UltraRapid Communication

Rapid Effect of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibition on Coronary Endothelial Function

Sven Wassmann, Anna Faul, Benno Hennen, Bruno Scheller, Michael Böhm, Georg Nickenig

Abstract—Treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) decreases cardiovascular event rates in hypercholesterolemic patients. Whether statins exert effects within 24 hours on the coronary vasculature in patients with endothelial dysfunction has not been elucidated. Twenty-seven patients with stable angina pectoris and average low-density lipoprotein cholesterol concentrations of 138±9 mg/dL at baseline were allocated to treatment with placebo (14 patients) or 40 mg/d pravastatin (13 patients) in a randomized, double-blind, prospective trial. Coronary endothelial function was assessed before and 24 hours after single treatment by quantitative coronary angiography during intracoronary infusion of nitroglycerin or increasing concentrations of acetylcholine (0.01, 0.1, and 1 μmol/L). Coronary blood flow reserve was measured by Doppler velocimetry during adenosine infusion. Intracoronary acetylcholine infusion induced abnormal vasoconstriction in both groups before treatment, indicating coronary endothelial dysfunction. Treatment with a single oral 40-mg dose of pravastatin significantly attenuated acetylcholine-mediated vasoconstriction after 24 hours (mean±SE decrease in luminal diameter before and after treatment: 0.01 μmol/L, 6.1±2.2% versus 3.0±1.2%; 0.1 μmol/L, 15.6±2.6% versus 7.4±1.8%; P<0.05; 1 μmol/L, 22.9±2.9% versus 13.2±2.6%; P<0.05). There was no significant difference in the response to acetylcholine in the placebo group (8.1±2.4% versus 9.7±2.4%, 16.1±2.9% versus 16.8±3.2%, and 21.4±3.9% versus 23.3±4.2%). The response to nitroglycerin infusion was not altered in both groups. Increase in coronary blood flow in response to adenosine and coronary flow reserve remained unchanged during placebo and statin treatment. Serum concentrations of blood lipids and high-sensitive C-reactive protein were not significantly altered after 24 hours in response to placebo or pravastatin therapy. Statin treatment improves endothelium-dependent coronary vasomotion within 24 hours in the absence of significant cholesterol reduction. The full text of this article is available online at http://www.circresaha.org. (Circ Res. 2003;93:e98-e103.)

Key Words: endothelial function ■ statins ■ coronary disease ■ acetylcholine ■ angiography

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) exert beneficial therapeutic effects in patients at risk for cardiovascular events, as demonstrated by various trials. Long-term treatment reduces the incidence of cardiovascular death, myocardial infarction, and stroke in patients with and without hypercholesterolemia. The antiatherogenic effects of these drugs may not be exclusively dependent on their lipid-lowering properties, which led to the view that statins may cause pleiotropic effects on vascular and cardiac cells independent of cholesterol synthesis. These putatively cholesterol-independent effects underscored the notion that statins could potentially exert more rapid effects than seen in the large lipid-lowering trials. Several studies were conducted in hypercholesterolemic patients to evaluate effects of statins within months on endothelial function. Moreover, trials are available in patients with acute coronary syndromes. However, treatment was usually initiated within 1 to 7 days after the acute disease onset, and outcomes were measured after several months. Although these studies showed that patients with acute coronary syndromes may benefit from statins, it was not investigated whether these positive results were related to the statin therapy per se or specifically caused by the relatively early initiation of therapy. In addition, it remained unclear whether statins lead to acute benefits within days in these unstable patients, which would favor an immediate beginning of statin therapy.

The vascular endothelium is involved in the pathogenesis of atherosclerosis. Endothelial dysfunction is characterized by an impaired function of the endothelium, associated with decreased production of nitric oxide, reduced vasorelaxation or abnormal vasoconstriction in response to acetylcholine, procoagulant, and proinflammatory processes. Endothelial dysfunction has prognostic implications by predicting adverse cardiac events and mortality in patients with and without manifested atherosclerosis. Although there are reports about statin effects on endothelial function of the forearm within 3 days to 1 month, it is unknown whether
Materials and Methods

Patient Population

Twenty-seven middle-aged, white patients were included in this study. Patients were eligible for the study if they were admitted for coronary angiography due to stable angina pectoris and had none of the following exclusion criteria: treatment with statins, left ventricular ejection fraction below 50%, acute coronary syndromes, coronary three-vessel disease or left main disease, revascularization procedures within the last 8 weeks, significant valvular heart disease, any liver disease, renal insufficiency (creatinine above 2.0 mg/dL), any severe disease at present or in the past, history of drug or alcohol abuse, or gravidity. Patients gave written informed consent, and a physical examination, an ECG recording, and blood pressure measurements (at least three times in the supine position) were undertaken. Routine chemical methods were used to determine serum concentrations of fasting cholesterol and triglycerides, liver enzymes, creatinine, and muscle creatinine kinase. Serum concentrations of high-sensitive C-reactive protein were assessed using a latex-enhanced turbidimetric assay (Roche).

Patients were randomized in a double-blind fashion to one of the two treatment groups, either placebo or pravastatin 40 mg once daily (Pravasin, Bristol-Myers Squibb), according to a random-number code list. Patients were treated with a single dose of the study medications. Measurements were performed before and 24 hours after treatment. The study was approved by the ethics committees of the University of Homburg/Saar, Germany. The study design was a prospective, double-blind, placebo-controlled, randomized, monocenter study.

Study Protocol

Only patients with at least one coronary vessel without limiting stenosis (luminal diameter reduction less than 50%), which was used as target vessel for intracoronary measurements, were recruited. After fasting overnight, routine diagnostic coronary angiography was performed. Then, 10 000 IU heparin and 500 mg acetylsalicylic acid were given intravenously, and a 7-French guiding catheter (Medtronic) was introduced into the left or right coronary artery. An over-the-wire balloon catheter (Maverick, Boston Scientific Scimed; 1.5-mm balloon [never inflated], catheter diameter 2.3 French [0.78 mm]) was advanced over a 0.014-inch (0.036 cm) Doppler wire catheter with an infusion pump (Perfusor secura, Braun) at a flow rate of 1 mL/min over 3 minutes. After infusion of saline and baseline measurements, acetylsalicylic infusion was started using stepwise increasing doses of 0.01, 0.1, and 1 μmol/L estimated final intracoronary concentrations. At the end of each infusion period of acetylsalicylic, coronary angiography was performed to assess coronary luminal diameter. After an interval with saline infusion, adenosine was infused at a concentration of 15 μg/min (1 μmol/L estimated final intracoronary concentration), and Doppler velocimetry was performed at the end of the infusion period to assess blood flow velocity, followed by coronary angiography. Finally, after another interval with saline infusion, nitroglycerin was infused at a concentration of 200 μg/min (15 μmol/L estimated final intracoronary concentration), and coronary angiography was performed.

Quantitative Coronary Angiography

Serial coronary angiograms were performed using the identical projection, tube and table height, and magnification. The non-ionic contrast medium iopromide (Ultravist 370, Schering) was used for angiography and was injected manually through the guiding catheter at low pressure. Biplane cineangiograms were recorded with the target vessel positioned near the isocenter. Overlapping of coronary segments was avoided. The film sequences were stored digitally for subsequent computer analysis. The coronary luminal diameter was analyzed by the use of an automated edge-detection software system (CAAS II, Pie Medical). The analysis was performed by a trained investigator who was unaware (blinded) of the investigated subject, of the study medication, and of the intracoronary infusions. The contrast medium-filled distal 7-French guiding catheter was used as the standard for calibration. The mean luminal diameter of the target vessel was measured at a segment proximal to the infusion catheter (control segment), at the tip of the Doppler wire before and after nitroglycerin infusion before and after adenosine infusion for the calculation of coronary blood flow, and in three subsequent vessel segments distal to the Doppler wire before and after administration of each successive dose of acetylsalicylic. For the comparison of the coronary response to adenosine, the combined response of all three analyzed vessel segments was used. The response of the vessel segment was calculated as percent change in mean luminal diameter compared with the baseline measurement. Negative values indicate decreases in luminal diameter.

Statistical Analysis

All results were compared before and after treatment. Data are shown as absolute change after treatment or as change in percentage, respectively. Continuous data are expressed as mean±SE. After testing for normal distribution, continuous variables were analyzed by two-tailed Student’s t test or by ANOVA followed by post-hoc comparisons using the Newman-Keuls procedure, where appropriate. Categorical variables were compared by the chi-squared test. Values of P<0.05 were considered to indicate statistical significance. The SPSS 10.0 software package (SPSS) was used for statistical analysis.

Results

Baseline Characteristics

Twenty-seven middle-aged, normotensive patients with average low-density lipoprotein (LDL) cholesterol concentrations calculated as the average peak velocity after adenosine administration divided by the average peak velocity at baseline. The measurements were performed in the left anterior descending artery (LAD; 21 patients), circumflex artery (CX; four patients), and in the right coronary artery (RCA; two patients).

Drug Administration

After a 15-minute equilibration period, resting measurements were performed. Drug administration was started when stable measurements under resting conditions were achieved. Saline (NaCl 0.9%), acetylsalicylic chloride (Miochol-E, Novartis Ophthalmics), adenosine (Adrekar, Sanofi-Synthelabo), and nitroglycerin (Trinitrosan, Merck) were administered through the inner lumen of the over-the-wire catheter with an infusion pump (Perfusor secura, Braun) at a flow rate of 1 mL/min over 3 minutes. After infusion of saline and baseline measurements, acetylsalicylic infusion was started using stepwise increasing doses of 0.01, 0.1, and 1 μmol/L estimated final intracoronary concentrations. At the end of each infusion period of acetylsalicylic, coronary angiography was performed to assess coronary luminal diameter. After an interval with saline infusion, adenosine was infused at a concentration of 15 μg/min (1 μmol/L estimated final intracoronary concentration), and Doppler velocimetry was performed at the end of the infusion period to assess blood flow velocity, followed by coronary angiography. Finally, after another interval with saline infusion, nitroglycerin was infused at a concentration of 200 μg/min (15 μmol/L estimated final intracoronary concentration), and coronary angiography was performed.

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Results

Baseline Characteristics

Twenty-seven middle-aged, normotensive patients with average low-density lipoprotein (LDL) cholesterol concentrations...
Blood pressure, mm Hg

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=14)</th>
<th>Pravastatin (n=13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>138.3±4.5</td>
<td>136.2±3.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81.3±1.4</td>
<td>78.8±3.8</td>
<td>0.57</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66.4±5.1</td>
<td>66.3±2.5</td>
<td>0.99</td>
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</tbody>
</table>

Data are shown as absolute change 24 hours after treatment compared with the baseline values, expressed as mean±SE. P values for the comparison between groups. There were no significant differences for the comparison before and 24 hours after treatment in both groups. Negative values indicate decreases.

Changes in Coronary Luminal Diameter in Response to Acetylcholine and Nitroglycerin

The response of epicardial coronary arteries to intracoronary infusion of acetylcholine was similar in both groups before treatment (P=0.50, P=0.90, and P=0.76 for 0.01, 0.1, and 1 μmol/L acetylcholine, respectively). Intracoronary acetylcholine infusion induced abnormal vasoconstriction in the subjects included in this study, indicating underlying coronary endothelial dysfunction in this population. The effect of treatment with either placebo or pravastatin on the coronary response to acetylcholine is shown in the Figure. The epicardial coronary response to acetylcholine remained unchanged after treatment in the placebo group (P=0.58, P=0.85, and P=0.69 for 0.01, 0.1, and 1 μmol/L acetylcholine, respectively; Figure, panel A). In contrast, 24 hours after a single oral administration of 40 mg pravastatin, the mean vasoconstrictive response to acetylcholine was significantly attenuated compared with the measurements before treatment (P=0.33, P=0.03, and P=0.04 for 0.01, 0.1, and 1 μmol/L acetylcholine, respectively; Figure, panel B) and compared with the placebo group (P=0.03, P=0.01, and P=0.04 for 0.01, 0.1, and 1 μmol/L acetylcholine, respectively. Analysis of coronary artery segments proximal to the infusion of acetylcholine (control segments) showed no significant response to acetylcholine in both groups before and after treatment (data not shown). The endothelium-independent epicardial coronary re-
response to nitroglycerin was similar at the initial measurements ($P=0.96$) and remained unchanged after treatment in both groups ($P=0.64$ for placebo and $P=0.81$ for pravastatin, respectively), as shown in Table 3.

**TABLE 3. Changes in Coronary Vessel Luminal Diameter and Coronary Blood Flow Before and 24 Hours After Treatment With Pravastatin (40 mg/d) or Placebo**

<table>
<thead>
<tr>
<th>Percent Change</th>
<th>Placebo (n=14)</th>
<th>Pravastatin (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>24 Hours</td>
</tr>
<tr>
<td>Luminal diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>+7.6±2.5</td>
<td>+5.4±2.4</td>
</tr>
<tr>
<td>Coronary blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>+154.4±28.5</td>
<td>+113.2±27.4</td>
</tr>
<tr>
<td>CFR (absolute values)</td>
<td>2.4±0.2</td>
<td>2.1±0.2</td>
</tr>
</tbody>
</table>

Data are shown as percentage of change in coronary luminal diameter or coronary blood flow compared with the baseline value before infusion of adenosine (1 $\mu$mol/L estimated final intracoronary concentration; 15 $\mu$g/min) or nitroglycerin (15 $\mu$mol/L estimated final intracoronary concentration; 200 $\mu$g/min), respectively, expressed as mean±SE. CFR indicates coronary blood flow reserve. There were no significant differences for the comparison before and 24 hours after treatment in both groups.

Changes in Coronary Blood Flow in Response to Adenosine

The coronary microvascular response to adenosine was investigated by assessment of coronary blood flow (CBF). There was no significant difference in adenosine-mediated increase in CBF between the groups at the initial measurements, as shown in Table 3 ($P=0.47$). Adenosine-mediated changes in CBF and coronary flow reserve (CFR) were not significantly influenced 24 hours after treatment with placebo ($P=0.55$ for CBF and $P=0.76$ for CFR, respectively) or pravastatin ($P=0.91$ for CBF and $P=0.70$ for CFR, respectively) (Table 3).

**Discussion**

Coronary endothelial dysfunction is associated with impaired endothelium-dependent vasodilatation or, more severely, with abnormal vasoconstriction in response to intracoronary infusion of acetylcholine.\(^{18,20}\) Accordingly, the acetylcholine-induced coronary vasoconstriction observed in our study indicates severe endothelial dysfunction in the patient population. Treatment with a single oral dose of 40 mg pravastatin rapidly improved acetylcholine-mediated epicardial coronary vasomotion, whereas endothelium-independent vasodilatation induced by nitroglycerin and coronary microvascular response to adenosine were not affected. Statin treatment led to a marked attenuation of vasoconstriction in response to acetylcholine but did expectedly not normalize endothelium-dependent vasomotion. This was also seen in other intervention studies\(^{11,12,26}\) and indicates that a longer, maybe more intense and multifactorial treatment is required to completely restore endothelial function. Because endothelial dysfunction is associated with increased cardiovascular event rate and mortality,\(^{19–21}\) the observed rapid improvement of endothelial function may be of importance for the patients’ prognosis.

The results of our study are in agreement with other studies showing an improvement of coronary endothelial function by statin treatment.\(^{10–12}\) In these studies, pretreatment LDL cholesterol concentrations were between 140 to 195 mg/dL and statin therapy was performed for 5 to 12 months, before follow-up measurements were performed. In contrast, our

Changes in coronary luminal diameter in response to increasing doses of acetylcholine before and 24 hours after treatment with pravastatin or placebo. Changes in coronary luminal diameter in response to acetylcholine at concentrations of 0.01, 0.1, and 1 $\mu$mol/L estimated final intracoronary concentration (combined analysis of 3 subsequent vessel segments) are expressed as percentage of change compared with the baseline values (mean±SE). Comparison of measurements before and 24 hours after treatment with either placebo (A) or pravastatin (40 mg/d) (B). *$P<0.05$ for the comparison before and after treatment by ANOVA. Negative values indicate vasoconstriction.
study shows a beneficial clinical effect of statin therapy on the coronary vasculature as early as 24 hours after initiation of treatment. Two studies demonstrated an early effect of statin treatment on endothelial function of the forearm within days.23,27 However, statin effects on the forearm and coronary vasculature may differ, and direct comparisons between forearm blood flow and coronary vasomotion have not been done. So far, the impact of statins within hours or days on the human coronary system had not been tested.

On the molecular level, endothelial dysfunction is characterized by an impaired bioactivity of nitric oxide (NO) within the vascular wall due to decreased NO production or accelerated degradation of NO by reactive oxygen species. Inhibition of the HMG-CoA reductase does not only lower plasma cholesterol concentrations, but also decreases intracellular levels of isoprenoid intermediates, which are important for the modification and functioning of numerous cellular factors.7–9 In vitro and in vivo studies identified various direct, cholesterol-independent, pleiotropic effects of statins in the vasculature that may beneficially influence vascular function.7–9 This includes, among many others, the rapid restoration of NO bioactivity by upregulation and activation of endothelial NO synthase and the enhancement of NO release as well as the reduction of oxidative stress by antioxidative properties.28–31 It is well possible that statins rapidly improve endothelial function by these lipid-independent mechanisms in the coronary vasculature of humans as well.

Inhibition of the HMG-CoA reductase is the key mechanism of action of all statins, and therefore, it seems likely that the observed rapid effect on coronary endothelial function is a class effect of statins. However, there are certain differences between the statins, and it cannot be excluded that these properties may lead to different effects on coronary endothelial function in humans.

In our study, the effect of statin treatment on endothelium-dependent vasomotion was observed although the herein measured serum concentrations of lipid subfractions were not significantly altered after 24 hours. Relevant changes in cholesterol levels are to be expected within days after initiation of statin therapy. This indicates a rapid beneficial effect of HMG-CoA reductase inhibition, which may be independent of the more sustained cholesterol-lowering. The results of a recent study in hypercholesterolemic pigs showed that statin treatment may restore coronary endothelial function in the absence of cholesterol-lowering.32 In two studies investigating forearm blood flow in humans, improvement of endothelial function was observed within days after initiation of statin therapy without a significant reduction of cholesterol levels.23,27 In addition, endothelial function was improved by statin treatment in patients with low pretreatment cholesterol concentrations.27,33 In the HPS trial and the ASCOT-LLA study, statin treatment prevented cardiovascular events irrespective of baseline cholesterol levels, including patients with LDL cholesterol concentrations below 116 mg/dL.5,34 In hypercholesterolemia, lowering of cholesterol concentrations itself has a major impact on endothelial function, and it may be argued that also in case of normocholesterolemia, the lipid-lowering properties of statins cause their beneficial effects. However, taking the findings of our and the other mentioned studies together with the molecular evidence of pleiotropic, cholesterol-independent actions of statins in vascular cells, it may be speculated that the observed rapid effect on endothelial function after HMG-CoA reductase inhibition is at least in part mediated by cholesterol-independent properties of statins. It has recently been shown that statins may act independent of the blockade of the HMG-CoA reductase in mononuclear and endothelial cells.36,37 At present, there is no valid tool available to test this possibility in humans in a clinical setting. Thus, it cannot be excluded that the observed effect on endothelial function is independent of HMG-CoA reductase inhibition.

Statins exert antiinflammatory effects and lower markers of inflammation, such as C-reactive protein, in patients with coronary artery disease.18,38 In our study, pravastatin did not reduce CRP levels. Treatment for 24 hours may be too short to find significant changes in CRP serum concentrations. In contrast, there was a nonsignificant trend toward increased CRP levels in both treatment groups, possibly related to coronary angiography and intracoronary measurements.

The presented findings demonstrate that statins may exert rapid beneficial effects on coronary endothelial function in humans with average cholesterol levels presenting with angina pectoris. This may have important implications. Endothelial dysfunction marks rapid disease progression and poor prognosis, and it is thought that proinflammatory and procoagulatory processes associated with the dysfunctional endothelium may also account for the development and the complications of acute coronary syndromes,16–21 in which immediate treatment is needed. Therefore, diagnosis of endothelial dysfunction may require efficient and rapid treatment regimen in order to reduce cardiovascular event rates in patients who are prone to adverse outcome. Statin treatment may possibly offer an additional option to rapidly improve vascular function, which may potentially have an impact on the rate of cardiovascular events in patients with endothelial dysfunction and acute coronary syndromes. The findings of the presented study need to be confirmed in a larger number of patients. An assessment of the statin effect on morbidity and mortality in correlation to the measurements of coronary endothelial function would probably allow to further define the role of statins in the treatment of endothelial dysfunction irrespective of the underlying risk factor and in acute treatment regimen for patients with coronary artery disease.

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References


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