Timing and Dose of Statin Therapy Define Its Impact on Inflammatory and Endothelial Responses During Myocardial Infarction

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Objective—Clinical trials of statins during myocardial infarction (MI) have differed in their therapeutic regimes and generated conflicting results. This study evaluated the role of the timing and potency of statin therapy on its potential mechanisms of benefit during MI.

Methods and Results—ST-elevation MI patients (n=125) were allocated into 5 groups: no statin; 20, 40, or 80 mg/day simvastatin starting at admission; or 80 mg/day simvastatin 48 hours after admission. After 7 days, all patients switched their treatment to 20 mg/day simvastatin for an additional 3 weeks and then underwent flow-mediated dilation in the brachial artery. As of the second day, C-reactive protein (CRP) differed between non–statin users (12.0±4.1 mg/L) and patients treated with 20 (8.5±4.0 mg/L), 40 (3.8±2.5 mg/L), and 80 mg/day (1.4±1.5 mg/L), and the daily differences remained significant until the seventh day (P<0.0001). The higher the statin dose, the lower the elevation of interleukin-2 and tumor necrosis factor-α, the greater the reduction of 8-isoprostane and low-density lipoprotein(−), and the greater the increase in nitrate/nitrite levels during the first 5 days (P<0.001). Later initiation of statin was less effective than its early introduction in relation to attenuation of CRP, interleukin-2, tumor necrosis factor-α, 8-isoprostane, and low-density lipoprotein(−), as well as in increase in nitrate/nitrite levels (P<0.0001). At the 30th day, there was no longer a difference in lipid profile or CRP between groups; the flow-mediated dilation, however, was proportional to the initial statin dose and was higher for those who started the treatment early (P=0.001).

Conclusion—This study demonstrates that the timing and potency of statin treatment during MI are key elements for their main mechanisms of benefit.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00906451.

Key Words: acute coronary syndromes ■ cytokines ■ endothelial function

Although there is no doubt that treatment with statins reduces cardiovascular mortality in the long term, it remains unclear whether this benefit can be maximized by their early prescription during the acute phase of coronary events. On one hand, a broad body of evidence indicates numerous beneficial mechanisms by which statins may positively influence the outcome after acute coronary syndromes (ACS).1 On the other hand, a metaanalysis of the clinical trials that were dedicated to this issue concluded that initiation of statin therapy within 14 days after onset of ACS has not been proven to reduce death, heart attack, or stroke up to 4 months.2 Although these findings, as they stand, are a nonconfirmation of a hypothesis, one may argue that the outline of these clinical trials would not be properly designed to support this interpretation. In particular, it is noteworthy to mention potential weaknesses, such as the late introduction of therapy (up to 10 days after onset of the ACS) and the use of doses based on targets for chronic preventive therapy.

During the first hours after onset of ACS, there is a massive generation of inflammatory mediators that negatively influence the remodeling of the arterial wall and endothelium-dependent vasomotor function in a systemic way.3 Consistently, the magnitude of the production of inflammatory mediators is strongly correlated with the recurrence of coronary events and incidence of sudden death.4,5 In a prior study, we found that the early use of statins at high doses rapidly reduces cholesterol-rich lipoproteins and inflammatory activ-

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the role of the timing of statin treatment initiation and of statin doses on the time course of inflammatory response during acute phase STEMI and its later effect on the endothelial vasomotor function.

Methods

Patients

For this prospective intervention trial, 125 consecutive patients with STEMI were enrolled. Inclusion criteria were as follows: (1) less than 24 hours after the onset of MI symptoms; (2) ST-segment elevation of a least 1 mm (frontal plane) or 2 mm (horizontal plane) in 2 contiguous leads; and (3) myocardial necrosis, as evidenced by increased creatine kinase-MB fraction and troponin levels. The exclusion criterion was the use of statins for at least 6 months before the MI event. The presence of diabetes mellitus was considered in patients with previous diagnosis and in those without diagnosis but with glycohemoglobin (HbA1c) >6.5%. The study was approved by the institutional ethics committee, and all patients signed an informed consent. This report satisfies the recommended reporting guidelines for clinical trials.8

Study Design

The experimental design of the study is illustrated in Figure 1. The study was designed with 2 consecutive phases of treatment. First, patients were allocated by rotating to a 7-day period with no statin (NS), 20 mg/day simvastatin (G20E), 40 mg/day simvastatin (G40E), or 80 mg/day simvastatin (G80E), all initiated at their admission; or 80 mg/day starting 48 hours after admission (G80L). Plasma C-reactive protein (CRP) was assessed daily, and the blood samples were drawn at the admission and at the fifth day for additional biochemical analyses. In the second phase, which started at the eighth day, all patients had their treatment regimes switched to G20E for an additional period of 3 weeks and then underwent brachial artery reactivity assay and a new determination of plasma lipid profile and CRP.

Biochemical Analyses

The following measurements were performed: blood glucose (Glucose GOD-PAP, Roche Diagnostics, Mannheim, Germany), total cholesterol (CHOD-PAP, Roche Diagnostics), triglycerides (GPO-PAP, Roche Diagnostics), high-density lipoprotein (HDL) cholesterol (HDL cholesterol without sample pretreatment, Roche Diagnostics), low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula. The area under the curve (AUC) of the CRP daily kinetics was calculated by the trapezoidal rule.

To evaluate NO production, the plasma levels of nitrite and nitrate (NOX) were measured by an NO chemiluminescence analyzer (model NOA, Sievers Instruments, Boulder, CO) after reduction with acidic vanadium (III) chloride. Plasma concentrations of electronegative LDL (LDL\(-\)) was determined by ELISA using an anti-LDL\(-\) human monoclonal antibody (mAb3D1036) produced by Dr Abdalla as described previously.9 All samples and standards were run in triplicate. The intraassay and interassay variations for this ELISA were 8% and 15%, respectively. All laboratory analyses were performed by technicians who were blinded to the statin treatment.

Brachial Artery Reactivity

Patients were studied in the fasting state, and vasoactive medications were withdrawn 24 hours before the study. After 10 minutes of rest in a quiet room with the temperature controlled around 22°C, the brachial artery was located above the elbow, and a longitudinal image of 6 to 8 cm was taken as the resting scan. A blood pressure cuff was placed on the forearm and inflated to 50 mm Hg above the systolic blood pressure for 5 minutes. The cuff was deflated, and the flow-mediated dilation (FMD) scan was obtained for 2 minutes. The second rest scan was taken after 10 minutes of rest, and endothelial-independent dilation was estimated by the vasodilator response 5 minutes after sublingual administration of 5 mg of isosorbide dinitrate. The percentage of diameter change for FMD and nitrate-mediated dilation was calculated in relation to the respective rest scans. Brachial artery reactivity was analyzed by experienced physicians who were blinded to the statin treatment. The interobserver agreement and intraobserver reproducibility were 93% and 95%, respectively.

Statistical Methods

The comparison between groups of the plasma values of the analytes measured at each blood sampling were performed by ANOVA (approximately normal variables) or the Kruskal-Wallis test (variables with skewed distributions). Analysis of covariance (ANCOVA) was used to assess the effect of treatments on CRP, IL-2, TNF-α, 8-isoprostane, LDL\(-\), NOX, and brachial artery reactivity parameters. Assumptions of the ANCOVA models (linearity, normality of distribution, and equal variance) were checked using histograms, normal probability plots, and residual scatter plots. Age and gender were included as covariates in all ANCOVA models. Additional adjustment for baseline values was also performed for comparison of mean change of these biochemical analytes across treatments. The effect of statin treatment on the 7-day plasma CRP kinetics was compared using the Friedman test for repeated measures. In addition, the CRP plasma levels at each treatment day and the AUC for the CRP kinetics were compared between treatments using the Kruskal-Wallis test. The Wilcoxon rank sum test was used for a posteriori comparison between the G80E and G80L groups. Frequencies in the categorical data were compared by the Fisher exact test. Data are presented as mean±standard deviation for normally distributed data or median and interquartile range for skewed data. Statistical analyses were performed using PASW for Windows, version 17.0.

Results

Baseline Data and the Short-Term Effect of Simvastatin Dose on Lipid Profile (Phase 1)

As shown in Table 1, there was no significant difference between patients enrolled in the study. Reperfusion therapy...
Table 1. Clinical and Laboratory Characteristics of the Enrolled Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>NS</td>
<td>G20E</td>
<td>G40E</td>
<td>G80E</td>
<td>G80L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simvastatin dose, mg/day</td>
<td>24</td>
<td>25</td>
<td>28</td>
<td>23</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapy initiation</td>
<td>Admission</td>
<td>Admission</td>
<td>Admission</td>
<td>48 hours</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>20 (84)</td>
<td>21 (84)</td>
<td>24 (86)</td>
<td>20 (87)</td>
<td>20 (85)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>56±15</td>
<td>59±11</td>
<td>58±11</td>
<td>60±12</td>
<td>57±10</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Prior myocardial infarction, n (%)</td>
<td>4 (17)</td>
<td>4 (16)</td>
<td>4 (15)</td>
<td>4 (17)</td>
<td>3 (12)</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>9 (37)</td>
<td>9 (36)</td>
<td>10 (36)</td>
<td>8 (35)</td>
<td>9 (36)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>6 (25)</td>
<td>6 (23)</td>
<td>7 (25)</td>
<td>5 (22)</td>
<td>6 (25)</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>No physical activity, n (%)</td>
<td>15 (63)</td>
<td>16 (64)</td>
<td>19 (68)</td>
<td>15 (66)</td>
<td>16 (63)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>5 (21)</td>
<td>5 (20)</td>
<td>7 (25)</td>
<td>5 (22)</td>
<td>6 (24)</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin, (%)</td>
<td>6.1±1.1</td>
<td>6.2±1.3</td>
<td>6.2±1.4</td>
<td>6.2±1.7</td>
<td>6.4±1.9</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Reperfusion therapy, n (%)</td>
<td>20 (83)</td>
<td>21 (84)</td>
<td>24 (86)</td>
<td>20 (87)</td>
<td>22 (88)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Symptoms to sampling, minutes</td>
<td>175 (120 to 287)</td>
<td>152 (130 to 265)</td>
<td>190 (154 to 210)</td>
<td>205 (75 to 330)</td>
<td>160 (145 to 285)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>TIMI risk score, points</td>
<td>3±2</td>
<td>4±2</td>
<td>4±2</td>
<td>3±2</td>
<td>3±2</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Creatine kinase–MB fraction peak, mg/dL</td>
<td>264±228</td>
<td>282±221</td>
<td>275±210</td>
<td>226±208</td>
<td>253±240</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Baseline LDL cholesterol, mg/dL</td>
<td>120±24</td>
<td>118±35</td>
<td>120±35</td>
<td>133±44</td>
<td>133±47</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Baseline HDL cholesterol, mg/dL</td>
<td>44±13</td>
<td>40±12</td>
<td>39±10</td>
<td>38±10</td>
<td>37±9</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Baseline triglycerides, mg/dL</td>
<td>159 (63 to 280)</td>
<td>154 (60 to 225)</td>
<td>146 (104 to 233)</td>
<td>150 (54 to 243)</td>
<td>154 (103 to 219)</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA was used to compare approximately normal variables expressed as mean±SD, and the Kruskal-Wallis test was used for variables with skewed distributions expressed as median (25th to 75th percentiles). TIMI indicates thrombolysis in myocardial infarction.

was obtained either by primary angioplasty (20%) or by the infusion of tenecteplase. There was no significant difference in the use of primary angioplasty (22.3% versus 20.5% versus 22.9% versus 22.0%; P=0.8), β-blockers (70.7% versus 73.8% versus 71.7% versus 74.1%; P=0.8), angiotensin-converting enzyme inhibitors (54.8% versus 56.0% versus 54.3% versus 58.0%; P=0.9), aspirin (92.9% versus 95.2% versus 100% versus 92.8%; P=0.9), or clopidogrel (33.7% versus 35.6% versus 31.2%; P=0.8) for non–statin users and those taking G20E. The differences did not reach statistical significance.

Effect of the Early Simvastatin Treatment on Inflammatory Response, Oxidative Stress, and NO Production

Figure 2 depicts the kinetics of daily changes in CRP during the first 7 days after hospital admission for MI. A rapid rise in CRP levels was observed within the first 24 hours, followed by a less marked increase, which peaked between the third and fourth days post-MI. In fact, as shown in Table 2, plasma CRP levels did not differ significantly between study groups at admission. As of the second day of treatment, however, a statistically significant difference was found between each group and remained significant until the end of the first 7 days after admission. A rapid rise in CRP levels was observed within the first 24 hours, followed by a less marked increase, which peaked between the third and fourth days post-MI. The attenuation in inflammatory response is distinct and proportional to the simvastatin dose.

In the group with the later initiation of statin treatment, we observed that CRP started to fall after the third day and thus followed by a less marked increase, which peaked between the third and fourth days post-MI. In fact, as shown in Table 2, plasma CRP levels did not differ significantly between study groups at admission. As of the second day of treatment, however, a statistically significant difference was found between each group and remained significant until the end of the first 7 days after admission. A rapid rise in CRP levels was observed within the first 24 hours, followed by a less marked increase, which peaked between the third and fourth days post-MI. The attenuation in inflammatory response is distinct and proportional to the simvastatin dose.
the 7-day treatment period. The Friedman test for repeated measures showed that the attenuation in inflammatory response was distinct and proportional to the simvastatin dose (p for trend <0.0001). Statistical analysis of the AUC values also revealed significant differences between groups (77 ± 9 versus 47 ± 21 versus 27 ± 22 versus 6 ± 5, for non–statin users and users of 20, 40, and 80 mg/day, respectively; P <0.0001). The difference between groups remained significant after adjustment for the admission levels of LDL cholesterol, gender, and age. Nevertheless, a weak association tended to occur between LDL cholesterol change and CRP change between admission and the fifth day after MI (r = 0.14; P = 0.075).

There was a significant increase in the proinflammatory cytokines IL-2 and TNF-α between admission and the fifth day after MI in all enrolled patients. Consistent with the kinetics of the CRP daily change, we observed that the treatment attenuated the increase of both IL-2 and TNF-α in direct proportion to the dose of simvastatin used (Table 3). Oxidative stress, estimated by plasma levels of LDL(−) and 8-isoprostane, reduced from first to fifth day in all patients, but this reduction was more pronounced in patients who used higher doses of simvastatin (Table 3). The NOx remained equivalent at baseline and on the fifth day in patients not treated by statins. In the remaining patients, there was an increase in NOx levels with a magnitude proportional to the dose of simvastatin used (Table 3). The comparisons of the changes in cytokines, NOx, and oxidative stress markers remained significant after adjustment for baseline values, age, and gender.

### Table 2. Change of Median Plasma CRP (mg/L) According to the Experimental Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>NS</th>
<th>G20E</th>
<th>G40E</th>
<th>G80E</th>
<th>G80L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First phase (7 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>0.6 (0.2 to 1.0)</td>
<td>0.7 (0.1 to 0.9)</td>
<td>0.7 (0.3 to 1.0)</td>
<td>0.6 (0.2 to 1.6)</td>
<td>0.6 (0.2 to 0.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>2nd day</td>
<td>12.6 (10.4 to 15.2)</td>
<td>8.1 (4.3 to 10.9)</td>
<td>3.4 (1.0 to 4.8)</td>
<td>1.2 (0.6 to 1.5)</td>
<td>11.4 (8.9 to 14.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>3rd day</td>
<td>14.3 (12.6 to 17.5)</td>
<td>8.9 (6.1 to 11.8)</td>
<td>3.7 (0.8 to 5.1)</td>
<td>2.4 (0.9 to 3.9)</td>
<td>14.1 (10.8 to 18.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>4th day</td>
<td>16.1 (14.0 to 18.0)</td>
<td>9.4 (7.9 to 14.1)</td>
<td>4.2 (0.8 to 5.8)</td>
<td>1.6 (0.6 to 2.4)</td>
<td>9.4 (6.8 to 13.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>5th day</td>
<td>15.8 (14.6 to 17.0)</td>
<td>7.2 (6.0 to 12.4)</td>
<td>3.2 (0.5 to 6.0)</td>
<td>0.8 (0.1 to 2.2)</td>
<td>7.1 (5.5 to 9.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>6th day</td>
<td>12.1 (11.0 to 14.0)</td>
<td>5.0 (4.3 to 12.0)</td>
<td>2.4 (0.4 to 8.7)</td>
<td>0.4 (0.1 to 1.2)</td>
<td>4.8 (3.4 to 8.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>7th day</td>
<td>10.0 (9.0 to 11.0)</td>
<td>2.9 (2.0 to 9.0)</td>
<td>1.9 (0.2 to 6.3)</td>
<td>0.1 (0.1 to 0.4)</td>
<td>2.1 (0.5 to 6.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Second phase (3 weeks)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>0.6 (0.2 to 3.9)</td>
<td>0.3 (0.2 to 2.4)</td>
<td>0.3 (0.2 to 5.3)</td>
<td>0.3 (0.1 to 2.6)</td>
<td>0.3 (0.2 to 5.3)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Kruskal-Wallis tests were used for comparisons, and the variables are arranged as median (25th to 75th percentiles).

### Table 3. Effect of the Treatments on Inflammatory and Oxidative Markers

<table>
<thead>
<tr>
<th>Variable</th>
<th>NS</th>
<th>20E</th>
<th>40E</th>
<th>80E</th>
<th>80L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL(−), mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>1.36±0.24</td>
<td>1.32±0.41</td>
<td>1.39±0.34</td>
<td>1.45±0.41</td>
<td>1.40±0.28</td>
<td>0.87</td>
</tr>
<tr>
<td>5th day</td>
<td>1.03±0.14</td>
<td>0.79±0.19</td>
<td>0.48±0.27</td>
<td>0.36±0.22</td>
<td>0.50±0.26</td>
<td>0.002</td>
</tr>
<tr>
<td>8-Isoprostane, pg/mL</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>47.1±7.7</td>
<td>46.1±6.9</td>
<td>48.3±14.8</td>
<td>48.1±9.1</td>
<td>49.1±8.2</td>
<td>0.94</td>
</tr>
<tr>
<td>5th day</td>
<td>47.9±6.6</td>
<td>39.9±6.6</td>
<td>32.3±6.4</td>
<td>12.9±5.4</td>
<td>34.8±6.4</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-2, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>2.4±0.3</td>
<td>2.3±0.7</td>
<td>2.3±0.4</td>
<td>2.4±0.3</td>
<td>2.3±0.8</td>
<td>1.00</td>
</tr>
<tr>
<td>5th day</td>
<td>19.9±3.9</td>
<td>8.7±3.6</td>
<td>5.1±4.2</td>
<td>2.9±2.1</td>
<td>5.3±2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>10.3±3.4</td>
<td>11.1±6.6</td>
<td>11.8±6.2</td>
<td>10.9±6.6</td>
<td>9.9±6.4</td>
<td>0.99</td>
</tr>
<tr>
<td>5th day</td>
<td>26.7±4.6</td>
<td>22.8±8.1</td>
<td>16.7±8.1</td>
<td>9.6±4.7</td>
<td>21.3±6.5</td>
<td>0.001</td>
</tr>
<tr>
<td>NOx, μmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>19.7±4.0</td>
<td>19.5±4.2</td>
<td>17.9±4.7</td>
<td>19.7±6.3</td>
<td>17.7±2.4</td>
<td>0.68</td>
</tr>
<tr>
<td>5th day</td>
<td>18.3±4.5</td>
<td>24.0±7.1</td>
<td>26.1±3.6</td>
<td>31.2±7.8</td>
<td>25.8±3.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ANCOVA adjusted for age and gender was used for comparisons, and the variables are arranged as mean±SD.
the inflammatory response, oxidative stress, and production of NO compared with responses obtained in patients whose treatment started at admission. Compared with patients not treated with statins, the late introduction of G80E promoted a 26% reduction in AUC of the CRP daily change (51/1006, \( P < 0.001 \)). However, the early introduction reduced the AUC threefold (92%), a difference that was statistically different from that obtained with the late introduction (\( P < 0.0001 \); Figure 1). Consistent with the CRP kinetic analysis, in the fifth day after MI, the increases in TNF-\( \alpha \) and IL-2 levels were higher and the decreases in LDL(\( \alpha \)) and 8-isoprostane lower in individuals enrolled in the G80L than in those on the G80E (Table 3). Finally, the increase in NO bioavailability between the admission and the fifth day was lower in the G80L than in the G80E (Table 3). The comparisons of the changes in cytokines, NOx, and oxidative stress markers between G80L and G80E were adjusted for baseline values, age, and gender. The change of NOx was significantly correlated with changes in 8-isoprostane (\( r = -0.35; P = 0.01 \)), LDL(\( \alpha \)) (\( r = -0.31; P = 0.035 \)), and IL-2 (\( r = -0.43; P = 0.005 \)).

**Effect of Statin Regimen at Phase 1 on Postdischarge Endothelial Function**

At the 30th day after MI and 3 weeks of the shift from acute phase statin treatment to G20E, there was no significant difference in plasma CRP between the treatment groups (Table 2). Likewise, there was no significant difference in LDL cholesterol (NS: 98±19 mg/dL; G20E: 103±18 mg/dL; G40E: 96±21 mg/dL; G80E 97±18 mg/dL; G80L: 99±20 mg/dL; \( P = 0.8 \)), HDL cholesterol (NS: 46±16 mg/dL; G20E: 48±9 mg/dL; G40E: 44±12 mg/dL; G80E: 37±17 mg/dL; G80L: 39±9; \( P = 0.35 \)), or triglycerides (NS: 117±27 mg/dL; G20E: 106±54 mg/dL; G40E: 114±91 mg/dL; G80E: 118±55 mg/dL; G80L: 107±29 mg/dL; \( P = 0.52 \)).

As shown in Figure 3, all groups of patients treated with simvastatin had higher FMD values than those who were not treated (\( P < 0.05 \)). FMD was higher in patients on G80E than in those on G40E (\( P < 0.01 \)), and it was higher in the latter group than in those on G20E (\( P < 0.05 \)). Individuals enrolled in G80L had an FMD 47% lower than that obtained in G80E (\( P < 0.001 \)), which was equivalent to that obtained in G40E group (\( P = 0.8 \)).

Nitrate-mediated dilation was higher in patients on early introduction of G80E than in those who did not receive statin (\( P < 0.05 \)). In the other intergroup comparisons, there was no significant difference. The difference in the FMD/nitrate-mediated dilation ratio was also significant and proportional to the simvastatin dose (NS: 0.17 [0.48 to 0.26]; G20E: 0.19 [0.12 to 0.33]; G40E: 0.29 [0.14 to 0.39]; G80E: 0.53 [0.40 to 0.64]; \( P = 0.001 \)). Again, the late introduction resulted in a significantly attenuated effect compared with the early initiation of G80E (G80L: 0.30 [0.16 to 0.41]; \( P = 0.01 \)).

**Discussion**

The present study establishes 2 mechanistically relevant parameters to be considered for statin therapy during the acute phase of MI. First, it shows that regardless of the LDL cholesterol level at admission, the higher the dose of statin therapy, the greater the benefit. For this latter, we mean the attenuation of oxidative stress and inflammatory activity and the improvement of NO production and endothelial vasomotor function. Second, it shows that to achieve maximum
benefit with statin therapy, its initiation is mandatory in the first hours after onset of MI.

Although inflammatory response is driven to healing MI lesions, it has been shown that excessive generation of systemic inflammatory mediators favors the extension and expansion of the infarcted area and thus the generation of systolic dysfunction, left ventricular aneurysm, and cardiac rupture.\textsuperscript{3,4,10} In addition, an intense inflammatory response after MI may also induce contractile dysfunction of noninfarcted myocytes and vasomotor endothelial dysfunction in coronary and systemic arteries, and it may intensify inflammation in atherosclerotic plaques.\textsuperscript{3} Accordingly, increased plasma levels of CRP after MI are related to the incidence of ventricular dysfunction, sudden death, and recurrent coronary events in the 2 years following the event.\textsuperscript{4,5}

Of note, we observed that the upregulation of systemic inflammatory activity is almost completely expressed (up to 80%), as estimated by CRP levels, in the first 24 hours after the onset of MI symptoms. It is extensively proven that statin treatment can reduce local and systemic inflammatory activities in chronic stable individuals. In keeping with this, we observed in NSTEMI\textsuperscript{7} and presently in STEMI that this antiinflammatory effect can be faster. Actually, the increase of the dose and anticipation of statin therapy significantly prevented the triggering of the inflammatory response. In addition, the early treatment naturally increased the duration of exposure to statins during the acute phase of STEMI. These 3 differences in the statin regimes must have contributed for the effect on inflammatory burden.

Besides the chronic antiinflammatory effect obtained by reducing the volume of circulating LDL, several other mechanisms may provide such extremely fast antiinflammatory effect induced by statins (see Sposito and Chapman\textsuperscript{1} for more details). Briefly, from the beginning, statins may reduce proinflammatory stimuli by reducing oxidative stress and blood volume of modified LDL, as documented in this study. In parallel, the inhibition of hydroxy-methylglutaryl coenzyme A reductase by statins deprives the intracellular content of mevalonate and isoprenyl radicals, which are important players in the inflammatory response. Finally, and probably the fastest mechanism, statins can bind directly to the lymphocyte function–associated antigen-1, thereby reducing the stimulation of T cells. Thus, through a set of mechanisms related and unrelated to the inhibition of hydroxy-methylglutaryl coenzyme A reductase, statins can markedly reduce the inflammatory response to an acute stimulus.

During ACS, endothelial dysfunction in the coronary and systemic arteries is caused or intensified by the release of vasoactive mediators from inflammatory response and thrombogenesis.\textsuperscript{1} The vasomotor endothelial dysfunction may persist for up to 6 months, a phenomenon called endothelial stunning, and has been found to be a determinant of clinical outcome.\textsuperscript{11,12} Such overall commitment of the coronary microvascular bed may favor the appearance of inappropriate constrictions and ischemia at the periphery of the infarcted area, thereby extending myocardial injury.\textsuperscript{13} Therefore, it seems highly probable that besides being a predictor of the clinical outcome, endothelial dysfunction is indeed involved in the pathophysiological basis for the recurrence of coronary events.

The shift of the phase 1 treatment to the same statin regime for all participants allowed us to evaluate the residual effect of the treatment during the acute phase. As expected, after 3 weeks under this new therapy, lipid profile and CRP levels became similar among the participants. Still, subjects who had higher statin doses during the acute phase presented a better endothelial vasomotor function 30 days after MI. In participants in the Framingham Study, FMD was inversely related to the plasma levels of CRP, IL-6, soluble intracellular adhesion molecule-1, and monocyte chemotactic protein-1.\textsuperscript{14} Consistent with this, we and others\textsuperscript{11} observed that the pool of NO is inversely proportional to the magnitude of the oxidative stress and systemic inflammatory activity. Furthermore, the spontaneous reduction of inflammatory activity is associated with the improvement of endothelium-mediated vasodilation.\textsuperscript{11} It is plausible that statin effect on inflammation or on the source of inflammation itself has contributed to the improvement or preservation of the endothelial function in the present study. In a recent study, reduced FMD was independently associated with recurrence of coronary events after STEMI.\textsuperscript{15} It is therefore possible that the increase in FMD with early and intensive treatment with statins acts favorably on the outcome of patients with STEMI.

Unexpectedly, endothelial-independent dilation was found to be greater in subjects treated with the maximum dose of simvastatin than in those who did not use this drug in the acute phase. Some studies have replicated this finding,\textsuperscript{16} but others have not.\textsuperscript{17} Potential mechanisms underlying this effect include statin action on sympathetic activity, on prenylation of proteins in vascular smooth muscle cells, and on endothelial potassium channels. Additional studies are required to clarify this issue.

Because the intensity of the inflammatory response during ACS is associated with adverse clinical outcomes, we hypothesized that besides the statin dose, the timing of the treatment initiation may also be essential for the benefit. In fact, according to the AUC of CRP daily change, patients who started the statin treatment 48 hours after onset of MI had an antiinflammatory effect one third as large as that obtained by the statin administration at admission. The difference was also apparent by the change in plasma values of IL-2 and TNF-\(\alpha\) and, as well, in the markers of oxidative stress 8-isoprostane and LDL(\(\sim\)). Moreover, the late start of the statin was less effective in improving the production of NO and endothelium-dependent vasodilation compared with its early introduction. In line with these findings, in an observational cohort with 10 484 consecutive STEMI patients, the use of statins in the first 24 hours was followed by a lower mortality compared with the late administration.\textsuperscript{18} As noted above, so far, the clinical trials aimed at investigating the effect of statins in ACS have started this treatment at a later period (up to 14 days after the coronary event). Thus, the present findings indicate that timing of the therapy must be considered in the design of future clinical trials to clarify the role of statin treatment during ACS.

Some limitations must be considered for this study. First, in this study, only STEMI patients were enrolled, and
findings can be quite distinct in another setting. Potentially, it is possible that the difference in FMD between groups would be lower if patients had milder activation of systemic inflammatory activity, such as that seen in patients with unstable angina or MI without ST-segment elevation. Thus, any extrapolation from these findings must be carefully considered. Second, the placebo effect cannot be completely discarded because the control group did not receive a dummy treatment. However, the experimental treatment was administered with the standard medications for STEMI, and the patient was blinded to the treatment.

In conclusion, our present findings demonstrate that the timing and the potency of statin treatment during acute phase of STEMI are key elements for the attenuation of oxidative stress, inflammatory activity, and endothelial dysfunction.

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Disclosures
None.

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ST분절 상승 급성 심근경색증 환자에서 스타틴의 조기, 고용량 투여가 염증억제와 혈관 내피 세포 기능 보전에 더 좋다

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Summary

목적
급성 심근경색증에서 스타틴을 사용한 연구들은 서로 다른 치료 방법을 사용하여 상이한 결과들을 보여 주고 있다. 본 연구의 목적은 급성 심근경색증에서 사용하는 스타틴의 용량과 사용 시기에 스타틴의 유익한 효과에 미치는 영향에 대한 평가이다.

방법 및 결과
ST분절 상승 심근경색증 환자 125명을 스타틴 비사용군과 음의 시작 후 simvastatin 20mg, 40mg, 80mg/day 사용한 3군, 그리고 음의 48시간 후 80mg/day를 시작한 1군의 총 5개 군으로 배정하였다. 이후 7일 후에는 모든 환자에서 simvastatin 20mg/day으로 전환하여 3주간 사용하고 FMD(flow-mediated dilatation)를 상완 동맥에서 측정하였다. 다음날 CRP를 측정한 결과 스타틴 비사용군에서는 120±41mg/L이었고 각 용량군에서는 8.5±4.0mg/L (20mg군), 3.8±2.5mg/L(40mg군), 1.4±1.5mg/L (80mg군)로 차이가 보였다. 그리고 이 차이는 7일째까지 유지되었다(P<0.001). 스타틴의 용량이 높을수록 처음 5일 동안 IL-2, TNF-α가 적게 상승하였고, 8-isoprostane과 low-density lipoprotein(LDL)은 많이 감소하였으며, nitrate/nitrite levels가 많이 증가하였다(P<0.001).

스타틴을 늦게 사용하는 것은 조기 사용보다 CRP, IL-2, TNF-α, 8-isoprostane, LDL의 감소와 nitrate/nitrite levels의 증가에 일 효과적이었다. 30일째의 lipid level, CRP에서는 각 군에서 더 이상 차이를 보이지 않았으나 FMD는 스타틴의 용량에 비례하여 조기 스타틴 사용군에서 더 떨어졌다(P=0.001).

결론
본 연구는 심근경색증에서 스타틴을 사용 시기와 용량이 유익한 효과의 중요한 기전 중의 하나임을 보여주고 있다.
본 연구는 스타틴을 고향용으로 조기에 사용하는 것이 자용량이나 심근경색증 발생 후 일정 시점(48시간) 이후 사용하는 것보다 향미중, 항산화 효과와 혈관내피세포 기능에 효과적이라는 사실을 보여주고 있다. 48시간 이후에 사용하는 것은 비록 고향용이라도 조기 자용량보다 효과가 미미하였다. 특히 흡취로운 것은 내피세포 기능보존인데, 자기 7일간 고향용 스타틴을 투여하고 이후 3주 동안은 통상적 용량을 투여했음에도 1개월째 혈관내피세포 기능은 저용량군이나 후기 고향용 투여군보다 활동히 잘 보존되고 있음을 보여주고 있다(Figure 1).

Figure 1. ST-분절 상승 급성 심근경색증 후 1개월째 상이동맥 내피세포 기능 평가 성(median brachial artery function [FMD] 스타틴 사용군에서 비스타틴군보다 FMD가 증가하였고 P<0.05), 고향용 사용군(800mg)에서 가장 크게 증가하였으며 증가량이 클수록 비해하며 증가하였다. 심근경색 48시간 이후에 고향용을 사용하지 시작한 후기 사용군(800mg)은 조기 고향용용보다 일부의 FMD 증가율을 보였고 (P<0.001).

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