Treatment with Simvastatin in Normocholesterolemic Patients with Alzheimer's Disease: A 26-Week Randomized, Placebo-Controlled, Double-blind Trial

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In a randomized, placebo-controlled, double-blind study, we investigated whether statins alter cholesterol metabolites and reduce Aβ levels in the cerebrospinal fluid of 44 patients with Alzheimer's disease. Individuals were given up to 80mg simvastatin daily or placebo for 26 weeks. Overall, simvastatin did not significantly alter cerebrospinal fluid levels of Aβ40 and Aβ42. In post hoc analysis, simvastatin significantly decreased Aβ40 levels in the cerebrospinal fluid of patients with mild Alzheimer's disease. The reduction of Aβ40 correlated with the reduction of 24S-hydroxycholesterol. These changes were not observed in more severely affected patients.

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There is emerging evidence linking cholesterol, Aβ, and Alzheimer's disease (AD).[1,2] The *apo*E4 allele of the apolipoprotein E gene is associated with higher cholesterol levels[3] and increases the risk of developing the disease.[4] Two recent retrospective epidemiological studies indicate that there is a decreased prevalence of dementia associated with the use of cholesterol-lowering drugs that inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[5,6] HMG-CoA is the rate-limiting enzyme in the cascade of cellular cholesterol biosynthesis. Experiments in cell culture and animals demonstrated that treatment with cholesterol-lowering drugs reduces the production of Aβ.[7–9] It therefore is possible that by reducing Aβ levels, statins might provide neuroprotective effects in patients with AD.[1,10] To analyze whether statins alter cerebral cholesterol metabolism and reduce Aβ levels in the cerebrospinal fluid (CSF) of humans with AD, we conducted a randomized, placebo-controlled and double-blind trial with 80mg per day simvastatin in patients with AD.

Patients and Methods

Patient Population

Patients were eligible if they fulfilled criteria for a diagnosis of probable AD, as outlined by the National Institute of Neurological and Communicative Disorders and AD and Related Disorders. We included patients with Mini-Mental State Examination (MMSE) scores of 12 to 26. For further analysis, patients were subgrouped into patients with mild (MMSE 21–26) and moderate (MMSE 12–20) AD. All patients had a computed tomography scan to rule out vascular encephalopathy as a cause of dementia. Furthermore, patients with a Hachinski score above 3 were excluded from the study. Patients were allowed to take donepezil or rivastigmine if the dose had been unchanged for the last 3 months before study entry and remained stable during the 26-week study period. Patients with a continuous intake of antiinflammatory drugs were excluded. Forty-four patients were randomized to placebo or simvastatin (Fig 1). Except for gender with more female than male patients in the simvastatin group ($p < 0.04$, Fisher's exact test), the two treatment groups were well matched at baseline (Table 1).

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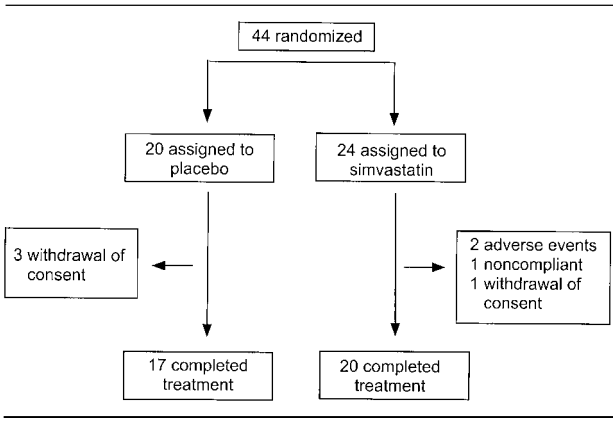

Fig 1. Trial profile.

Study Medication and Randomization

A randomization list was computer generated. Two copies of the randomization list were prepared: one was used by the packaging department of the study medication or placebo (Merck Sharp & Dohme, Munich, Germany), the other was kept in a locked location until the study was completed. All personnel directly involved in the conduct of the study remained unaware of the treatment groups until all patients had completed the trial and all data had been retrieved. Serum concentrations of total cholesterol, low-density lipoprotein cholesterol, creatinine, creatine kinase, electrolytes, and liver transaminases were controlled monthly by a physician (data not shown), who was otherwise not involved in the study; this guaranteed that all the investigators were kept blinded.

Outcome Measures

CSF samples were collected at baseline and after 26 weeks at the end of the study. The CSF was aliquoted and immediately frozen at −70°C until assayed. Concentrations of Aβ40 and Aβ42[11] were analyzed by enzyme-linked immunosorbent assay. Concentrations of lathosterol, cholesterol, and 24S-hydroxycholesterol in the CSF were analyzed using combined gas chromatography–mass spectrometry.[12] Cognitive performance was evaluated by MMSE and AD Assessment Scale–Cognitive Portion at the beginning and the end of the study.

Ethics

The study was approved by the local ethical committee on human experimentation. All precedures were in accordance with the Helsinki Declaration as revised in 1989. All patients gave informed consent.

Statistics

Data are presented as mean ± standard deviation. Statistical analyses were performed by using JMP 4.02 (SAS Institute). For statistical analysis, we used a multivariate analysis of variance (MANOVA) with repeated-measures analysis (pretreatment vs posttreatment) and a drug factor (simvastatin vs placebo). For correlation studies, we used Spearman's rank correlation. Fisher's test was used to detect gender differences.

Results

Safety, Tolerability, and Exclusions

Thirty-seven patients completed the study (see Fig 1). Muscle pain without an elevation of creatine kinase led to the discontinuation of therapy in one patient of the simvastatin group. One patient was withdrawn, because creatine kinase was elevated. There were no other reports of adverse effects in the simvastatin group. One patient was considered noncompliant because his serum low-density lipoprotein cholesterol level decreased to less than 10%. Two patients receiving placebo did not complete the study. One patient from the placebo-treated group and one patient from the simvastatin-treated group refused to have a second spinal tap at the end of the study.

Efficacy

Lipid concentrations in the serum showed few changes in the placebo group (Table 2). After 4 weeks of taking 40mg per day simvastatin orally, the dose was increased to 80mg per day simvastatin for the following 22 weeks in the simvastatin group, leading to a 52% reduction of serum low-density lipoprotein cholesterol on average. Compared with baseline, treatment with simvastatin reduced the CSF concentrations of the cholesterol precursor lathosterol and the brain-specific cholesterol metabolite 24S-hydroxycholesterol but not of cholesterol (see Table 2).

In the CSF, mean change of Aβ40 concentration from baseline was 5.4 ± 14.4% in the placebo group. In the total simvastatin group, Aβ40 was reduced by −4.0 ± 9.4% ($p = 0.06$, MANOVA with repeated-measures analysis). Post hoc analysis showed that in patients with a mild form of AD, Aβ40 was reduced by

Table 1. Patient Baseline Demographics

Baseline Demographics	Placebo (n = 20)	Simvastatin (n = 24)
Women/men [%]	47/53	63/37
Age (yr)	68.5 ± 8	68.0 ± 9
Disease duration (yr)	2.8 ± 1.3	2.6 ± 1.4
MMSE (points)	17.1 ± 4.9	17.8 ± 5.0
ADAS–cog score (points)	33.2 ± 11.3	29.4 ± 10.4
Serum LDL cholesterol (mg/dl)	134 ± 32	137 ± 42
CSF Aβ40 (pM)	5,797 ± 1724	6,237 ± 1,655
CSF Aβ42 (pM)	775 ± 92	694 ± 79
CSF lathosterol (μg/dl)	0.47 ± 0.13	0.37 ± 0.09
CSF cholesterol (mg/dl)	0.49 ± 0.12	0.40 ± 0.11
CSF 24S-hydroxy-cholesterol (ng/ml)	3.1 ± 1.0	2.8 ± 1.0

MMSE = Mini-Mental State Examination; ADAS–cog = Alzheimer's Disease Assessment Scale–Cognitive Portion; LDL = low-density lipoprotein; CSF = cerebrospinal fluid.

Table 2. Changes from Baseline at 26 Weeks

Measures	MMSE 12–26		MMSE 12–20		MMSE 21–26	
	Placebo (n = 17)	Simvastatin (n = 20)	Placebo (n = 11)	Simvastatin (n = 12)	Placebo (n = 6)	Simvastatin (n = 8)
Serum						
ΔLDL cholesterol (%)	−5.9 ± 16.5	−51.9 ± 16.2[a]	−0.7 ± 14.3	−56.1 ± 13.1[a]	−16.6 ± 15.3	−43.6 ± 19.3[b]
CSF						
ΔAβ40 (%)	5.4 ± 14.4	−4.0 ± 9.4	4.3 ± 14.2	−2.8 ± 10.3	6.8 ± 13.2	−5.7 ± 6.5[a]
ΔAb42 (%)	3.6 ± 11.3	−0.9 ± 13.9	4.0 ± 9.0	2.3 ± 15.2	−0.9 ± 9.7	−5.6 ± 9.5
ΔLathosterol (%)	3.6 ± 33.5	−9.6 ± 20.9[a]	4.4 ± 38.1	−8.7 ± 15.9	19.3 ± 45.6	−10.0 ± 28.0
ΔCholesterol (%)	11.1 ± 23.8	−4.6 ± 15.8	11.3 ± 25.9	−3.6 ± 18.4	13.9 ± 35.4	−6.1 ± 19.4
Δ24S-hydroxy-cholesterol (%)	3.7 ± 12.6	−10.3 ± 13.7[a]	1.7 ± 10.1	−7.8 ± 13.5	6.2 ± 16.3	−15.2 ± 12.6[b]

[a] $p < 0.05$, [b] $p < 0.01$, repeated-measures analysis compared with the pretreatment condition (baseline).

MMSE = Mini-Mental State Examination; LDL = low-density lipoprotein; CSF = cerebrospinal fluid.

−5.7 ± 6.5% in the simvastatin group (n = 8; $p < 0.05$ for simvastatin treatment, MANOVA, repeated-measures analysis) compared with 6.8 ± 13.2% in the placebo (n = 6) group (Fig 2A; see Table 2). In these patients, the reduction of Aβ40 correlated with the reduction of 24S-hydroxycholesterol (see Fig 2B). In moderately affected AD patients, there was no significant reduction of Aβ40 (see Fig 2A) and no correlation with changes in 24S-hydroxycholesterol levels (see Fig 2B). As observed for Aβ40, we found that Aβ42 was not significantly decreased in the CSF compared with vehicle. In patients with a mild form of AD, there was a trend toward a reduction in simvastatin-treated patients compared with placebo-treated patients (see Table 2; $p = 0.11$).

The average MMSE score in the placebo group decreased from 17.1 ± 4.9 to 14.4 ± 5.6 ($p < 0.05$, MANOVA, repeated-measures analysis). In the simvastatin group, the MMSE score was only slightly reduced from 17.8 ± 5.0 to 17.2 ± 4.8. The MMSE scores at the end of the treatment period were different between the placebo and simvastatin-treated groups ($p < 0.02$, ANOVA). The mean change in the AD Assessment Scale–Cognitive Portion was not different between the simvastatin-treated group (+4.1 ± 6.5) and the placebo-treated group (+3.4 ± 7.0 points).

Discussion

We have shown previously that simvastatin efficiently reduces Aβ production in cultured hippocampal neurons and in the CSF and brain homogenate of guinea pigs.[7,8] Experiments in cell culture suggest that β-secretase processing is reduced by decreasing cholesterol levels.[7] Because β-secretase is found in cholesterol-rich microdomains, lipid rafts, its activity might critically depend on cholesterol.[1] We therefore hypothesized that reducing cholesterol via de novo synthesis may decrease Aβ production and may slow the progression of AD. Treatment with 80mg per day simvastatin for 26 weeks led to a small but significant reduction of Aβ40 in the CSF of mild but not moderate AD patients. Aβ40 was primarily evaluated, because it is the major component of total Aβ-amyloid in the CSF and more soluble than Aβ42. Furthermore, Aβ42 preferentially aggregates into amyloid plaques and appears to be reduced during disease progression.[11,13]

We selected simvastatin for our study, because as compared with the more hydrophilic statins, including pravastatin, and atorvastatin, the lipophilic HMG-CoA reductase inhibitors simvastatin and lovastatin pass the blood–brain barrier more efficiently.[14,15] Cholesterol of the central nervous system originates almost entirely from in situ synthesis.[16] To determine cholesterol synthesis and turnover in the brain, we measured the concentrations of the cholesterol precursor lathosterol and the formation of the cholesterol metabolite 24S-hydroxycholesterol in the CSF.[12,17] In humans, 24S-hydroxycholesterol is almost exclusively produced in the brain.[17,18] Treatment with simvastatin resulted in the reduction of both lathosterol and 24S-hydroxycholesterol in the CSF, indicating that the de novo synthesis of cholesterol in brain was reduced. Because of its long, calculated half-life of approximately 6 months,[19] the total cholesterol content appeared to not yet be affected, or its reduction did not result in decreased CSF concentrations of cholesterol. Similarly, administration of high-dose simvastatin to guinea pigs for 3 weeks diminished de novo cholesterol synthesis in the brain followed by reduced concentrations of Aβ without altering total cerebral cholesterol content.[8]

Although Aβ concentrations were drastically reduced in the CSF of guinea pigs, we observed only a small decrease of Aβ40 and Aβ42 in the CSF of patients with a mild form of AD. Possible reasons are (1) the

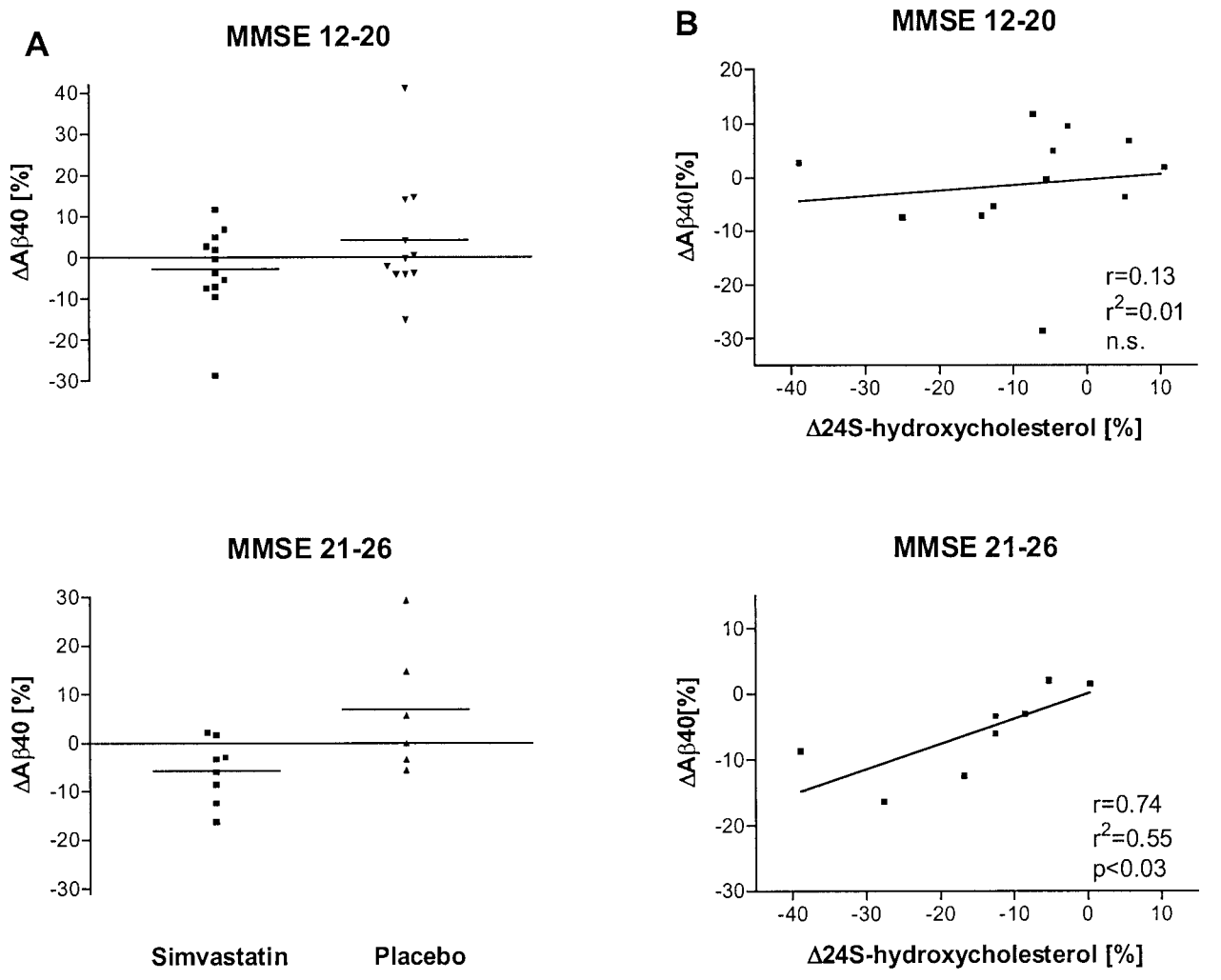

Fig 2. Effects of treatment with 80mg simvastatin per day on Aβ40 concentrations in the cerebrospinal fluid (CSF). CSF was collected at day 0 and at week 26 of treatment with simvastatin or placebo. (A) Treatment with simvastatin significantly ($p < 0.05$, MANOVA with repeated-measures analysis) reduced Aβ in the CSF in patients with a mild form of AD (Mini-Mental State Examination [MMSE] 21–26) but not in patients with a more severe dementia (MMSE 12–20). (B) In the CSF, reduction of Aβ40 correlated with the reduction of 24S-hydroxycholesterol in the patients with a mild form of AD, but not in patients with a more severe form. n.s. = not significant.

smaller reduction of serum cholesterol that was achieved in AD patients (52%) compared with the reduction in guinea pigs (85%), and (2) the large proportion of Aβ that is deposited into amyloid plaques in AD patients, which therefore may escape detection in the CSF. In patients with a mild form of AD, Aβ40 levels were reduced more pronounced than in moderate AD. Patients with mild AD may have a lower plaque load, and Aβ therefore might enter the CSF more easily. The reduction of Aβ40 correlated with the reduction of 24S-hydroxycholesterol in mild AD patients, suggesting that a stronger reduction in cholesterol de novo synthesis results in a more pronounced reduction of Aβ. Because small increases in Aβ production apparently can lead to some forms of hereditary AD and Down's syndrome,[20] a subtle reduction of Aβ in AD patients may be sufficient to slow the progression of the disease.

Repeated-measures analysis of the MMSE but not the AD Assessment Scale–Cognitive Portion showed a slower progression in simvastatin than in placebo-treated AD patients over the study period of 26 weeks. Long-term trials in a larger population will have to clarify whether statins slow the progression of cognitive symptoms in AD.

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