Effects of Initiation and Acute Withdrawal of Statins on the Neurovascular Coupling Mechanism in Healthy, Normocholesterolemic Humans

Bernhard Rosengarten, MD; Dieter Auch, MD; Manfred Kaps, MD

Background and Purpose—Recent clinical trials imply increased risk of vascular events after statin withdrawal. There is evidence that this observation relates to an impaired nitric oxide system. The present analysis investigates the effect of initiation and withdrawal of statin therapy on resting and functionally activated cerebral hemodynamics in healthy young volunteers.

Methods—Sixteen healthy students (aged 23.7 ± 3.3 years, 10 male) were subjected to a placebo-controlled, double-blind crossover study with a washout phase between blocks of 4 weeks. In the verum group, 20 mg pravastatin was taken for 2 weeks followed by 40 mg for 4 weeks. Withdrawal effects were investigated the day after discontinuation. Total cholesterol levels, blood pressure, resting and evoked hemodynamic responses due to a visual stimulation task in the posterior cerebral artery were obtained at baseline and then weekly and the day after discontinuation.

Results—In the verum group, cholesterol levels significantly decreased after 2 weeks (from 183 ± 30 to 150 ± 28 mg/dL; \( P < 0.001 \)) and then remained nearly stable (147 ± 21 mg/dL after 6 weeks). Blood pressure, resting and evoked hemodynamic responses remained constant throughout the study. The day after statin withdrawal, evoked flow velocity responses were significantly lower (11 ± 4% versus 13 ± 5% at baseline; \( P < 0.01 \)) indicating inappropriate blood supply of active neurons.

Conclusions—Reduction in evoked flow velocity responses reflects reduced nitric oxide bioavailability and therefore supports molecular findings of acute statin withdrawal. Questions arise if the present data might give a link to reports of increased vascular events in patients at vascular risk after acute statin withdrawal. (Stroke. 2007;38:3193-3197.)

Key Words: cerebral blood flow • endothelium • HMG-CoA reductase inhibitors • neurovascular coupling • nitric oxide

Inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (statins) are potent cholesterol-lowering drugs. Additionally, they improve endothelial function and have antithrombotic and antiinflammatory effects.\(^1\)\(^-\)^\(^5\) In clinical trials and meta-analyses, statins have demonstrated a significant reduction in myocardial infarction and ischemic stroke.\(^6\)\(^-\)^\(^9\) In the last years, there is growing evidence that acute statin withdrawal can increase cerebrovascular and coronary ischemic events in patients at vascular risk,\(^10\)\(^-\)^\(^12\) whereas such an effect was not seen in stable cardiac patients.\(^13\) However, in men with normocholesterolemia, initiation of statin therapy led to an improved endothelium-dependent forearm blood flow as assessed by venous occlusion plethysmography.\(^14\) The effect was abolished 24 hours after acute withdrawal of atorvastatin. Vasoregulative improvement under statin therapy was assumed to be caused by an improvement of the nitric oxide (NO) system. On several mechanisms, it is assumed that statins inhibit RhoA membrane translocation and guanosine–triphosphate hydrolase activity, whereas they upregulate RhoA expression.\(^15\)\(^,\)^\(^16\) Because RhoA is a negative regulator of endothelial NO synthase, inhibition of the RhoA leads to increased bioavailability of NO and possibly improved vasoreactivity. After acute discontinuation of statin treatment, inhibition of geranylgeranylation of RhoA GTPase is abolished. This results in an overt membrane-binding and activation of RhoA and decreased bioavailability of NO.\(^15\)\(^,\)^\(^16\) Another mechanism is massive Rac-1 translocation from the cytosol to the membrane after statin withdrawal. Membrane-bound Rac1 is an essential subunit of the NADPH-oxidase complex leading to oxidative burst and scavenging of NO by superoxide anions.\(^17\) Besides effects on peripheral vasculature, measurements of cerebral vasoreactivity have not been undertaken to investigate effects of initiation and withdrawal of statin therapy in humans. We investigated effects on basal and functionally activated cerebral hemodynamics in humans using a func-
tional transcranial Doppler test with a visual stimulation task.18–24

Materials and Methods

Study Design

Sixteen healthy students (aged 23.7±3.3 years, 10 male) without family or personal history of migraine, premature vascular disease, hypertension, diabetes mellitus, or hyperlipidemia were included in the randomized, double-blind, placebo-controlled crossover study. All volunteers were clinically healthy, nonsmokers, and did not take regular medication. Absence of vessel stenosis was an inclusion criterion, which was examined by an extra- and transcranial Duplex scan. The study was approved by the institutional review committee and each volunteer gave written informed consent. The tests were performed in a quiet room at approximately 25°C while the subjects were sitting comfortably. Before each test, volunteers had abstained from caffeine overnight (10 to 14 hours) and venous blood samples were drawn. The arterial blood pressure was measured noninvasively and the functional transcranial Doppler test was performed in all subjects after sitting for 10 minutes.

Volunteers were randomly assigned to one of 2 test blocks. In the first block, volunteers received orally 20 mg pravastatin (Bristol-Myers Squibb, München, Germany) daily for 2 weeks and then received 40 mg per day for an addition 4 weeks. In the second group, volunteers took placebo. To assure double blindness, verum and placebo were crushed, color-adjusted, and newly packed in a capsule.

Between blocks, a time span of more than 1 month was chosen. The design of the study is illustrated schematically in Figure 1.

Vascular Studies

Two 2-MHz probes were mounted on an individually fitted headband. In all cases, the P2-segment of the left posterior cerebral artery and the right middle cerebral artery in its M1 segment were insonated. The middle cerebral artery was recorded to determine nonspecific effects in the test situation. The procedure of finding and verifying the vessels followed the descriptions of published protocols.18–20,25 Peak systolic (versus) blood flow velocities were recorded using a Multidop T2 Doppler device (DWL). The reason for the crossover design.

Figure 1. Study design. At baseline conditions, blood pressure, resting flow velocities, hemodynamic responses, total cholesterol levels, and safety parameters (see text) were measured. After random assigning, volunteers to placebo or verum group measurements were repeated weekly (black arrows) up to 6 weeks (2 weeks 20 mg pravastatin followed by 4 weeks 40 mg pravastatin in the verum group). The day after withdrawal, measurements were repeated (gray arrow). After 4 weeks, measurements were repeated according to the crossover design.

angle, and to allow comparisons between volunteers, absolute data were transformed into relative changes of cerebral blood flow velocity in relation to baseline. The baseline was calculated from the blood flow velocity averaged for a time span – 5 to 0 seconds before the beginning of the stimulation phase. The method and algorithm for analyzing the data sets in terms of a control system are described in detail in an earlier work.26,27 The following parameters were specified: $K$ represents the gain, $T$, the rate time, $o$, the undamped natural angular frequency (natural frequency), and $\xi$ the attenuation parameter of the system. Additionally, the time delay $T$ was calculated. The parameters describe different dynamic features of the assumed regulative principle of the neurovascular coupling. Because the parameters all derived from a mathematical approximation, they are at first glance theoretical and do not have a direct correlation to physiological processes. However, as compared with the specification of the overshoot, the evaluation allows description of the dynamic aspects of flow velocity adaptation.

Laboratory Assays

In the morning, fasting blood probes were taken. Venous blood samples were collected in tubes containing edetic acid. Samples were centrifuged within 10 minutes at 4000 rpm for 10 minutes. The plasma was then separated and stored at −70°C until analysis.

Plasma total cholesterol levels were analyzed by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Creatinine, myoglobin, liver enzymes, pancreatic enzymes, and lactate dehydrogenase were also obtained according to laboratory routine procedures for safety issues.

Statistical Analysis

Data are expressed as means±SD. Statistical comparisons between chemical measurements, resting blood flow velocities, and each of the independent control system parameters was performed using a one-way analysis of variance for repeated measurements. Time points of each test were assumed as the fixed treatment according to analysis of variance model 1. Statistical significance was inferred at $P<0.05$. However, because 4 functional parameters were compared, resembling a multiple testing situation, a Bonferroni correction was applied and the significance level was set to $p/4$, which gives $P<0.0125$. When statistical significance occurred, Scheffe’s post hoc test was performed. Test for normal distribution of data was done by the F-test.

Results

All volunteers completed the study. Blood pressure levels, creatinine, myoglobin, liver enzymes, pancreatic enzymes, and lactate dehydrogenase remained unaffected from medication or its acute withdrawal. Because we did not find position effects of verum or placebo blocks, we pooled data from both groups. Figure 2 shows the total cholesterol data for the verum and placebo groups. With initiation of statin therapy, the total cholesterol levels decreased from 183±30
mg/dL to 150±28 mg/dL at the end of the second week, which was statistically significant (P<0.001). The increase in dose of pravastatin to 40 mg did not lower the cholesterol levels significantly. The day after statin withdrawal, levels were still in the lower range but then normalized in the next 4 weeks before the placebo phase started.

Data for resting flow velocities and hemodynamic responses are given in the Table. Post hoc analysis demonstrated that the gain parameter significantly dropped after acute pravastatin withdrawal (11±4% versus 13±5% at baseline; P<0.01), whereas it remained stable during the preceding treatment phase. The other parameters remained constant throughout the study. Reproducibility of the data in the placebo phase underlines retest reliability of the technique.

Discussion
Stability of resting blood flow velocities in healthy volunteers under statin treatment is in good agreement with the literature. The NO effects, therefore, might not be as strong as compared with L-arginine infusion, which improved basal cerebral blood flow in humans. It was shown that only high doses of simvastatin (20 mg/kg) led to significant higher levels of basal cerebral blood flow in rats, whereas lower concentrations of 2 mg/kg did not change the flow significantly. However, a different picture may emerge under pathologic conditions. Patients with subcortical small vessel disease showed improved cerebral vasomotor reactivity under pravastatin treatment. The constellation of decreased evoked but stable resting flow velocities under NO inhibition was also previously reported in humans. Whereas evoked flow velocities decline under NO inhibition, resting flow velocities in basal arteries remained constant even under infusion of 6 mg/kg L-NMMA, a nonselective NO synthase antagonist. The findings were supported by Xe-133 inhalation single photon emission CT. However, as concluded from blood pressure effects, L-NMMA inhibition was much more potent than statin withdrawal; NO inhibition led to an increase in mean arterial blood pressure of 20%. Compared with the NO inhibition, we and others did not find a significant change in blood pressure due to statin withdrawal.

Although the Doppler technique measures flow velocity rather than blood flow, it is well accepted that flow velocity changes are a good indicator of flow changes. Performing investigations on the visual cortex the low spatial resolution of the Doppler technique is not a limitation because the visual

Table. Resting Flow Velocities and Hemodynamic Parameters Given as Mean±SD*

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=32)</th>
<th>Pravastatin (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1</td>
</tr>
<tr>
<td>Resting flow velocity, cm/s</td>
<td>60±10</td>
<td>59±7</td>
</tr>
<tr>
<td>Gain, %</td>
<td>13±4</td>
<td>13±6</td>
</tr>
<tr>
<td>Natural frequency, 1/s</td>
<td>0.23±0.05</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>Attenuation</td>
<td>0.48±0.21</td>
<td>0.45±0.16</td>
</tr>
<tr>
<td>Rate time, s</td>
<td>3.6±2.5</td>
<td>4±1.6</td>
</tr>
</tbody>
</table>

*Compared with baseline values, the gain parameter changed significantly after acute statin withdrawal.
†P<0.01.
cortex is supplied by the posterior cerebral artery.\textsuperscript{18–20} We used the Doppler technique because of its high temporal resolution and accuracy in determining flow velocity changes, which is needed to assess dynamic flow velocity changes.\textsuperscript{20} Unfortunately, we did not perform additional investigations after cessation of medication. From the present crossover study, we can only conclude that evoked flow velocity responses normalized with beginning of the new block.

The effects of statin withdrawal on the neurovascular coupling cannot be explained readily. According to the literature, there is little or no active participation by endothelium in the coupling of blood flow to neuronal activity, although this issue has not been addressed directly.\textsuperscript{23,24} Nevertheless, the possibility that the endothelium participates in the coupling has been proposed. An effect on other structures related to the neurovascular coupling, ie, the neuronal NO synthase from perivascular NO neurons, has not been investigated but is rather unlikely because of the hydrophilic nature of pravastatin and the tightness of the blood–brain barrier. Assuming mainly endothelial effects to explain the present findings, the most likely explanation might be given by the function of RhoA. RhoA plays an important role in shear stress-mediated vasoregulation.\textsuperscript{33} The reduced gain parameter might then be explained by a reduced shear stress-initiated vasodilation of cerebral vessels under conditions of functionally stimulated blood flow. A reduction in shear stress-related vasoregulation was seen in peripheral vasculature.\textsuperscript{14,15} Whereas the molecular interpretation of the present findings has to remain speculative, the observation of the decreased flow velocity responses resembles a situation of reduced blood supply of active neurons and an uncoupling of the neurovascular coupling mechanism. Although changes were not associated with neurological signs in healthy volunteers, there is growing evidence that even slight deviations from the ideal blood supply of active neurons might be clinically relevant. It was shown that a mismatch of approximately 10\% leads to disturbance of protein synthesis in neurons if conditions lasted for several hours.\textsuperscript{35} Therefore, the question arises if our findings might be linked to reports on an increased vascular risk of patients in which statin treatment was stopped. Data from a prospective study in patients with acute stroke indicated neurological deterioration in the group in which statin therapy was discontinued.\textsuperscript{36} The rapid effect after statin withdrawal is explained by the short plasma half-life of pravastatin, which is approximately 1.8 hours.\textsuperscript{37} Effects on the endothelium and the stability of evoked flow velocity responses under medication might be explained by the 10- to 100-fold higher plasma levels of pravastatin compared with other statins partially compensating for the short half-life of the substance.\textsuperscript{37} However, our present study demonstrates that irregular medication might abrogate any preventive effect of statins and therefore, agents with a longer half-life might be recommended.

The study relies on a relatively new analytical method that is capable of describing the influence of different disease processes on the hemodynamic responses. However, a limitation of the approach is that it is mainly descriptive because the complex physiological mechanisms of the neurovascular coupling are not very well understood. The simplicity of the vasoregulative characteristics, which can be expressed in terms of a second-order model, contrast to the complexity of the mechanism. Therefore, it appears that the control system parameters only describe features of regulator systems rather than single signaling pathways. However, our post hoc finding of a reduced gain parameter nicely matches the reports of others as already mentioned, whereas reports regarding statin effects on the other parameters are missing. According to the mathematical and physiological theory, a change of only one parameter rather than the total system might have been anticipated corroborating the present findings. Mathematically, the parameters are independent from each other because they describe different regulative features of the system. Physiologically, it has been shown that different mediator systems govern different aspects of the coupling, which are not affected by the statin withdrawal.

Summary

Abrupt statin withdrawal led to vasoregulative dysfunction as concluded from the uncoupling of the neurovascular coupling mechanism. Further studies have to prove if the uncoupling might contribute to the reported higher vascular risk of patients after statin withdrawal.

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Disclosures

None.

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