Reduction of Plasma 24S-Hydroxycholesterol (Cerebrosterol) Levels Using High-Dosage Simvastatin in Patients With Hypercholesterolemia

Evidence That Simvastatin Affects Cholesterol Metabolism in the Human Brain

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Background: Previous studies have shown that patients with early onset of Alzheimer disease and vascular dementia have higher levels of circulating brain-derived 24S-hydroxycholesterol (cerebrosterol). Two recent epidemiological studies indicated that treatment with inhibitors of cholesterol synthesis (statins) reduces the incidence of Alzheimer disease.

Objective: To test the hypothesis that treatment with high-dosage simvastatin reduces circulating levels of 24S-hydroxycholesterol.

Design: Prospective, 24-week treatment trial for lowering of cholesterol levels. We conducted assessments at baseline, week 6, and week 24.

Setting: An academic outpatient clinical study.

 Patients: Eighteen patients who met the criteria for hypercholesterolemia.

 Intervention: Treatment with 80 mg/d of simvastatin at night.

Main Outcome Measures: Plasma lipoprotein levels were measured enzymatically; lathosterol, by means of gas chromatography; and 24S-hydroxycholesterol, by means of gas chromatography–mass spectrometry.

Results: Simvastatin reduced total plasma cholesterol levels by 36% and 35% after 6 and 24 weeks, respectively (P < .001). Lathosterol levels were reduced by 74% and 72%, respectively, and the ratio of lathosterol to cholesterol, an indicator of whole-body cholesterol synthesis, was reduced by 60% and 61%, respectively (P < .001). Plasma 24S-hydroxycholesterol levels were lowered by 45% and 53%, respectively (P < .001). The ratio of 24S-hydroxycholesterol to cholesterol also decreased significantly (~12% [P = .01] and ~23% [P < .002], respectively). The further reduction of 24S-hydroxycholesterol levels and its ratio to cholesterol from weeks 6 to 24 was also significant (P = .02 for both).

Conclusions: The greater reduction of plasma concentrations of 24S-hydroxycholesterol compared with cholesterol indicates that simvastatin in a dosage of 80 mg/d reduces cholesterol turnover in the brain. The present results might describe a possible mechanism of how long-term treatment with statins could reduce the incidence of Alzheimer disease.

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PATIENTS AND METHODS

PATIENTS

Outpatients with primary hypercholesterolemia were enrolled in 4 centers for the study. The study was in accordance with the Helsinki Declaration, and approvals were obtained by all local ethical committees. Written informed consent was obtained from all patients after the nature of the procedure had been fully explained. Inclusion criteria for enrollment in the study were as follows: age between 21 and 70 years, low-density lipoprotein (LDL) cholesterol level of at least 160 mg/dL (4.1 mmol/L), and triglyceride level of no greater than 350 mg/dL (4.0 mmol/L). None of the patients had had impaired renal or liver function, diabetes mellitus, thyroid dysfunction, acute coronary heart disease, myocardial infarction, or coronary bypass surgery within the previous 3 months, and none had received drug therapy for lowering of lipid levels during the past 6 weeks.

STUDY DESIGN AND INTERVENTIONS

Eighteen patients (12 men and 6 women; mean [SD] age, 50±12 years; mean [SD] body mass index [calculated as weight in kilograms divided by the square of height in meters], 26±3) were enrolled in this prospective study. After a 4-week placebo run-in period with a diet low in cholesterol intake (<300 mg/d), patients were treated with 80 mg/d of simvastatin at night for 24 weeks. Fasting blood samples for the analysis of plasma lipoprotein levels were obtained after an overnight fast after the 4-week placebo run-in period and after 24 weeks of treatment. Samples for determination of plasma cholesterol, lathosterol, and 24S-hydroxycholesterol levels were obtained at the end of the 4-week placebo run-in period and after 6 and 24 weeks of treatment. Total cholesterol and triglyceride levels were determined enzymatically using commercially available kits (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol level was determined enzymatically after precipitation of apolipoprotein B–containing particles with phosphotungstic acid; LDL cholesterol level was calculated using the formula of Friedewald et al; and lathosterol level was quantified by means of gas-liquid chromatography and flame-ionization detection using 5α-cholestan as an internal standard. Level of 24S-hydroxycholesterol was analyzed by means of an isotope dilution method using gas chromatography–mass spectrometry. The variability of the measurements of lathosterol and 24S-hydroxycholesterol levels was assessed by means of 6-fold workup of a single serum sample. The coefficient of variation for lathosterol was 6.3% (mean [SD], 0.063±0.004 mg/dL; n=6); for 24S-hydroxycholesterol, 5.9% (mean [SD], 68±4 mg/mL; n=6).

STATISTICAL ANALYSIS

We used Wilcoxon matched-pair signed rank test to compare differences at baseline and after 6 and/or 24 weeks of treatment. P values of lower than .05 were considered significant.
different place of action. The reduction of total and LDL cholesterol levels during the present study supports the effect of simvastatin on plasma lipoprotein concentrations as described in a previous study with an identical dosage of simvastatin.26 Plasma cholesterol level is lowered as a result of the inhibition of cholesterol synthesis in the liver and subsequent increased expression of LDL receptors, which result in an up-regulated catabolic rate for plasma LDL. The decreased cholesterol synthesis was confirmed by the reduction of the ratio of lathosterol to cholesterol, an indicator of hepatic27 and total cholesterol synthesis.28 During treatment with simvastatin, the lowering effect on plasma 24S-hydroxycholesterol level was significantly more pronounced than that on plasma cholesterol level, suggesting that high-dosage simvastatin also affects cholesterol metabolism in the brain. Indeed, administration of simvastatin to guinea pigs diminishes de novo cholesterol synthesis in the brain, followed by reduced concentrations of β-amyloid, without altering total cholesterol content.29 Maintaining the high-dosage simvastatin treatment for a total of 24 weeks did not lead to a more pronounced decrease in the plasma concentrations of total cholesterol or lathosterol or the ratio of lathosterol to cholesterol. In contrast, 24S-hydroxycholesterol level was additionally reduced by 12% during the following 18 weeks, and the ratio of 24S-hydroxycholesterol to cholesterol, by 8%. Whether long-term treatment with lower dosages of simvastatin or other statins also reduces 24S-hydroxycholesterol levels remains to be elucidated.

Previous studies16,17 have shown that lower plasma concentrations of 24S-hydroxycholesterol in severely affected patients with AD is a peripheral marker for loss of cholesterol and/or cholesterol 24S-hydroxylase in the brain. Thus, early detection of predicted candidates for AD (early-onset AD) by means of elevated levels of plasma 24S-hydroxycholesterol or its ratio to cholesterol should initiate a protective measure to prove the beneficial therapy using statins for prevention of AD and VD.

Although the reduction of plasma cholesterol level may be responsible for the lower incidence of AD, the recent results from Jick et al15 suggest that only statins, and no other drug that lowers lipid levels, exhibit this preventive effect. Thus, the results of the present study might provide the pharmacological basis for a possible mechanism of action of statins in preventing AD and VD. However, only prospective randomized studies can prove our hypothesis that the reduction in plasma 24S-hydroxycholesterol concentrations by means of statins, indicating impaired cholesterol metabolism in the brain, is a method for preventing AD and/or VD.

### Levels of Serum Lipids, Lathosterol, and 24S-Hydroxycholesterol, and Their Ratio to Cholesterol Before and During Treatment With Simvastatin*

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 6</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL†</td>
<td>361 ± 81</td>
<td>227 ± 48‡</td>
<td>233 ± 52‡</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL†</td>
<td>280 ± 79</td>
<td>ND</td>
<td>158 ± 48‡</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL†</td>
<td>50 ± 13</td>
<td>ND</td>
<td>54 ± 15§</td>
</tr>
<tr>
<td>Triglycerides, mg/dL]</td>
<td>153 ± 71</td>
<td>ND</td>
<td>107 ± 38§</td>
</tr>
<tr>
<td>Lathosterol, mg/dL]</td>
<td>0.528 ± 0.202</td>
<td>0.139 ± 0.062‡</td>
<td>0.146 ± 0.057‡</td>
</tr>
<tr>
<td>Lathosterol-cholesterol, µg/mg</td>
<td>1.469 ± 0.486</td>
<td>0.605 ± 0.211‡</td>
<td>0.630 ± 0.187‡</td>
</tr>
<tr>
<td>24S-Hydroxycholesterol, ng/mL</td>
<td>114 ± 37</td>
<td>63 ± 21‡</td>
<td>56 ± 19†</td>
</tr>
<tr>
<td>24S-Hydroxycholesterol-cholesterol, ng/mg</td>
<td>32 ± 9</td>
<td>27 ± 7§</td>
<td>24 ± 5‡</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; and ND, not performed.
†To convert to the Système International Unit of millimoles per liter, multiply by 0.0259.
‡P < .005, compared with baseline.
§P < .05, compared with baseline.
∥To convert to the Système International Unit of millimoles per liter, multiply by 0.0113.
¶P < .02, compared with 6-week measurement.

![Figure 1](https://example.com/figure1.png) Plasma concentration of 24S-hydroxycholesterol at baseline (week 0) and after 6 and 24 weeks of treatment with simvastatin (80 mg/d) in 18 patients with hypercholesterolemia.

![Figure 2](https://example.com/figure2.png) The ratio of 24S-hydroxycholesterol to cholesterol in plasma at baseline (week 0) and after 6 and 24 weeks of treatment with simvastatin (80 mg/d) in 18 patients with hypercholesterolemia.
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Author contributions: Study concept and design (Drs Locatelli, Lütjohann, Schmidt, Otto, Beisiegel, and von Bergmann); acquisition of data (Drs Locatelli, Lütjohann, and von Bergmann); analysis and interpretation of data (Drs Locatelli, Lütjohann, and von Bergmann); drafting of the manuscript (Drs Locatelli, Lütjohann, and von Bergmann); critical revision of the manuscript for important intellectual content (Drs Locatelli, Lütjohann, and von Bergmann); statistical expertise (Drs Locatelli, Lütjohann, and von Bergmann); obtaining funding (Drs Locatelli, Lütjohann, and von Bergmann); administrative, technical, or material support (Drs Otto, Schmidt, and Beisiegel).

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REFERENCES


