control group and the statin group preoperatively in BUN. BUN was dramatically increased postoperatively both in the control group and the statin group (P<0.05). However, statin reduced BUN concentration in the statin group compared with the control group at 24, 48, and 72 hours postoperatively (P<0.05; Figure 3A). There was no significant difference between the control group and the statin group preoperatively in serum creatinine. However, creatinine was dramatically increased postoperatively in the control group but not in the statin group (P<0.05). Statin reduced creatinine release compared with the control group at 24, 48, and 72 hours postoperatively (P<0.05). Interestingly, creatinine at 7 days postoperatively is even lower than that of preoperation in the statin group (P<0.05; Figure 3B). The increase of plasma IL-6 and IL-8 postoperatively was dramatically less in the statin group compared with the control group (Figure 3C and 3D).

NO Generation
There was a tendency for reduction in the control group and a tendency of enhancement in the statin group in concentration of NO2− +NO3− postoperatively. Furthermore, the concentration of NO2− +NO3− generation was significantly higher in the statin group than that of the control group both preoperation and postoperation (Figure 3E). Additional experiment showed similar result after using NG-methyl-L-arginine monoacetate to block NO3− release from fresh heart tissues (Figure I in the online-only Data Supplement).

Inotropic Requirement and Left Ventricular Ejection Fraction
There was a significantly greater inotropic requirement in the control group compared with the statin group at both 6 hours (5.5±3.2 μg/kg per minute versus 4.1±2.6 μg/kg per minute, P=0.008) and 12 hours (4.9±3.2 μg/kg per minute versus 3.5±2.5 μg/kg per minute, P=0.005) postoperatively. There was no significant difference in left ventricular ejection fraction between the control group and the statin group preoperatively (Table 1). Ejection fraction was higher in the statin group than 65.3±7.3% in the control group in 1 month postoperation (P=0.029) and 69.5±6.4% in the statin group than 65.3±7.3% in the control group in 1 month postoperation (P=0.029).

Akt-eNOS, p38 MAPK, Caveolin-1, Hsp90 Expression, and the Association eNOS With Hsp90
Both eNOS expression and phosphorylation at serine 1177 in the heart were dramatically increased in the statin group preoperatively and postoperatively. eNOS phosphorylation at serine 1177 was increased even more after than before surgery. eNOS phosphorylation at threonine 495 was increased

### Table 2. Details of Surgical Procedures

<table>
<thead>
<tr>
<th>Type of Procedure</th>
<th>Statin Group</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVR, n (%)</td>
<td>17 (25)</td>
<td>12 (18.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>AVR, n (%)</td>
<td>10 (14.7)</td>
<td>6 (9.4)</td>
<td>0.43</td>
</tr>
<tr>
<td>MVP, n (%)</td>
<td>2 (2.9)</td>
<td>3 (4.7)</td>
<td>0.67</td>
</tr>
<tr>
<td>ASDR, n (%)</td>
<td>4 (5.9)</td>
<td>2 (3.1)</td>
<td>0.68</td>
</tr>
<tr>
<td>VSDR, n (%)</td>
<td>6 (8.8)</td>
<td>8 (12.5)</td>
<td>0.58</td>
</tr>
<tr>
<td>TVR, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>MVR+AVR, n (%)</td>
<td>7 (10.3)</td>
<td>11 (17.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>MVR+AVR+TVP, n (%)</td>
<td>1 (1.5)</td>
<td>3 (4.7)</td>
<td>0.35</td>
</tr>
<tr>
<td>MVR+TVP, n (%)</td>
<td>8 (11.8)</td>
<td>3 (4.7)</td>
<td>0.21</td>
</tr>
<tr>
<td>MVR+AVP, n (%)</td>
<td>1 (1.5)</td>
<td>1 (1.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>AVR+TVP+MVP, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>MVR+ radiofrequency ablation of atrial fibrillation, n (%)</td>
<td>2 (2.9)</td>
<td>0</td>
<td>0.50</td>
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<tr>
<td>MVR+ left atrial thrombectomy, n (%)</td>
<td>1 (1.5)</td>
<td>0</td>
<td>1.0</td>
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<tr>
<td>ASD+MVP, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Repair of TOF, n (%)</td>
<td>1 (1.5)</td>
<td>2 (3.1)</td>
<td>0.61</td>
</tr>
<tr>
<td>Repair of TOF + ligation of PDA, n (%)</td>
<td>2 (2.9)</td>
<td>0</td>
<td>0.50</td>
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<tr>
<td>DORV, n (%)</td>
<td>1 (1.5)</td>
<td>1 (1.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>VSD + repair of right ventricular outflow obstruction, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Repair of partial endocardial cushion defect, n (%)</td>
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<td>2 (3.1)</td>
<td>0.23</td>
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<tr>
<td>Correction of coarctation aorta+ MVP, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Removal of left atrial myxoma, n (%)</td>
<td>4 (5.9)</td>
<td>3 (4.7)</td>
<td>1.0</td>
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<tr>
<td>Bentall procedure, n (%)</td>
<td>1 (1.5)</td>
<td>1 (1.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Bentall procedure +MVP, n (%)</td>
<td>0</td>
<td>1 (1.6)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

| MVR indicates mitral valve replacement; AVR, aortic valve replacement; MVP, mitral valvuloplasty; ASDR, atrial septal defect repair; VSDR, ventricular septal defect repair, AVP, aortic valvuloplasty; TVR, tricuspid valve replacement; TVP, tricuspid valvuloplasty; TOF, tetralogy of Fallot; PDA, patent ductus arteriosus; DORV, double outlet right ventricle. |

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Akt phosphorylation was decreased postoperatively in the control group and increased both preoperatively and postoperatively in the statin group (Figure 4B). Statin inhibited p38 mitogen–activated protein kinase (MAPK) phosphorylation postoperatively (Figure 4C). There was no difference in caveolin-1 expression before and after surgery in the control group and between the control group and the statin group preoperatively. However, Hsp90 expression was significantly increased postoperatively in the statin group (Figure 4D). The Hsp90 association with eNOS was reduced postoperatively in the control group. There was no difference in Hsp90 association with eNOS before and after surgery in the statin group and between the control group and the statin group preoperatively. The Hsp90 association with eNOS was higher in the statin group than the control group postoperatively (Figure 4E).

**Discussion**

This is the first single-blinded, randomized controlled trial exploring the effects of perioperative statin therapy in patients undergoing noncoronary artery cardiac surgery with CPB.
Figure 3. Simvastatin decreased blood urea nitrogen (BUN), creatinine concentrations, plasma interleukin L (IL)-6, and IL-8 and increased nitric oxide (NO) production in the heart. Plasma BUN, creatinine, IL-6, and IL-8 before surgery and at different time points after surgery were measured according to the manufacturer’s instructions. A, BUN was dramatically increased postoperatively both in the control group and the statin group (*vs control preoperatively (preop); & vs statin preop; P < 0.05). Statin reduced BUN postoperatively (&vs control group; P < 0.05). B, Creatinine was dramatically increased postoperatively in control group but not in the statin group (*vs control preop; P < 0.05). Statin reduced creatinine postoperatively (*vs control group; P < 0.05). Creatinine at 7 days postoperatively is lower than that at 7 days preoperatively in statin group (*vs statin preop; P < 0.05). C and D, Simvastatin reduced the increase of plasma IL-6 and IL-8 (*P < 0.05). E, NO generation was measured by detecting the nitrite+nitrate concentration in the right atrium. Simvastatin increased NO production in the heart both in preoperation and postoperation (*vs control; P < 0.05) (mean±SEM).
Figure 4. Simvastatin activated Akt–endothelial nitric oxide (eNOS) and attenuated p38 mitogen–activated protein kinase (MAPK) pathway in heart. Proteins expression and association in right atrium were measured. **A**, Statin increased eNOS phosphorylation at serine 1177 (S1177) site (*vs control; *vs statin preop; *P* <0.05). eNOS phosphorylation in threonine 495 (T495) site was increased postoperatively and decreased by statin (*vs control; *vs control preoperatively (preop); *P* <0.05). Statin increased eNOS expression (*vs control; *P* <0.05). **B**, Akt phosphorylation was decreased postoperatively and statin increased Akt phosphorylation both preoperatively and postoperatively (*vs control; *vs control preop, *P* <0.05). **C**, Statin decreased p38 phosphorylation postoperatively without altering p38 expression (*vs control; *vs statin preop; *P* <0.05). **D**, Statin significantly decreased caveolin-1 expression and slightly increased heat shock protein (Hsp90) expression postoperatively (*vs control; *vs statin preop; *P* <0.05). **E**, Hsp90 association with eNOS in the heart was reduced postoperatively and was restored by statin (*vs control; *vs control preop, *P* <0.05) (n=64–68; mean±SEM). p-eNOS indicates phosphorylated eNOS; p-Akt, phosphorylated Akt; postop, postoperatively; IB, immunoblot.
Figure 4. (continued)
In light of the fact that the patients, surgeons, anesthetist, perfusionist, ultrasound physicians, and individuals collecting and analyzing the blood samples and analyzing the data were also all blinded, this randomized controlled study closely approached a double-blind randomized trial in design. Previous studies indicate that elevations in TnT and CKMB after cardiac surgery are associated with poor short- and long-term clinical outcomes.\(^1\) The present study found that perioperative simvastatin therapy significantly decreases plasma TnT, CKMB, CRP, IL-6, IL-8, BUN, creatinine, the requirement of inotropic and improves left ventricular ejection fraction, suggesting that perioperative simvastatin therapy can reduce myocardial injury and inflammatory response and improve cardiac and renal function in noncoronary artery cardiac surgery with CPB.

Beneficial effects of statin pretreatment have already been demonstrated in various studies in patients undergoing both coronary artery surgery and cardiac percutaneous interventions.\(^2\) However, although these studies indicate that statins reduce myocardial injury during the intervention of coronary artery disease, it is not possible to conclude from those reports whether the reduction in myocardial injury was associated with the decrease in lipid level because statins are designed to treat coronary artery disease. In other words, can statins protect against myocardial injury during CPB? Indeed, 1 large, retrospective study showed that statins had nothing to do with myocardial protection during cardiac surgery. To clarify this controversy, 15 dyslipidemia patients were excluded from the analysis in the current study and similar results were attained. Therefore, although simvastatin decreased cholesterol level, our findings can demonstrate that the myocardial protective effect of statins during cardiac surgery maybe independent of their ability to reduce lipid levels as the patients’ cholesterol levels are normal before administration of simvastatin.

The mechanism by which statins reduce myocardial injury remains unclear. It is known that excessive systemic inflammatory responses unleashed by CPB leads to myocardial injury. Some studies showed that statins decreased the systemic inflammatory response, which, in turn, may contribute to a decrease in myocardial injury.\(^9,13\) However, Florens et al\(^10\) reported that acute preoperative statin therapy failed to limit the inflammatory response to CPB in a small randomized trial; this study only used a single dose statin. In the present study, we found that perioperative simvastatin therapy reduced concentration of IL-6, IL-8, and CRP, demonstrating that statins can inhibit systemic inflammatory response to reduce myocardial injury.\(^14,15\) It is well known that the p38 MAPK pathway participate in the inflammatory response.\(^16,17\) Our data showed that simvastatin decreased p38 MAPK phosphorylation, suggesting that one of the mechanisms by which statins reduces myocardial injury is by limiting the inflammatory response by attenuating the p38 MAPK pathway in noncoronary artery cardiac surgery with CPB.

Previous studies showed that statins can improve endothelial dysfunction by protecting tissues from the damage that results from ischemia and reperfusion during cardiac surgery.\(^18\) Statin is able to increase vascular endothelial NO production (by modulating NOS expression) and diminish myocardial necrosis after ischemia and reperfusion in mice, contributing to cardioprotective effects.\(^19,21\) In the current study, our data showed that eNOS phosphorylation at T495 site was increased and Hsp90 association with eNOS was decreased postoperatively in control, suggesting that not only is the eNOS activation inhibited, but eNOS is also uncoupled during cardiac surgery with CPB.\(^11,22,23\) As a result, eNOS may generate superoxide instead of NO, which leads to endothelial dysfunction and myocardial injury. Importantly, simvastatin increased eNOS expression and phosphorylation at serine 1177 site, decreased eNOS phosphorylation at T495 site preoperatively and postoperatively, demonstrating that statin is able to activate eNOS. Meanwhile, we found that Hsp90 association with eNOS was restored by simvastatin postoperatively, suggesting eNOS was maintained in coupling status to maintain NO generation during cardiac surgery.\(^23,24\) We further found that Hsp90 expression was increased and caveolin-1 expression was decreased by simvastatin. As Hsp90 is the chaperone of eNOS and positively regulates eNOS and caveolin-1 negatively regulates eNOS, our findings further support the idea of statin’s ability in activating eNOS and maintaining eNOS uncoupled activity to generate NO instead of superoxide.\(^11,24,25\)

Although NO decreased during surgery in the control group, it did not reach statistical significance and there was a tendency for the reduction of NO production postoperatively (P=0.08). More importantly, simvastatin was able to stimulate NO generation compared with control group. Recent findings from Antoniades et al\(^26\) showing that perioperative statin therapy reduced myocardial superoxide generation in cardiac surgery also support our findings. Taken together, our findings demonstrated that statin can stimulate the heart to generate NO and decrease superoxide generation to limit oxidative stress in the heart and avoid injury before and during cardiac surgery. Such findings can explain why statins reduce myocardial injury and improve cardiac and renal function during cardiac surgery.

It is known that Akt phosphorylation is the key step of eNOS activation.\(^27,28\) We found that Akt phosphorylation in heart was decreased postoperatively and was increased by simvastatin preoperatively and postoperatively. These data demonstrate that statin can activate Akt-eNOS signaling pathway before and after cardiac surgery.

As simvastatin can improve endothelial function, it is possible that perioperative simvastatin therapy also improves the systemic vascular endothelial function. Our data showing less inotropic requirement postoperatively in the statin group indicate that simvastatin can not only reduce myocardial injury but may improve systemic vascular function.

In summary, our study shows that perioperative simvastatin therapy significantly reduced myocardial injury and inflammatory response and improved cardiac and renal function in patients undergoing noncoronary artery cardiac surgery with CPB by activating Akt-eNOS and attenuating p38 MAPK signaling pathway. Such cardiac protection of simvastatin is independent of its ability to reduce lipid levels. Our findings may provide a novel approach to reduce myocardial injury during cardiac surgery with CPB.

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Disclosures
None.

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Simvastatin reduces myocardial injury undergoing noncoronary artery cardiac surgery: A randomised controlled trial

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Online supplemental information for additional details on some methods

Surgical Procedures

Operative and anesthetic techniques were standardized in both groups. All surgical procedures were performed by the same group of surgeons. The anesthesia was similar for both groups. Intraoperative myocardial protection techniques were standardized in both groups, using the same cardioplegic solution (hyperkalemic blood cardioplegia). The right atrial appendage tissue was harvested before cross-clamping the aorta to perfuse cardioplegic solution into the heart to stop the heart beating. The right atrial appendage tissue was harvested again at 10 minutes after removing the aorta cross-clamp to re-perfuse the heart to beating when finishing the major surgical procedures in the heart. To avoid impairing the right atrial appendage tissue, superior vena cava cannulation was used to set up the cardiopulmonary bypass. The heart tissues were put in liquid nitrogen immediately after removal and stored in -80 °C later for future use. At the end of surgery, patients
were transferred to the intensive care unit (ICU) where a standardized protocol was followed. Patients were transferred to a ward until discharged from hospital.

**NO measurement in heart:**

Frozen heart samples from -80 °C were pulverized and homogenized five times on ice (10 s with a 30 s intervals between homogenizations). The homogenates were then centrifuged at 2000 r/min for 8 min at 4°C. The supernatant was transferred to a cold microcentrifuge tube for NO assay and protein concentrations determined by a bicinchoninic acid protein assay (Merck, Whitehouse Station, NJ, USA). NO was determined spectrophotometrically by measuring total nitrate plus nitrite (NO$_3^-$ plus NO$_2^-$) with a NO detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, nitrate was enzymatically converted into nitrite by nitrate reductase, and nitrite was quantified using Griess reagent at an absorbance of 550 nm as previously described.$^{1, 2}$ Results were expressed as μmol/g protein.

To rule out the contribution of background nitrite and nitrate, we have performed addition study in fresh right atrial appendage tissues from ten patients with or without prescribed simvastatin before surgery. After removed right atrial appendage, the tissues were immediately put in PBS. And then, the tissues were cultured in HBSS buffer with or without L-NMMA (1 mM, final concentration) for 1 hour as previously described.$^3$ The heart tissues were washed three times with HBSS and cut into small pieces and homogenized. The homogenates were then centrifuged at 2000 r/min for 8 min at 4°C. The supernatant was also transferred to a cold microcentrifuge tube for NO assay and protein concentrations determined by a bicinchoninic acid protein assay.
as mentioned above. The results were showed as L-NMMA inhibitable nitrite production. Results were expressed as μmol/g protein (Figure I, n=5). Our data showed similar results with frozen heart samples (Figure 3E in manuscript) and demonstrated that simvastatin was able to stimulate NO generation compared with control group.

![Figure I Simvastatin increased L-NMMA inhibitable nitrite production in the heart.](image)

**Western Analysis**

Western analysis and protein association in heart (right atrial appendage) were
performed using standard protocol as our previous described \(^4\). Each patient has two samples, one from before cross-clamping the aorta, another from 10 minutes after removal of the aorta cross-clamp. Frozen heart samples from -80 °C were pulverized and placed in a modified RIPA buffer. \(^5\) The mixture was then homogenized, sonicated to break the cells, and the cell debris was removed by centrifugation at 14000g for 10 min at 4°C. The supernatant was transferred to a cold microcentrifuge tube and protein concentrations were determined by a bicinchoninic acid protein assay. The proteins were used for western blot analysis and immunoprecipitation.

For western blot analysis, aliquots (20 µL/mL) were combined with an equal volume of Laemmeli buffer and heated (95°C, 5 min) and stored on ice until fractionated by sodium dodecyl sulfate-polyacrylamide (10%) electrophoresis gel (SDS–PAGE). The proteins were transferred to nitrocellulose membranes and blotted with following antibodies. Anti-eNOS and anti-Hsp90 were from Santa Cruz Biotechnology(USA). Anti-Ser1177-phospho-eNOS(P-eNOS S1177), anti-Thr495-phospho-eNOS(T495), anti-AKT, anti-Thr308-phospho-AKT(P-Akt), Anti-P38 and phosphorylation of P38, anti-caveolin-1 were from Cell Signaling Technology(USA). Anti-GAPDH was from Proteintech Group(USA). Bands were visualized using horseradish peroxidase (HRP)-linked secondary antibodies and a western blotting luminol reagent (Santa Cruz Biotechnology, USA). Images of the bands of interest in the autoradiograms were obtained with an EPSON scanner and Adobe PhotoShop elements 4.0. Densities of the bands were quantified from the scanned images with NIH Image 1.63 software. Relative changes were determined as follows. The band densities for eNOS, Akt, P38,
caveolin-1 and Hsp90 were divided by the band densities for the corresponding GAPDH. The band densities for P-eNOS S1177 and T495 were divided by the band densities for the corresponding eNOS. The band densities for P-Akt were divided by the band densities for the corresponding Akt. The band densities for P-P38 were divided by the band densities for the corresponding P38. The resulting ratios of P-eNOS S1177/eNOS, T495/eNOS, P-Akt/Akt, P-P38/P38, and eNOS/GAPDH, Akt/GAPDH, P38/GAPDH, caveolin-1/GAPDH and Hsp90/GAPDH for the controls (pre-op) were used to calculate relative ratios for all experimental groups, after setting the control ratio to 1. Data are represented mean ± SEM.

To determine the protein interactions between HSP90 and eNOS, an aliquot was removed (500 µg) and the volume adjusted to 100 µg cell protein/100 µl. Aliquots of the lysates were precleared with protein A-Sepharose beads for 2 h to minimize nonspecific binding of cell proteins to the beads used to isolate the eNOS immunocomplex. The precleared supernatant fractions were transferred to another microfuge tube and incubated 24 h with the anti-eNOS antibody, H32 (Enzo Life Sciences, USA; 1 µg/100 µl of lysate) or the anti-eNOS antibody from BD Biosciences (USA; 1 µg/100 µl of lysate) to immunoprecipitate eNOS. The antibody-eNOS immunocomplex was isolated by incubation for 2 h with 60 µl of a 50% slurry of protein A-Sepharose beads. The beads were washed three times with 750 µl of Tris-HCL-buffer saline (TBS), mixed with Laemmeli buffer (60 µl), heated (95°C, 5 min), mixed, and stored on ice until fractionated by 10% SDS–PAGE (20 µl/lane). The separated proteins in the gel were electrotransferred to a nitrocellulose
membrane. The membrane was blocked with 5% nonfat milk in TBS-Tween-20 (0.1%) and immunoblotted for eNOS (Santa Cruz Biotechnology) and Hsp90 (Santa Cruz Biotechnology). Bands were visualized using horseradish peroxidase (HRP)-linked secondary antibodies and a western blotting luminol reagent (Santa Cruz Biotechnology, USA). Images of the bands of interest in the autoradiograms were obtained with an EPSON scanner and Adobe PhotoShop Elements 4.0. Densities of the bands were quantified from the scanned images using NIH Image 1.63 software. Relative changes in Hsp90 association with eNOS in eNOS immunoprecipitates were determined as follows. The band densities for Hsp90 were divided by the band densities for the corresponding eNOS. The resulting ratios for Hsp90/eNOS for the controls (pre-op) were used to calculate relative ratios for the four experimental groups, thereby making Control 1.0. Relative band densities, normalized to control, represent mean ± SEM. 3

References
관동맥 수술이 아닌 심장 수술 환자에서 심바스타틴은 심근 손상을 줄인다.

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Summary

배경
심장 수술 도중의 심근 손상은 수술 전후의 사망과 합병증의 주 원인이다. 수술 전후의 스타틴 사용이 비관상동맥 심장 수술 환자에서 심근 보호 효과가 있는지와 가능한 기전에 대해 연구하였다.

방법 및 결과
비관상동맥 심장 수술을 시행 받은 151명의 환자가 무작위로 스타틴 군(77명)과 대조군(74명)으로 배정되었다. 심바스타틴 20 mg 이 수술 전후로 투여되었다. 혈장 troponin과 CK isoenzyme (isoenzyme of creatine kinase), CRP (C-reaction protein), IL6 (interleukin-6), IL8 (interleukin-8), creatinine (Cr)과 BUN (blood urea nitrogen)이 측정되었고 심장 초음파도 함께 시행되었다. 심장의 endothelial nitric oxide synthase (eNOS), Akt, p38, heat shock protein 90, caveolin-1, nitric oxide (NO)도 측정되었다. 심바스타틴은 혈장 troponin과 CK isoenzyme, CRP, BUN, Cr, IL6, IL8과 수술 후의 승압제 사용의 필요성을 감소시켰다. 또한 NO와 eNOS의 발현과 serine 1177에서의 인산화, AKT의 인산화, heat shock protein 90의 발현, eNOS와 연관된 heat shock protein 90의 발현을 증가시켰다. 반면 Threonine 495에서의 eNOS의 인산화, p38의 인산화, caveolin-1의 발현은 감소시켰고 심바스타틴은 수술 후의 심장 기능을 개선시켰다.

결론
수술 전후의 스타틴 치료는 비관상동맥 심장 수술을 시행 받은 환자에서 Akt-eNOS 활성화와 p38 신호 전달 체계의 악화를 통하여 염증반응과 심근 손상을 줄이고, 심장과 신장 기능을 향상시킨다.
본 연구는 심장 수술(관상동맥수술 제외) 환자에서 수술 전후의 심바스타틴 사용이 수술로 인한 심근 손상을 감소시키고, 그 주된 기전이 Akt-eNOS, p38 MAPK signaling에 의한 것임을 주장하고 있다. 스타틴의 LDL-C 감소 효과 이외에 소위 pleiotropic effect는 잘 알려져 있는데, 주 기전이 항산화, 항염증, 항혈관관련 고위험 환자의 일차, 이차 예방은 물론, 저위험 환자의 일차 예방으로부터 고위험군인 ST부절 상승 심근경색 환자의 심근 손상 감소 가능성 등 매우 폭넓은 스타틴의 효과들이 확인되었다. 특히 최근의 연구들은 급성기의 심근 손상 즉, ACS (acute coronary syndrome) 환자에서 고용량 스타틴이 효과적임 또는 여전한 가능성을 보인다. 즉, 단기간 고용량 스타틴 사용을 통하여 PCI (percutaneous coronary intervention)를 시행 받은 STEMI, NSTEMI 환자에서 시술로 인한 심근경색의 크기 감소 및 예후 개선 가능성을 보인바 있으며 추가적으로 그 환자들이 심기능 저하 예방에서도 효과적임 가능성을 제기하고 있다. 이는 주로 심근경색증과 같은 질병 상태에서의 급격한 혈액학적 변화와 심한 염증반응에 의한 상태에서 관상동맥 조영술을 시행하는 경우, 조영제 유발 신독성이 병발하게 되어 심기능 심장 수측 기능 등이 저하되며, 이로 인한 심기능 저하에도 스타틴의 단기간 고용량 사용이 효과적이 될 수 있다는 증거들이 발표되고 있는 것이다.

Coronary bypass 수술, 특히 cardiopulmonary bypass를 시행하는 수술에서도 수술 전후의 스타틴 사용이 수술 후 심근 기능을 보존하는데 도움을 준다는 후방적 관찰 연구 및 소규모 전향적 연구들이 있어왔으나, 판막 수술이나 심장 중앙 수술, 성인 선천성 심기형 수술과 같은 비관상동맥 심장 수술에서 도 과연 효과적일지는 잘 알려져 있지 않았다. 이론적으로도 관상동맥질환의 주 기전이 즉시성과 고 혈압의 억제 기능을 스타틴이 가지고 있는바, 관상동맥 수술에서의 효과는 당연한 것일 수 있으나 과연 비관상동맥 수술에서 효과적일지는 의문시 되었고 이를 증명한다면 스타틴의 pleiotropic 효과를 다시 한번 증명할 수 있는 것이다. 본 연구는 그런 의미에서 스타틴의 pleiotropic 효과를 간접적으로 입증하고 심근 손상과 신장 손상 감소시킨 결과를 보여서 향후 임상진료에도 영향을 끼칠 수 있는 중요한 연구 성과이다.

이전의 소규모 후향적 연구들의 대립되는 스타틴의 효과는 스타틴의 투여 방법, 기간, 종류가 달라 상이한 결과를 나왔으리라 생각되며, 대개는 소규모 연구라는 점 때문에 다양한 결과가 나왔다고 판단된다. 본 연구도 비록 적은 환자를 대상으로 하였으나 이 연구를 동시에 바탕내 이론적인 평가를 하였기 때문에 향후 대규모 outcome 연구를 시행한다면 긍정적 결과가 나올 가능성이 있다고 생각한다. 향후 스타틴 이외에 수술 전후 심근을 보호하기 위한 mechanical한 방법으로 preconditioning법과의 비교와 병용 예방법 연구도 좋은 연구 주제라고 생각하며, 여러 추가적인 긍정적 연구 결과가 나온다면 심장 수술 후의 심근 손상 예방을 위한 스타틴의 임지는 보다 확고해질 것이다.

REFERENCE
Simvastatin Reduces Myocardial Injury Undergoing Noncoronary Artery Cardiac Surgery
A Randomized Controlled Trial

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Objective—Myocardial injury during cardiac surgery is a major cause of perioperative morbidity and mortality. We determined whether perioperative statin therapy is cardioprotective in patients undergoing noncoronary artery cardiac surgery and the potential mechanisms.

Methods and Results—One hundred fifty-one patients undergoing noncoronary artery cardiac surgery were randomly assigned to either a statin group (n=77) or a control group (n=74). Simvastatin (20 mg) was administered preoperatively and postoperatively. Plasma were analyzed for troponin T, isoenzyme of creatine kinase, C-reaction protein, interleukin-6, interleukin-8, creatinine, and blood urea nitrogen. Cardiac echocardiography was performed. Endothelial nitric oxide synthase (eNOS), Akt, p38, heat shock protein 90, caveolin-1, and nitric oxide (NO) in the heart were detected. Simvastatin significantly reduced plasma troponin T, isoenzyme of creatine kinase, C-reaction protein, blood urea nitrogen, creatinine, interleukin-6, interleukin-8, and the requirement of inotropic postoperatively. Simvastatin increased NO production, the expression of eNOS and phosphorylation at serine1177, phosphorylation of Akt, expression of heat shock protein 90, heat shock protein 90 association with eNOS and decreased eNOS phosphorylation at threonine 495, phosphorylation of p38, and expression of caveolin-1. Simvastatin also improved cardiac function postoperatively.

Conclusion—Perioperative statin therapy can improve cardiac function and renal function by reducing myocardial injury and inflammatory response through activating Akt-eNOS and attenuating p38 signaling pathways in patients undergoing noncoronary artery cardiac surgery. (Arterioscler Thromb Vasc Biol. 2012;32:2304-2313.)

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01178710.

Key Words: cardiopulmonary bypass ☐ cardiac surgery ☐ myocardial injury ☐ statin ☐ endothelial nitric oxide synthase

Despite the advances in cardiopulmonary bypass (CPB) technology and myocardial protection techniques, morbidity and mortality remain high in patients with poor preoperative cardiac function, long surgical times, complicated surgical procedures, or incomplete correction of the anatomic malformation. Myocardial injury during cardiac surgery is the primary contributor to these high mortality and morbidity rates. It is, therefore, necessary to search for approaches to reduce myocardial injury during cardiac surgery.

Statins are well known for their ability to decrease hyperlipidemia and are used in the prevention and treatment of coronary artery disease. In addition to lowering hyperlipidemia, statins also exert other pleiotropic effects, such as improving endothelial dysfunction, increasing nitric oxide (NO) bioavailability, enhancing antioxidant effects and strengthening anti-inflammatory properties. These properties of statins suggest that these drugs have the potential ability to attenuate myocardial injury in patients undergoing cardiac surgery with CPB.

Some retrospective studies have shown that perioperative statin therapy reduced myocardial injury, morbidity, and mortality in patients undergoing both coronary artery bypass grafting and valvular heart surgery. Statin decreased mortality in patients undergoing cardiac surgery even in the face of normal cholesterol levels, suggesting that the cardiac protective effect of statins may be independent of their ability to reduce lipid levels. In contrast, another large retrospective study reported that preoperative statin use was not associated with a reduction of in-hospital mortality or major morbidity after cardiac surgery.
Some small, prospective, randomized studies demonstrated that statins reduced myocardial injury; however, all of those studies were performed in patients undergoing coronary artery bypass grafting.\textsuperscript{8} Because patients with coronary artery disease may also benefit from statin’s ability to lower hyperlipidemia, it is difficult to confirm that statins really reduce myocardial injury in patients undergoing cardiac surgery with CPB. In addition, although several reports showed that statins decreased the systemic inflammatory response during CPB,\textsuperscript{3} Florens et al\textsuperscript{10} reported that acute preoperative statin therapy failed to limit the inflammatory response to CPB in a small randomized trial. Those results prompted the study’s authors to indicate that whether statins protect myocardium during cardiac surgery with CPB remains unclear. The purpose of this single-blinded, randomized, controlled trial was to determine whether statins had cardioprotective effects in patients undergoing cardiac surgery with CPB independent of their ability to reduce lipid levels.

**Patients and Methods**

**Patients, Randomization, and Masking**

Patients referred for elective noncoronary artery cardiac surgery between September 2010 and June 2011 were recruited. Patients with coronary artery disease, contraindications to statins treatment, and women who were gestating or lactating were excluded. Patients <10 years of age were also excluded because it is unknown whether statins are safe for these young patients. Patients with noncyanotic congenital heart disease without pulmonary hypertension were also excluded because these patients can be safely treated with current CPB and myocardial protective techniques. One hundred fifty-one patients were eligible and were randomly assigned to either the statin group (n=77) or control group (n=74) by a random number produced by a computer. Simvastatin (20 mg) was administered every day for the 5 to 7 days preoperatively, but not on the day of surgery in the statin group. Then simvastatin was readministered on the second day postoperatively. The control group was administered all of the same routine medications as the statin group, such as digoxin, furosemide, but without statin therapy. The patients, surgeons, anesthetists, perfusionists, ultrasound physicians, the individuals collecting the samples, and people performing data analysis were all blinded. This randomized controlled trial was approved by The First Affiliated Hospital, Sun Yat-sen University Ethics Review Board. Informed consent was obtained from all patients enrolled in this trial and the procedures followed were in accordance with institutional guidelines. The trial profile has been summarized in Figure 1.

**Blood Analysis**

All patients included in the study underwent routine preoperative examinations. Additionally, plasma troponin-T (TnT) and the isoenzyme of creatinine kinase (CKMB) levels were measured preoperatively and at 6, 12, 24, and 72 hours postoperatively via electrochemiluminescence technology (Roche Diagnostics GmbH, Mannheim, Germany). C-reaction protein (CRP), blood urea nitrogen (BUN), and serum creatinine were measured preoperatively and at 24, 48, 72 hours and 7 days postoperatively via turbidimetry technology (Orion Diagnostica, Espoo, Finland), speed enzyme coupling method (Biosino Biotechnology and Science Inc, Beijing, China) and enzymatic method (Sekisui Medical Co Ltd, Tokyo, Japan), respectively. Serum interleukin-6 (IL-6) and IL-8 were assayed preoperatively and 6 hours postoperatively by commercially available enzyme-linked immunosorbent assay kits (Multisciences, Shanghai, China) and (eBioscience, San Diego, CA), respectively, according to the manufacturer’s instructions.

**Surgical Procedures**

Operative and anesthetic techniques were standardized in both groups. All surgical procedures were performed by the same group of surgeons. The anesthesia was similar for both groups. Intraoperative myocardial protection techniques were standardized in both groups, using the same cardioplegic solution (hyperkalemic blood cardioplegia). Heart biopsies were taken in the right atrial appendage before cross-clamping the aorta, at 10 minutes after removal of the cross-clamp when finishing the major surgical procedures in the heart. At the end of surgery, patients were transferred to the intensive care unit where a standardized protocol was followed. Patients were then transferred to a ward until discharged from the hospital.

**Cardiac Function Measurement**

Cardiac echocardiography was performed in patients preoperatively and again 7 days and 1 month postoperatively.

**Nitric Oxide Measurement**

Frozen right atrial appendages from −80°C were pulverized and homogenized for NO measurement. NO was determined spectrophotometrically by measuring total nitrate plus nitrite (NO$^+$ + NO$^−$) with a NO detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions using Griess reagent at an absorbance of 550 nm, as previously described.\textsuperscript{11} Results were expressed as µmol/g protein.

**Western Analysis**

Western analysis and protein association in the heart (right atrial appendage) were performed using standard protocol, as previously described.\textsuperscript{11} Anti-eNOS and anti–heat shock protein 90 (Hsp90)
were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti–Ser1177-phospho-eNOS (P-eNOS S1177), anti–Thr495-phospho-eNOS (T495), anti-AKT, anti–Thr308-phospho-AKT (P-Akt), and Anti-P38, and phosphorylation of P38 and anti–caveolin-1 were from Cell Signaling Technology (Danvers, MA). Anti-GAPDH was from Proteintech Group (Chicago, IL). eNOS was immunoprecipitated with H-32 antibody (Enzo Life Sciences, Farmingdale, NY) or with anti-eNOS (BD Biosciences, San Jose, CA). Proteins were visualized using a Western blotting luminol reagent (Santa Cruz Biotechnology).

**Statistical Methods**

Data were presented as means, SD, and IQR. Comparisons between treatment groups were made with the unpaired Student t test or repeated measures ANOVA where appropriate. Categorical data were expressed as frequency and percentage and compared with the Fisher exact test where appropriate. The total area under the curve was calculated using the trapezoidal method for each patient and compared using the t test. Estimations were presented with 95% CIs. A value of \( P<0.05 \) was regarded as significant. All statistical analyses was performed using SPSS for windows, version 17.0 (Chicago, IL) and MedCalc (version 11.0.0.0, Frank Schoonjans, Mariakerke, Belgium).

See the online-only Data Supplement for additional details in the Methods section.

**Results**

**General Preoperative Comparison**

One hundred fifty-one patients were randomly assigned to either the statin group (n=77) or control group (n=74). In total, 132 patients (68 in the statin group and 64 in the control group) completed the study. Baseline characteristics have been summarized in Table 1.

**Operative Characteristics and Postoperative Outcomes**

There were no significant differences in the operative characteristics between the 2 groups (Table 1). Preoperative atrial fibrillation was identified in 15 patients in the statin group and 13 patients in the control group. There were no differences in cholesterol levels between the 2 groups before administration of simvastatin. There was a tendency for reduction of total cholesterol and low-density lipoprotein levels in 1 week postoperatively. Dyslipidemia (high levels of total cholesterol, low-density lipoprotein, or triglyceride) was diagnosed in 8 patients in the statin group and 7 patients in the control group. Diabetes mellitus was diagnosed in 4 patients in the statin group and 2 patients in the control group. Erythrocyte sedimentation rate, lactate dehydrogenase, and the lipid profiles were the same in both the groups. No statistically significant differences in terms of type and number of surgical procedures were found between the statin and control groups (Table 2). Postoperative development of atrial fibrillation occurred in 1 patient in the control group. One case of preoperative atrial fibrillation disappeared postsurgically in the statin group.

**Plasma TnT**

Preoperatively, plasma TnT concentrations were <0.01 µg/L in both the groups and increased postoperatively—patients administered perioperative simvastatin therapy released less TnT than the patients who did not receive perioperative statins. Specifically, the mean TnT level was 0.88 µg/L (SD, 0.56; IQR, 0.49–1.12) versus 1.35 µg/L (SD, 1.56; IQR, 0.56–1.48) \( (P=0.020) \) at 6 hours, 0.81 µg/L (SD, 0.55; IQR, 0.40–1.12) versus 1.22 µg/L (SD, 1.47; IQR, 0.53–1.32) \( (P=0.037) \) at 12 hours, 0.59 µg/L (SD, 0.44; IQR, 0.29–0.76) versus 0.91 µg/L (SD, 1.08; IQR, 0.38–0.99) \( (P=0.031) \) at 24 hours, and 0.36 µg/L (SD, 0.29; IQR, 0.15–0.42) versus 0.61 µg/L (SD, 0.74; IQR, 0.29–0.69) \( (P=0.013) \) at 72 hours in the statin and control groups, respectively (Figure 2A). The total TnT released 72 hours postoperatively was reduced by 36.7%, from 60.33 µg/L (SD, 71.12) in the control group to 38.16 µg/L (SD, 26.58) in the statin group (mean difference was 22.17 [SD, 7.28]; 95% CI, 3.89–40.45; \( P=0.018 \)). After excluding the 15 patients with dyslipidemia, similar results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statin Group</th>
<th>Control Group</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Mean age (SD), y</td>
<td>45.5 (14.5)</td>
<td>41.5 (18.7)</td>
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<tr>
<td>Sex</td>
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<td>Men, n (%)</td>
<td>30 (44.1)</td>
<td>35 (54.7)</td>
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<td>Women, n (%)</td>
<td>38 (55.9)</td>
<td>29 (45.3)</td>
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<tr>
<td>Weight, mean (SD), kg</td>
<td>57.6 (20.7)</td>
<td>53.6 (12.8)</td>
<td>0.19</td>
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<td>Cardiac function NYHA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I, n (%)</td>
<td>8 (11.8)</td>
<td>7 (10.9)</td>
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<tr>
<td>II, n (%)</td>
<td>11 (16.2)</td>
<td>12 (18.8)</td>
<td></td>
</tr>
<tr>
<td>III, n (%)</td>
<td>48 (70.6)</td>
<td>43 (67.2)</td>
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</tr>
<tr>
<td>IV, n (%)</td>
<td>1 (1.5)</td>
<td>2 (3.1)</td>
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</tr>
<tr>
<td>Chronic AF, n (%)</td>
<td>15 (22.1)</td>
<td>13 (20.3)</td>
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<tr>
<td>Diabetes mellitus, n (%)</td>
<td>4 (5.9)</td>
<td>2 (3.1)</td>
<td>0.68</td>
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<tr>
<td>Dyslipidemia, n (%)</td>
<td>8 (11.8)</td>
<td>7 (10.9)</td>
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<tr>
<td>Hypertension, %</td>
<td>9 (13.2)</td>
<td>6 (9.4)</td>
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<td>Ejection fraction (SD), %</td>
<td>62.4 (10.9)</td>
<td>64.3 (8.4)</td>
<td>0.23</td>
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<tr>
<td>Erythrocyte sedimentation rate (SD), mm/L</td>
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<td>19.4 (17.1)</td>
<td>0.32</td>
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<tr>
<td>Lactic acid salt dehydrogenase(SD), U/L</td>
<td>215.9 (75.2)</td>
<td>233.3 (103.9)</td>
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<tr>
<td>Total operative time (SD), min</td>
<td>257.2 (69.6)</td>
<td>269.6 (100.25)</td>
<td>0.41</td>
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<tr>
<td>Total cardiopulmonary bypass time (SD), min</td>
<td>109.7 (41.3)</td>
<td>119.8 (53.9)</td>
<td>0.23</td>
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<td>Aortic cross-clamp time (SD), min</td>
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<td>72.7 (32.4)</td>
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<td>Postoperative AF, n (%)</td>
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<td>Usage of intraaortic balloon assistance, %</td>
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<td>1 (1.6)</td>
<td>1</td>
</tr>
<tr>
<td>Postsurgery cardiac tamponade, n (%)</td>
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<td>0.48</td>
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<tr>
<td>Postsurgery AF disappear, n (%)</td>
<td>1 (1.5)</td>
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AF indicates atrial fibrillation; NYHA, New York Heart Association.
similar results were obtained (data not shown). Again, when all patients with dyslipidemia were excluded, concentrations of CRP between both the groups. In contrast, there were no significant differences in preoperative plasma CKMB concentrations was not significantly different between the 2 groups (P=0.05). Postoperatively, a significant reduction in plasma CKMB concentrations was identified in patients treated with simvastatin compared with the control group (Figure 2B). The total CKMB released 72 hours postoperatively was reduced by 27.6%, from 1618.68 µg/L (SD, 1232.15) in the control group to 1171.55 µg/L (SD, 776.86) in the statin group. The mean difference was 447.12 µg/L (SD, 31.99; 95% CI, 94.61–799.64; P=0.013). Again, when all patients with dyslipidemia were excluded, similar results were obtained (data not shown).

### Plasma CRP

There were no significant differences in preoperative plasma concentrations of CRP between both the groups. In contrast, less amounts of CRP release were observed in the statin group in comparison with the control group postoperatively (Figure 2C). After excluding the patients with dyslipidemia, the same significant differences were obtained (data not shown).

### Blood Urea Nitrogen, Serum Creatinine, Plasma IL-6, and IL-8

There were no significant differences between the control group and the statin group preoperatively in BUN. BUN was dramatically increased postoperatively both in the control group and the statin group (P<0.05). However, statin reduced BUN concentration in the statin group compared with the control group at 24, 48, and 72 hours postoperatively (P<0.05; Figure 3A). There was no significant difference between the control group and the statin group preoperatively in serum creatinine. However, creatinine was dramatically increased postoperatively in the control group but not in the statin group (P<0.05). Statin reduced creatinine release compared with the control group at 24, 48, and 72 hours postoperatively (P<0.05). Interestingly, creatinine at 7 days postoperatively is even lower than that of preoperation in the statin group (P<0.05; Figure 3B). The increase of plasma IL-6 and IL-8 postoperatively was dramatically less in the statin group compared with the control group (Figure 3C and 3D).

### NO Generation

There was a tendency for reduction in the control group and a tendency of enhancement in the statin group in concentration of NO2−+NO3− postoperatively. Furthermore, the concentration of NO2−+NO3− generation was significantly higher in the statin group than that of the control group both preoperation and postoperation (Figure 3E). Additional experiment showed similar result after using NG-methyl-L-arginine monococetate to block NO3− release from fresh heart tissues (Figure I in the online-only Data Supplement).

### Inotropic Requirement and Left Ventricular Ejection Fraction

There was a significantly greater inotropic requirement in the control group compared with the statin group at both 6 hours (5.5±3.2 µg/kg per minute versus 4.1±2.6 µg/kg per minute, P=0.008) and 12 hours (4.9±3.2 µg/kg per minute versus 3.5±2.5 µg/kg per minute, P=0.005) postoperatively. There was no significant difference in left ventricular ejection fraction between the control group and the statin group preoperatively (Table 1). Ejection fraction was higher in the statin group (60.4±11.0%) than in the control group (56.8±9.3%) at 7 days postoperation (P=0.029) and 69.5±6.4% in the statin group than 65.3±7.3% in the control group in 1 month postoperation (P=0.002).

### Akt-eNOS, p38 MAPK, Caveolin-1, Hsp90 Expression, and the Association eNOS With Hsp90

Both eNOS expression and phosphorylation at serine 1177 in the heart were dramatically increased in the statin group preoperatively and postoperatively. eNOS phosphorylation at serine 1177 was increased even more after than before surgery. eNOS phosphorylation at threonine 495 was increased.
postoperatively in the control group and decreased both preoperatively and postoperatively in the statin group (Figure 4A). Akt phosphorylation was decreased postoperatively in the control group and increased both preoperatively and postoperatively in the statin group (Figure 4B). Statin inhibited p38 mitogen–activated protein kinase (MAPK) phosphorylation postoperatively (Figure 4C). There was no difference in caveolin-1 expression before and after surgery in the control group and between the control group and the statin group preoperatively. Caveolin-1 expression was decreased postoperatively in the statin group (Figure 4D). There was no difference in Hsp90 expression before and after surgery in the control group and between the control group and the statin group preoperatively. However, Hsp90 expression was significantly increased postoperatively in the statin group (Figure 4D). The Hsp90 association with eNOS was reduced postoperatively in the control group. There was no difference in Hsp90 association with eNOS before and after surgery in the statin group and between the control group and the statin group preoperatively. The Hsp90 association with eNOS was higher in the statin group than the control group postoperatively (Figure 4E).

**Discussion**

This is the first single-blinded, randomized controlled trial exploring the effects of perioperative statin therapy in patients undergoing noncoronary artery cardiac surgery with CPB.
Figure 3. Simvastatin decreased blood urea nitrogen (BUN), creatinine concentrations, plasma interleukin L (IL)-6, and IL-8 and increased nitric oxide (NO) production in the heart. Plasma BUN, creatinine, IL-6, and IL-8 before surgery and at different time points after surgery were measured according to the manufacturer’s instructions. A, BUN was dramatically increased postoperatively both in the control group and the statin group (*vs control preoperatively (preop); vs statin preop; \( P < 0.05 \)). Statin reduced BUN postoperatively (&vs control group; \( P < 0.05 \)).

B, Creatinine was dramatically increased postoperatively in control group but not in the statin group (*vs control preop; \( P < 0.05 \)). Statin reduced creatinine postoperatively (&vs control group; \( P < 0.05 \)). Creatinine at 7 days postoperatively is lower than that at 7 days preoperatively in statin group (#vs statin preop; \( P < 0.05 \)).

C and D, Simvastatin reduced the increase of plasma IL-6 and IL-8 (*\( P < 0.05 \)).

E, NO generation was measured by detecting the nitrite+nitrate concentration in the right atrium. Simvastatin increased NO production in the heart both in preoperation and postoperation (*vs control; \( P < 0.05 \)) (mean±SEM).
Figure 4. Simvastatin activated Akt–endothelial nitric oxide (eNOS) and attenuated p38 mitogen–activated protein kinase (MAPK) pathway in heart. Proteins expression and association in right atrium were measured. A, Statin increased eNOS phosphorylation at serine 1177 (S1177) site (* vs control; # vs statin preop; \( P < 0.05 \)). eNOS phosphorylation in threonine 495 (T495) site was increased postoperatively and decreased by statin (* vs control; \( P < 0.05 \)). Statin increased eNOS expression (* vs control; \( P < 0.05 \)).

B, Akt phosphorylation was decreased postoperatively and statin increased Akt phosphorylation both preoperatively and postoperatively (* vs control; # vs control preop, \( P < 0.05 \)).

C, Statin decreased p38 phosphorylation postoperatively without altering p38 expression (* vs control; # vs statin preop; \( P < 0.05 \)).

D, Statin significantly decreased caveolin-1 expression and slightly increased heat shock protein (Hsp90) expression postoperatively (* vs control; # vs statin preop; \( P < 0.05 \)).

E, Hsp90 association with eNOS in the heart was reduced postoperatively and was restored by statin (* vs control; # vs control preop; \( P < 0.05 \)) (n=64–68; mean±SEM). p-eNOS indicates phosphorylated eNOS; p-Akt, phosphorylated Akt; postop, postoperatively; IB, immunoblot.
Figure 4. (continued)
In light of the fact that the patients, surgeons, anesthetist, perfusionist, ultrasound physicians, and individuals collecting and analyzing the blood samples and analyzing the data were also all blinded, this randomized controlled study closely approached a double-blind randomized trial in design. Previous studies indicate that elevations in TnT and CKMB after cardiac surgery are associated with poor short- and long-term clinical outcomes.13 The present study found that perioperative simvastatin therapy significantly decreases plasma TnT, CKMB, CRP, IL-6, IL-8, BUN, creatinine, the requirement of inotropic and improves left ventricular ejection fraction, suggesting that perioperative simvastatin therapy can reduce myocardial injury and inflammatory response and improve cardiac and renal function in noncoronary artery cardiac surgery with CPB.

Beneficial effects of statin pretreatment have already been demonstrated in various studies in patients undergoing both coronary artery surgery and cardiac percutaneous interventions.8 However, although these studies indicate that statins reduce myocardial injury during the intervention of coronary artery disease, it is not possible to conclude from those reports whether the reduction in myocardial injury was associated with the decrease in lipid level because statins are designed to treat coronary artery disease. In other words, can statins protect against myocardial injury during CPB? Indeed, 1 large, retrospective study showed that statins had nothing to do with myocardial protection during cardiac surgery. To clarify this controversy, 15 dyslipidemia patients were excluded from the analysis in the current study and similar results were attained. Therefore, although simvastatin decreased cholesterol level, our findings can demonstrate that the myocardial protective effect of statins during cardiac surgery may independent of their ability to reduce lipid levels as the patients’ cholesterol levels are normal before administration of simvastatin.

The mechanism by which statins reduce myocardial injury remains unclear. It is known that excessive systemic inflammatory responses unleashed by CPB leads to myocardial injury. Some studies showed that statins decreased the systemic inflammatory response, which, in turn, may contribute to a decrease in myocardial injury.11,12 However, Flores and al10 reported that acute preoperative statin therapy failed to limit the inflammatory response to CPB in a small randomized trial; this study only used a single dose statin. In the present study, we found that perioperative simvastatin therapy reduced concentration of IL-6, IL-8, and CRP, demonstrating that statins can inhibit systemic inflammatory response to reduce myocardial injury.14,15 It is well known that the p38 MAPK pathway participate in the inflammatory response.16,17 Our data showed that simvastatin decreased p38 MAPK phosphorylation, suggesting that one of the mechanisms by which statins reduces myocardial injury is by limiting the inflammatory response by attenuating the p38 MAPK pathway in noncoronary artery cardiac surgery with CPB.

Previous studies showed that statins can improve endothelial dysfunction by protecting tissues from the damage that results from ischemia and reperfusion during cardiac surgery.18 Statin is able to increase vascular endothelial NO production (by modulating NOS expression) and diminish myocardial necrosis after ischemia and reperfusion in mice, contributing to cardioprotective effects.19-21 In the current study, our data showed that eNOS phosphorylation at T495 site was increased and Hsp90 association with eNOS was decreased postoperatively in control, suggesting that not only is the eNOS activation inhibited, but eNOS is also uncoupled during cardiac surgery with CPB.11,22 As a result, eNOS may generate superoxide instead of NO, which leads to endothelial dysfunction and myocardial injury. Importantly, simvastatin increased eNOS expression and phosphorylation at serine 1177 site, decreased eNOS phosphorylation at T495 site preoperatively and postoperatively, demonstrating that statin is able to activate eNOS. Meanwhile, we found that Hsp90 association with eNOS was restored by simvastatin postoperatively, suggesting eNOS was maintained in coupling status to maintain NO generation during cardiac surgery.23,24 We further found that Hsp90 expression was increased and caveolin-1 expression was decreased by simvastatin. As Hsp90 is the chaperone of eNOS and positively regulates eNOS and caveolin-1 negatively regulates eNOS, our findings further support the idea of statin’s ability in activating eNOS and maintaining eNOS uncoupled activity to generate NO instead of superoxide.11,24,25

Although NO decreased during surgery in the control group, it did not reach statistical significance and there was a tendency for the reduction of NO production postoperatively (P=0.08). More importantly, simvastatin was able to stimulate NO generation compared with control group. Recent findings from Antoniadis et al26 showing that perioperative statin therapy reduced myocardial superoxide generation in cardiac surgery also support our findings. Taken together, our findings demonstrated that statin can stimulate the heart to generate NO and decrease superoxide generation to limit oxidative stress in the heart and avoid injury before and during cardiac surgery. Such findings can explain why statins reduce myocardial injury and improve cardiac and renal function during cardiac surgery.

It is known that Akt phosphorylation is the key step of eNOS activation.27,28 We found that Akt phosphorylation in heart was decreased postoperatively and was increased by simvastatin preoperatively and postoperatively. These data demonstrate that statin can activate Akt-eNOS signaling pathway before and after cardiac surgery.

As simvastatin can improve endothelial function, it is possible that perioperative simvastatin therapy also improves the systemic vascular endothelial function. Our data showing less inotropic requirement postoperatively in the statin group indicate that simvastatin can not only reduce myocardial injury but may improve systemic vascular function.

In summary, our study shows that perioperative simvastatin therapy significantly reduced myocardial injury and inflammatory response and improved cardiac and renal function in patients undergoing noncoronary artery cardiac surgery with CPB by activating Akt-eNOS and attenuating p38 MAPK signaling pathway. Such cardiac protection of simvastatin is independent of its ability to reduce lipid levels. Our findings may provide a novel approach to reduce myocardial injury during cardiac surgery with CPB.

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Disclosures
None.

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