Plasma Levels of β-Amyloid(1-40), β-Amyloid(1-42), and Total β-Amyloid Remain Unaffected in Adult Patients With Hypercholesterolemia After Treatment With Statins

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**Background:** Epidemiological studies suggest that statins reduce the risk of developing Alzheimer disease. Cell and animal experiments have revealed a connection between cholesterol metabolism and the processing of amyloid precursor protein. To our knowledge, the mechanism for statins in risk reduction of Alzheimer disease is unknown.

**Objective:** To test the effect of statin treatment on β-amyloid (Aβ) metabolism in humans.

**Design:** A prospective, randomized, dose-finding 36-week treatment trial with statins. Plasma samples were taken at baseline (week 0) and at weeks 6, 12, and 36.

**Setting:** Outpatient clinical study at a university hospital.

**Patients:** Thirty-nine patients who met the criteria for hypercholesterolemia.

**Interventions:** Patients were randomized to oral treatment with either simvastatin or atorvastatin calcium according to the following regimen: simvastatin, 40 mg/d, or atorvastatin, 20 mg/d, for 6 weeks; followed by simvastatin, 80 mg/d, or atorvastatin, 40 mg/d, for 6 weeks; and finally, simvastatin, 80 mg/d, or atorvastatin, 80 mg/d, for 24 weeks.

**Main Outcome Measures:** Plasma levels of Aβ(1-40) and Aβ(1-42) were measured using 2 enzyme-linked immunosorbent assays, and total Aβ was quantified by Western blotting.

**Results:** Treatment with both statins reduced total plasma cholesterol levels by 56% (P = .00). The plasma levels of Aβ(1-40), Aβ(1-42), and total Aβ were stable in individual patients during the treatment period. No significant change in the level of Aβ(1-40), Aβ(1-42), or total Aβ was found.

**Conclusion:** This study questions the effect of statins on the processing of amyloid precursor protein in humans.

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It is believed that cholesterol in the brain mainly results from de novo synthesis of cholesterol in the cells. The efflux of cholesterol is in the form of 24S-hydroxycholesterol. Two recent studies have demonstrated that patients with early-onset AD and vascular dementia have elevated concentrations of 24S-hydroxycholesterol in their plasma, indicating an overproduction of cholesterol in the brain. Support for this hypothesis has been given in 2 studies showing that cholesterol metabolism in the human brain was affected by treatment with statins and effects were seen after 6 weeks of treatment.

To test the effect of statins on \( \beta \)-amyloid metabolism in humans, we examined the plasma levels of \( \beta \)-amyloid(1-40), \( \beta \)-amyloid(1-42), and total \( \beta \)-amyloid in longitudinal samples from patients with hypercholesterolemia who were treated with statins. We observed that pharmacologically attainable concentrations of atorvastatin calcium or simvastatin did not affect the plasma levels of \( \beta \)-amyloid(1-40), \( \beta \)-amyloid(1-42), or total \( \beta \).

**METHODS**

This study is an extension of a substudy of a larger multicenter investigation. In the present study, 39 subjects (27 men and 12 women) were included, and all had completed the entire treatment; 19 patients were treated with simvastatin, and 20 with atorvastatin. The mean age of the 39 subjects was 55 years (range, 28-67 years). Eight patients were excluded from the multicenter investigation because no samples were available. Inclusion criteria were the same as for the main study, published separately. To qualify, all patients were required to have a low-density lipoprotein cholesterol level of 162.2 mg/dL or more (\( > 4.19 \) mmol/L) and a triglyceride level of 351.5 mg/dL or less (\( \leq 3.97 \) mmol/L). At baseline, the mean (SD) plasma level of total cholesterol was 301.5 (54.2) mg/dL (7.80 [1.40] mmol/L). The medical history of all patients was studied, and all patients underwent a thorough clinical examination to exclude cognitive impairment. Following the start of a 4-week run-in period, the patients were randomized to 1 of 2 treatments: simvastatin, 40 mg/d, or atorvastatin, 20 mg/d, for 6 weeks; followed by simvastatin, 80 mg/d, or atorvastatin, 40 mg/d, for 6 weeks; and finally, simvastatin, 80 mg/d, or atorvastatin, 80 mg/d, for 24 weeks. Other ongoing lipid-lowering treatment was discontinued at least 8 weeks before randomization. The EDTA-plasma samples were collected at baseline (week 0) and at the end of each treatment period (weeks 6, 12, and 36), and then frozen. All samples analyzed by an enzyme-linked immunosorbent assay (ELISA) were analyzed on the same day. The study was approved by the local ethics committees, and patients were included after giving informed consent. The study was performed according to the Declaration of Helsinki.

**BIOCHEMICAL ANALYSIS**

The plasma \( \beta \)-amyloid(1-40) concentration was determined using the high-sensitivity format of one assay (INNOTEST \( \beta \)-AMYLOID(1-42); INNOGENETICS NV) with a detection limit of 7.8 pg/mL. The assay and its characteristics have previously been described in detail. The method is based on the antibody 21F12, specific for the carboxyl terminus of \( \beta \)-amyloid, and the biotinylated detection antibody 3D6, specific for the amino terminus, including amino acid D1 of \( \beta \)-amyloid(1-42).

The concentration of \( \beta \)-amyloid(1-42) plasma levels was determined by a research prototype version of another assay (INNOTEST \( \beta \)-AMYLOID(1-40); INNOGENETICS NV) with a detection limit of 7.8 pg/mL. The method is based on the antibody R293, specific to the carboxyl terminus of \( \beta \)-amyloid(1-40), and the biotinylated detection antibody 3D6.

The total levels of \( \beta \)-amyloid were measured using an advanced version of the sensitive Western blot assay according to an internally developed protocol (ABETA GmbH). The method is based on the monoclonal antibody WO-2 (ABETA GmbH), which recognizes the 4-10 residue of \( \beta \)-amyloid. Quantification was performed using computer software (Quantity One; BioRad Laboratories, Hercules, Calif). The synthetic peptide from the assay used (INNOTEST \( \beta \)-AMYLOID(1-42)) was used as a standard. A control sample was loaded onto all gels, and was quantified and used subsequently as an internal quality control. Wiklund et al provide further information on the characterization of lipids and lipoproteins.

**STATISTICAL ANALYSIS**

All statistical procedures were performed using a commercially available software program (SPSS for Windows; SPSS Inc, Chicago, Ill). The Pearson product moment correlation coefficient was used for correlations. Comparison between groups was performed using the nonparametric Kruskal-Wallis test. We used the Wilcoxon matched-pair signed rank test to compare differences at baseline and after 6, 12, or 36 weeks of treatment. \( P < .05 \) was considered significant.

**RESULTS**

**LIPIDS**

Treatment with either atorvastatin or simvastatin for 36 weeks produced, as expected, significant effects on total cholesterol levels (even after just 6 weeks of treatment). For the combined groups, total cholesterol levels were reduced by 56% (\( P = .00 \)) after 36 weeks of treatment. In the group treated with atorvastatin, the reduction was 53% and in the simvastatin group, 57% (\( P = .00 \) for both). Changes in total cholesterol levels are given in the Table. The major adverse effect was gastrointestinal symptoms; however, no patient in either treatment group experienced myopathy.

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**Table**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Cholesterol, mg/dL</th>
<th>( \beta )-amyloid(1-40), pg/mL</th>
<th>( \beta )-amyloid(1-42), pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atorvastatin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>263 (244-305)</td>
<td>141 (117-183)</td>
<td>23 (21-31)</td>
</tr>
<tr>
<td>6 wk, 40 mg/d</td>
<td>170 (155-182)</td>
<td>138 (124-169)</td>
<td>25 (21-28)</td>
</tr>
<tr>
<td>12 wk, 40 mg/d</td>
<td>151 (143-170)</td>
<td>147 (124-178)</td>
<td>25 (22-28)</td>
</tr>
<tr>
<td>36 wk, 80 mg/d</td>
<td>143 (135-155)</td>
<td>143 (121-175)</td>
<td>25 (23-28)</td>
</tr>
<tr>
<td><strong>Simvastatin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>325 (244-305)</td>
<td>148 (137-161)</td>
<td>35 (21-46)</td>
</tr>
<tr>
<td>6 wk, 40 mg/d</td>
<td>201 (155-182)</td>
<td>153 (144-184)</td>
<td>34 (23-48)</td>
</tr>
<tr>
<td>12 wk, 80 mg/d</td>
<td>186 (174-205)</td>
<td>163 (146-179)</td>
<td>36 (27-48)</td>
</tr>
<tr>
<td>36 wk, 80 mg/d</td>
<td>186 (135-205)</td>
<td>157 (135-178)</td>
<td>30 (24-44)</td>
</tr>
</tbody>
</table>

Abbreviation: \( \beta \)-amyloid.

**SI conversion factor:** To convert total cholesterol to millimoles per liter, multiply by 0.0259.

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Aβ(1-40) AND Aβ(1-42)

Neither simvastatin nor atorvastatin administration significantly affected the plasma levels of Aβ(1-40) (P = .99) or Aβ(1-42) (P = .10). At baseline, the mean (SD) plasma level of Aβ(1-40) for the combined groups (N = 39) was 148 (44) pg/mL; after 36 weeks of treatment, it was 152 ± 36 pg/mL. Mean (SD) levels of Aβ(1-42) for the combined groups (N = 39) were as follows: 33 (23) pg/mL at baseline and 32 (22) pg/mL after 36 weeks of treatment.

Values of Aβ(1-40) and Aβ(1-42) in plasma at baseline and after 6, 12, and 36 weeks of treatment are given in the Table. Figure 1 depicts the levels of Aβ(1-40) and Aβ(1-42) for the combined groups at baseline and after 6, 12, and 36 weeks of treatment. There were broad variations between individuals, but the levels were stable between samples taken at baseline and after 6, 12, or 36 weeks of treatment in individual subjects. Individual plasma levels of cholesterol and Aβ(1-42), before and during treatment, are illustrated in Figure 2. The levels of Aβ(1-42) and Aβ(1-40) corresponded, ie, patients with higher levels of Aβ(1-42) also had higher levels of Aβ(1-40), as recognized by the correlation coefficients (rweek 0 = 0.42 [P < .001], rweek 6 = 0.40 [P < .001], rweek 12 = 0.45 [P < .04], and rweek 36 = 0.35 [P < .001]).

TOTAL Aβ

Because of the limited sample volume (due to an earlier analysis), selected samples only (n = 14) were analyzed by Western blotting. Neither simvastatin nor atorvastatin treatment significantly changed the levels of total Aβ (P = .59). For the combined treatment groups (n = 14), the mean ± SD baseline level of Aβ was 228 (150) pg/mL; after 36 weeks of treatment, it was 219 (172) pg/mL. Broad variations between individuals were seen, but the levels were relatively stable between samples taken at baseline and after 6, 12, or 36 weeks of treatment in individual subjects.

COMMENT

At several time points after treatment with 2 different statins, simvastatin or atorvastatin, no significant change in the levels of either Aβ(1-40) or Aβ(1-42) was observed compared with baseline levels. The levels of Aβ(1-40) and Aβ(1-42) measured at baseline and after 6, 12, or 36 weeks of treatment were remarkably stable within each patient, and there was no significant difference between the 2 treatment groups. There was no control group in this study. However, the CSF levels of Aβ(1-42) have been shown to be stable in untreated patients with AD.25

Although ELISA is sensitive, one potential drawback when measuring Aβ is that the measurement can be affected if other proteins bind to Aβ26-28 and mask the epitope of antibodies used in the assay. Therefore, selected plasma samples were also quantified using an advanced sensitive Western blot; where possible, protein complexes were separated by subjecting the samples to sodium dodecyl sulfate and heat treatment. The results corresponded well with the ELISA measurements, and no significant change in plasma Aβ level was seen during treatment with statins. Thus, it is unlikely that the lack of effect on plasma Aβ(1-40) and Aβ(1-42) measured by the present ELISA (INNOGENETICS NV) is due to methodological shortcomings.

To our knowledge, this is the first longitudinal treatment study of patients with hypercholesterolemia in which the plasma levels of Aβ have been studied. In a case-control study,26 the CSF level of Aβ in patients with hypercholesterolemia, treated with statins or untreated, was compared with that of healthy controls. In agreement with our findings, no effect on Aβ metabolism was seen. It was demonstrated earlier that levels of Aβ(1-42) in the CSF and plasma were unchanged in patients with AD treated with simvastatin for 12 weeks. However, the levels of sAPP (α-cleaved soluble APP) and βsAPP (β-cleaved soluble APP) were significantly reduced, which could indicate that altered APP processing is not reflected in plasma or CSF Aβ levels. In a placebo-controlled double-blind study,31 patients with AD were treated with simvastatin for 26 weeks. In the total AD group, simvastatin did not significantly alter CSF levels of Aβ(1-40) or Aβ(1-42), in agreement with our results. However, a post hoc analysis revealed that the levels of Aβ(1-40) were slightly (−5.7% ± 6.5%), but significantly (n = 8; P < .05), reduced in patients with mild AD.

In the present study, the plasma level of Aβ was measured. Buxbaum and colleagues32 recently published a study on serum levels of Aβ in patients with hypercholesterolemia who were treated for 3 months with control-released lovastatin. They observed a concentration-dependent decrease in Aβ using a different ELISA, based on different antibodies: 4G8, specific to amino acids 17 to 24 of the Aβ peptide; and 6E10, specific to amino acids 5 to 10 of the Aβ peptide. The serum level of Aβ has

Figure 1. Histogram illustrating the levels of 2 β-amyloids (Aβs), Aβ(1-40) and Aβ(1-42), in plasma, measured by an enzyme-linked immunosorbent assay, at baseline and after 6, 12, and 36 weeks of treatment with atorvastatin calcium or simvastatin. Data are given as mean (SD) levels for the combined groups (N = 39).
been considered to be under the detection limit using our assay (INNOTEST \( \beta \)-AMYLOID(1-42)).

In agreement with the results in the present study, there are 2 prospective studies\(^{15,16} \) that do not support the connection between statin treatment and reduced prevalence of AD. In these 2 studies, no effect on developing dementia was seen after statin treatment. However, in both of these studies, patients with cognitive impairment or dementia were excluded, by excluding either patients with a Mini-Mental State Examination score below 24\(^{15} \) or patients with dementia\(^{16} \).

The dosages of statins given in the clinical studies mentioned were between 20 and 80 mg/d. However, in animal studies\(^{12,13,33} \) and cell culture experiments,\(^{12,13,33} \) the dosage of statins exceeds these dosages many times over. The differences in dosage between animal/cell culture studies and human studies may explain the lack of effect of pharmacological dosages of statins on A\( \beta \) in the present study.

Although cell culture and animal studies suggest that statins reduce A\( \beta \) production, the present and other clinical studies show no effect or only a minor effect on plasma and CSF levels of A\( \beta \) after statin treatment. Because statins also have other nonlipid effects, such as anti-inflammatory or neuroprotective effects,\(^{34} \) the potential protective effect of statins on AD seen in epidemiological studies may not be through the inhibition of cholesterol synthesis.

The origin of A\( \beta \) in plasma is still unclear. No correlation between CSF and plasma levels of A\( \beta \) has been seen,\(^{23,35} \) nor is there a correlation between CSF, A\( \beta_{42} \), and blood-brain barrier deficits, which suggests that plasma A\( \beta_{42} \) is not derived from the brain but is secreted
peripherally. Despite a marked reduction in cholesterol levels, we did not observe any change in plasma levels of Aβ. It is possible that statins may affect Aβ metabolism in the brain, but not peripherally. However, several studies have found no change in CSF levels or only minor effects in mild AD cases after statin treatment. In conclusion, further studies are needed to examine the mechanism by which statins may reduce the risk of developing AD.

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REFERENCES