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.

Arch Neurol. 2004;61:333-337
from the 25th percentile to the 75th percentile. Multiply by 0.0259.

A overproduction of cholesterol in the brain. Support for hydroxycholesterol in their plasma, indicating an Alzheimer dementia have elevated concentrations of 24S-hydroxycholesterol.18 Two recent studies18,19 have demonstrated that patients with early-onset AD and vascular dementia level of 351.5 mg/dL or less (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.

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.18 Two recent studies18,19 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 studies20,21 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 Aβ metabolism in humans, we examined the plasma levels of Aβ(1-40), Aβ(1-42), and total Aβ 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 Aβ(1-40), Aβ(1-42), or total Aβ.

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.

### METHODS

This study is an extension of a substudy of a larger multicenter investigation.21 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,21 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 (≤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,
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 (r week 6 = 0.42 [P<.001], r week 12 = 0.40 [P<.001], r week 12 = 0.45 [P<.04], and r week 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 Aβ 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 earlier30 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 αsAPP (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 colleagues25 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
been considered to be under the detection limit using our assay (INNOTEST β-AMYLOID(1-42)).

In agreement with the results in the present study, there are 2 prospective studies 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 or patients with dementia.

The dosages of statins given in the clinical studies mentioned were between 20 and 80 mg/d. However, in animal studies and cell culture experiments, 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 Aβ in the present study.

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

The origin of Aβ in plasma is still unclear. No correlation between CSF and plasma levels of Aβ has been seen, nor is there a correlation between CSF, Aβ_{42}, and blood-brain barrier deficits, which suggests that plasma Aβ_{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.

Accepted for publication October 28, 2003.

Author contributions: Study concept and design (Ms Hoglund and Drs Wiklund and Blennow); acquisition of data (Ms Hoglund and Drs Wiklund and Eikenberg); analysis and interpretation of data (Ms Hoglund and Drs Vanderstichele, Eikenberg, Vanmeechelen, and Blennow); drafting of the manuscript (Ms Hoglund and Drs Vanderstichele and Blennow); critical revision of the manuscript for important intellectual content (Ms Hoglund and Drs Wiklund, Vanmeechelen, and Blennow); statistical expertise (Dr Blennow); obtained funding (Ms Hoglund and Drs Wiklund, Vanmeechelen, and Blennow); administrative, technical, and material support (Drs Vanderstichele, Eikenberg, Vanmeechelen, and Blennow); study supervision (Drs Wiklund and Blennow).

This study was supported by projects 11560, 12103, and 14002 of the Swedish Medical Research Council, Stockholm, Sweden; Alzheimerfonden, Lund, Sweden; and Alzheimerfonden, Stockholm, Sweden; Alzheimerfonden, Lund, Sweden; and Stiftelse for Gamla Tjanarinnor, Stockholm, Sweden. Administrative, technical, and material support (Ms Hoglund and Drs Wiklund and Eikenberg); drafting of the manuscript (Ms Hoglund and Drs Wiklund and Blennow); and for sharing his scientific knowledge; and Inge Tomic, Mareike Grees, and Tobias Hartmann, PhD, for introducing us to the blotting method for Aβ, and for sharing his scientific knowledge; and Inge Tomic, Mareike Grees, and Monique Beumer for their skilful technical assistance. Corresponding author: Kina Hoglund, MSc, Experimental Neuroscience Section, Institute of Clinical Neuroscience, NeuroLab, SU/Molndal, S-431 80 Molndal, Sweden (e-mail: kina.hoglund@neuro.gu.se).

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