DOI 10.1007/s00702-004-0273-1
J Neural Transm (2005) 112: 1057–1071

Journal of Neural Transmission
Printed in Austria

Statins: drugs for Alzheimer's disease?

Review

G. P. Eckert[1], **W. G. Wood**[2], and **W. E. Müller**[1]

[1] Department of Pharmacology, Biocenter Niederursel, ZAFES, University of Frankfurt, Germany
[2] Department of Pharmacology, University of Minnesota School of Medicine and Geriatric Research, Education and Clinical Center, VA Medical Center, Minneapolis, MN, USA

Received September 13, 2004; accepted November 20, 2004
Published online January 31, 2005;

Summary. Evidences from cell culture experiments and animal studies suggest a strong link between cholesterol and Alzheimer's disease (AD). This relationship is supported by retrospective epidemiological studies demonstrating that statin treatment reduced the prevalence of AD in patients suffering from hypercholesterolaemia. The alternative processing of the amyloid-precursor protein (APP) in the brain of AD patients leads to the production of the neurotoxic amyloid-beta protein (Aβ), a causative factor for AD pathology. *In vitro*, this mechanism is modulated by alterations in cellular cholesterol levels. Moreover, lowering cholesterol in animal experiments reduced the production of Aβ in most but not all studies. These findings led to prospective clinical trials of cholesterol-lowering statins in AD patients, even if many studies do not support elevated cholesterol levels in serum and brain as a risk factor for Alzheimer's disease. Most of these studies were negative. Thus, up to date there is insufficient evidence to suggest the use of statins for treatment in patients with AD.

Keywords: Cholesterol, statins, brain, Alzheimer's disease.

Cholesterol in the pathogenesis of Alzheimer's disease

Neuritic plaques and neurofibrillary tangles in brain are characteristic neuropathological features of Alzheimer's disease (AD). Amyloid-beta protein (Aβ) is a primary component of neuritic plaques. Aβ is 39–43 amino acid residues long and is derived in part from the transmembrane region of the amyloid-precursor protein (APP) (Bossy-Wetzel et al., 2004). Observations that Aβ was neurotoxic in cells provided first evidence that Aβ might be directly involved in neurodegeneration in individuals with AD (Yankner et al., 1990; Pike

et al., 1991). Today, Aβ's initial pathophysiological role in AD is widely agreed on (Hardy and Selkoe, 2002). Mounting body of data indicate that brain cholesterol homeostasis is strongly coupled with brain amyloid metabolism (Michikawa, 2003; Wood et al., 2003), although the clear role of cholesterol within the pathogenetic cascade of excessive Aβ deposition in the brain of AD patients is yet unknown. Membrane cholesterol controls the direction of the processing of the amyloid-precursor protein (APP) *in vitro* (Frears et al., 1999; Bodovitz and Klein, 1996; Simons et al., 1998; Fassbender et al., 2001). Vice versa, Aβ peptides itself influence the cellular cholesterol homeostasis (Gong et al., 2002; Yao and Papadopoulos, 2002). The intake of cholesterol-rich diets as well as the medication with cholesterol-lowering drugs were shown to influence central steps of brain amyloid pathogenesis in animals (Fassbender et al., 2001; Refolo et al., 2000, 2001). In humans, HMG-CoA reductase inhibitors (statins) were reported to lower the incidence of AD in comparable epidemiological studies (Wolozin et al., 2000; Jick et al., 2000; Hajjar et al., 2002) and to affect CNS cholesterol homeostasis (Fassbender et al., 2002). Based on a recent review about studies examining cholesterol levels in Alzheimer's patients and control subjects the conclusion was reached that little evidence exists to support the idea of elevated cholesterol levels in serum and brain as a risk factor for AD (Wood et al., 2004). The aim of this article is to evaluate whether the reported *in vitro* findings on cholesterol and APP/Aβ-metabolism offer a causal explanation for the observed *in vivo* brain effects of statins in animal studies as well as in clinical trials.

Pharmacological characteristics of statins

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase that represents the rate-limiting enzyme in cholesterol biosynthesis (Hamelin and Turgeon, 1998). Thereby, statins induce significant plasma cholesterol reductions (Lousberg et al., 2001). The inhibition of HMG-CoA reductase not only prevents cholesterol biosynthesis but also affects the isoprenoid pathway that accounts for the pleiotropic effects of statins (reviewed in Bellosta et al., 2000). Statins are used therapeutically either as free acids or as lactones. The prodrug lactone form needs transformation to the acid form that represents the active motive responsible for its pharmacological effects. This transformation occurs predominantly in the liver. Both, prodrug lactone and active acid forms differ in their pharmacological profile. The main difference regards to their water solubility. The lactone forms (e.g. lovastatin, simvastatin, cerivastatin) are very lipophilic while the acid forms are hydrophilic (e.g. atorvastatin, fluvastatin, pravastatin) (Corsini et al., 1999). This difference accounts for the different brain penetration properties. Lipophilic substances are expected to enter the brain, whereas hydrophilic compounds, apart from selective transport mechanisms, do not readily cross the blood-brain-barrier (BBB) (Saheki et al., 1994; Botti et al., 1991). Recently, brain availability of lovastatin, simvastatin and pravastatin was tested in a mouse model (Eckert et al., 2004). Considerable levels of simvastatin and lovastatin in the prodrug lactone as well as in the respective active acid form were detected in mouse brain after single oral

administration. Measured pravastatin concentrations in the brain, however, are close to the limits of quantification of the applied HPLC-MS/MS method (Eckert et al., 2004). Latter findings confirm an earlier report that lovastatin and simvastatin are detectable and pravastatin is undetectable in the CSF (Botti et al., 1991). Although, pravastatin obviously does not readily enter the brain, it affects cholesterol homeostasis and gene regulation in the brain (Kirsch et al., 2003b; Johnson-Anuna et al., 2005). Comprising, clear data on statins' possible pharmacological effects in the brain are still lacking although their profile in the peripheral systems has been thoroughly investigated (reviewed in Farnier and Davignon, 1998).

Cholesterol and APP processing: evidence from cell culture studies

Cholesterol is the most prominent modulator of the integrity and the functional activity of biological membranes (Wood et al., 2003) and hence plays an essential role in the regulation of synaptic function and membranous signal transduction pathways (Koudinov and Koudinova, 2001). Cholesterol intercalates the fatty acid residues of membrane phospholipids and modulates the fluidity of the surrounding membrane environment. Thereby, the intracellular distribution of cholesterol is not uniform (Wood et al., 2002). Cholesterol content of subcellular compartments successively increases from the cell interior to the plasma membrane (Liscum and Munn, 1999). Within the plasma membrane, cholesterol is strictly organized into structural and kinetic pools like leaflet domains, lipid rafts or caveolae (Wood et al., 2002). Lipid rafts are specialized plasma membrane micro domains floating on the exofacial side of the membrane bilayer (Simons and Ehehalt, 2002). They clearly differ from the adjacent non-raft lipid environment (Eckert et al., 2003a). Statin treatment of mice lowered flotillin, a widely used lipid raft marker protein, pointing to specific statin-induced alterations in raft structure and distribution (Kirsch et al., 2003b). APP processing is likely to occur in raft domains since APP and a substantial fraction of intracellular Aβ are co-localized with cholesterol and APP-cleaving secretases in such detergent-insoluble membrane domains (Wahrle et al., 2002). Several studies demonstrated that the cleavage of APP can be modulated by altering membrane cholesterol levels *in vitro* (Frears et al., 1999; Parvathy et al., 2004; Buxbaum et al., 2001; Kojro et al., 2001; Bodovitz and Klein, 1996; Simons et al., 1998; Fassbender et al., 2001). Statins were used to inhibit cholesterol synthesis in cell culture experiments (Table 1). Interestingly, it was reported that statins protect cortical neurons from excitotoxicity (Zacco et al., 2003). Another experimental approach to deplete cholesterol in cells uses methyl-β-cyclodextrin (MβCD), a water-soluble cyclic oligosaccharide (Table 1). In contrast, cholesterol-cyclodextrin-complexes can be used to enrich cellular cholesterol (Eckert et al., 2003b). Cholesterol depletion with lovastatin as a lipophilic inhibitor of the HMG-CoA reductase enzyme alone or in combination with MβCD induced a significant decrease in cellular Aβ-load in neurons *in vitro* (Frears et al., 1999; Simons et al., 1998; Fassbender et al., 2001). Thereby, significant reduction of cellular Aβ-levels with lovastatin occurred

Table 1. Effects of cholesterol lowering on Aβ production in cells culture experiments

Cell/media	Treatment	Cholesterol/Aβ	Reference
HEK 293-cells; 2% UltroserG (''cholesterol free'') media	Lovastatin [20 μM], 40 h	Chol: −50%; Aβ: −50%	(Frears et al., 1999)
Rat primary hippocampal neuronal cells; serum-free N2 media	Lovastatin [4 μM] + MβCD [5 mM]; 48 & 72 hr	Chol: n.d.; Aβ: −29–50%	(Fassbender et al., 2001)
	Simvastatin [4 μM] MβCD [5 mM]; 48 & 72 hr		
HEK ADAM10 and SH-SY5Y-cells; serum-free DMEM	Lovastatin [1 μM]; 20 hr	Chol: −50%; Aβ: −20%	(Kojro et al., 2001)
	MβCD [10 mM], 30 min	Chol: −67%; Aβ: −40–50%	
Rat primary hippocampal neuronal cells; serum-free MEM media	Lovastatin [4 μM]; 4 days + MβCD [5 mM], 20 min. Lovastatin alone: no effect!	Chol: −70%; Aβ: −70%	(Simons et al., 1998)
CHO- and MDCK-cells, DMEM media containing 10% FCLPDS	Lovastatin acid [0.5–5 μM], 4 hr	Chol: n.d. %; Aβ: −50–60%	(Buxbaum et al., 2001)

when cholesterol was depleted of about 30–40% (Frears et al., 1999). Under similar experimental conditions, reduced Aβ-levels were found to be accompanied by increased α-secretase activity (Kojro et al., 2001), suggesting that membrane cholesterol variations are coupled with activity shifting of APP-cleaving membrane-bound secretases (Wolozin, 2001).

It was further reported that statin-induced decreased release of Aβ was not due to its accumulation in the cell, but rather due to decreased formation of Aβ (Buxbaum et al., 2001). Cholesterol depletion possibly favours a non-raft lipid environment in which β- and γ-secretase cleavage of APP does not efficiently work (Wolozin, 2001). Reversely, cholesterol loading enhanced amyloidogenic processing of membranous APP and hence Aβ-load *in vitro* under conditions where cellular cholesterol content reached twice and more of the normal cellular level (Frears et al., 1999; Bodovitz and Klein, 1996). The idea that these external membrane cholesterol modulations affect raft assembly and in a second step raft-associated APP cleavage is supported by recent data indicating that MβCD and its cholesterol inclusion complexes affect transbilayer distribution of cholesterol in synaptosomal plasma membranes of mice in an opposite manner (Kirsch et al., 2002). MβCD was reported to disrupt membrane raft domains which are known to reside in the outer membrane leaflet (Kabouridis et al., 2000).

Thus, *in vitro* investigations clearly link cholesterol to APP processing. Vice versa, the product of the APP processing, Aβ, affects cellular cholesterol metabolism and intracellular cholesterol distribution (Gong et al., 2002; Igbavboa et al., 2003). However, effects of cholesterol modulation on Aβ-generation in the reported cell culture studies have been usually generated under rather

unphysiological conditions that are probably not feasible *in vivo*. Furthermore, in some studies statins alone did not to affect APP processing. Only the combination of statins with MβCD and/or serum free conditions led to significant effects (Table 1). Moreover, recent findings emphasise an intracellular production of Aβ when the isoprenoid-pathway is inhibited by statins (Cole et al., 2004). Statin-induced suppression of geranylgeranylpyrophosphate formation causes changes in the microtubule-stabilizing protein tau leading to transient increase in tau phosphorylation and finally cell death (Meske et al., 2003). Tau phosphorylation ends up in the intracellular formation of paired helical filaments representing another hallmark of AD (Ohm et al., 2003). Thus, lowering cellular cholesterol decreases Aβ levels *in vitro*, but toxic effects in cell culture studies were also observed.

Cholesterol and Aβ generation: evidence from animal studies

Peripheral and brain cholesterol homeostasis are regulated independently of each other. Both pools are strictly separated via the blood-brain-barrier (BBB) (Dietschy and Turley, 2004). Although 24S-hydroxycholesterol, the metabolic elimination product of brain cholesterol, is cleared from the CNS via the BBB (Bjorkhem et al., 1998), no relevant cholesterol flux from the periphery into the CNS seems to take place (Dietschy and Turley, 2004). This notion is supported by recent data obtained from a cholesterol feeding trial (Kirsch et al., 2003a). Feeding of rats with a 2% cholesterol enriched diet for at least 6 months had no effect on total brain cholesterol levels within the cortex, the cerebellum, the pons or the hypothalamus compared to control animals, whereas plasma and liver cholesterol levels significantly increased (Kirsch et al., 2003a). Cholesterol demand in the CNS is sufficiently met by *in situ* synthesis within the brain, which was further shown to decrease with age (Dietschy and Turley, 2004). However, significant species differences have to be considered when studying cholesterol homeostasis. For example, cholesterol turnover in the mouse is both quantitatively and qualitatively different from other animal species, particularly primates (Dietschy and Turley, 2002). The biosynthesis rate for cholesterol is approximately 16-fold higher in mice compared to humans (Dietschy and Turley, 2002). There is also a marked difference in the handling of cholesterol carried in circulating LDL. The liver is the primary site for the removal of LDL from the plasma in all species. Whereas the rate of entry of cholesterol into the LDL pool in the mouse is only 4-fold higher than in the human, the rate of hepatic LDL clearance in this animal is 40-fold greater than in the human. Consequently, the steady-state concentration of cholesterol carried in LDL in the mouse is approximately 14-fold lower compared to humans (Dietschy and Turley, 2002). Regarding the central nervous system, 0.02% of the cholesterol pool in the human brain turns over every day, while turnover rate of mouse brain cholesterol is 0.4% (Dietschy and Turley, 2004). Despite these variations, however, most studies in AD research are preformed in the mouse, since genetically modified animals are available in which proteins of interest have been functionally introduced or deleted.

Recently, Refolo and colleagues have published two interesting studies on peripheral cholesterol balance and brain amyloid pathology in transgenic mice (Refolo et al., 2000, 2001). They reported that diet-induced hypercholesterolaemia resulted in significantly increased levels of formic acid-extractable Aβ peptides in the CNS, whereas the levels of total Aβ were strongly correlated with the levels of both plasma and CNS total cholesterol (Refolo et al., 2000). This report confirmed earlier findings that rabbits fed a 2% cholesterol-containing diet exhibit an increasing accumulation of intracellular immunolabeled Aβ in the brain (Sparks et al., 1994). Another study reported that elevated dietary cholesterol led to significant reduction in brain levels of secreted APP derivatives, including sAPPalpha, sAPPbeta, $A\beta_{1-40}$, and $A\beta_{1-42}$, while having little to no effect on cell-associated species, including full-length APP and the COOH-terminal APP processing derivatives (Howland et al., 1998). It has been shown that the cholesterol-lowering drug BM15.766 reduced plasma cholesterol, brain Aβ peptides, as well as brain Aβ-load by greater than twofold (Refolo et al., 2001). Again, a positive correlation between the amount of plasma cholesterol and Aβ was observed (Refolo et al., 2001). However, both studies reported only marginal alterations in total brain cholesterol levels of about 11–13%, which were not corrected for cholesterol present in the vascular compartment. These small changes can hardly account for the observed pronounced effects on brain Aβ metabolism (Refolo et al., 2000, 2001) (Table 2). Recently, it was shown that RPR107393 decreased Aβ production in Thy1-hAPP751SL transgenic mice (Pradier et al., 2004). RPR107393 lowers cholesterol synthesis upstream to HMG-CoA reductase and isoprenoid synthesis by inhibiting squalen epoxidase. This indicates that reduced cholesterol production accounts for the observed effects on Aβ production in the brain rather than a clearance of isoprenoid derivatives.

Petanceska et al. (2002) initiated a study aimed to determine the effects of atorvastatin on brain Aβ deposition in the PS/APP transgenic mouse model of Alzheimer's amyloidosis. The results indicate that Lipitor treatment markedly attenuated Aβ deposition in their animal model. On average, Lipitor treatment

Table 2. Effects of cholesterol lowering on Aβ production in animal experiments

Model	Treatment	Brain cholesterol/Aβ	Reference
PSAPP	BM15.766 [250 mg/kg p.o.], 5 weeks	Chol: −12%; Aβ: −30–40%	(Refolo et al., 2001)
PSAPP	Atorvastatin [30 mg/kg p.o.], 8 weeks	Chol: −9%; Aβ: −48–62%	(Petanceska et al., 2002)
Guinea pig	Simvastatin [0.5% in diet], 3 weeks	Chol: no changes; Aβ: −47–62%	(Fassbender et al., 2001)
APP (Tg2576)	Simvastatin [50 mg/kg p.o.], 3 months	Chol: n.d.; Aβ: no changes	(Li et al., 2004)
$APP_{K670,M671L}$ (Tg2576)	Lovastatin [100 mg/kg in diet]	Chol: n.d.; Aβ: +50–60%	(Park et al., 2003)
Female Thy1-hAPP751SL mice	RPR107393 [30 mg/kg p.o.], 4.5 days	Chol: n.d.; Aβ: −39%	(Pradier et al., 2004)

n.d. not determined; *p.o.* per oral; *Chol* brain cholesterol

reduced total serum cholesterol by about 59%, but had no effect on total brain cholesterol content as determined in cortex and cerebellum (Petanceska et al., 2002). However, opposite data were also published: Park et al. (2003) reported that lovastatin enhances Aβ production and senile plaque deposition in brains of female TG2596 mice. Using guinea pigs, Fassbender et al. (2001) showed that high-dosed simvastatin treatment led to strong reduction of $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in brain homogenates without clearly affecting total brain cholesterol levels. The cholesterol precursor lathosterol in the brain was significantly reduced and hence, it was concluded that brain cholesterol synthesis was altered (Fassbender et al., 2001). However, changes in lathosterol levels were in the nanomolar range, whereas total cholesterol brain levels are 10^6 – fold higher.

A recent study by Li et al. (2004) showed that simvastatin treatment corrected learning and memory deficits in Tg2576 mice, and more intriguingly, it significantly enhanced learning and memory in normal non-transgenic mice. In this study, statin treatment did not affect cerebral Aβ levels suggesting possible mechanisms that are independent of Aβ/APP metabolism. Accordingly, pleiotropic effects of statins on gene expression in cerebral cortex of mice were recently published (Johnson-Anuna et al., 2005). Latter results suggest that statins act on multiple pathways in addition to cholesterol synthesis (Johnson-Anuna et al., 2005). These pathways involve apoptosis, neuronal growth, glucose homeostasis, cell motility and differentiation. Thus, effects of statins in brain may be both neuroprotective and regenerative, and could lead to a new direction in understanding the potential therapeutic efficacy of statins in AD.

Taken together, these *in vivo* results suggest that bulk changes of the cholesterol content in the central compartment *per se* are not the only decisive mechanism regulating the APP cleavage process and brain Aβ generation. Studies on synaptosomal plasma membranes showed that statins induce discrete changes in cholesterol micro-domains within the membrane including transbilayer distribution and lipid rafts (Eckert et al., 2001; Kirsch et al., 2003b). In this study, pravastatin was active although it only poorly crosses the BBB (Kirsch et al., 2003b). This finding among others raises the question by which signalling mechanism peripheral cholesterol and brain are connected. Although the peripheral and the central cholesterol pool are mainly independent form each other, both compartments are linked by 24S-hydroxycholesterol (Dietschy and Turley, 2004). In the brain, cholesterol is metabolised by the enzyme cholesterol 24-hydroxylase (Cyp46) leading to the formation of 24S-hydroxycholesterol that is secreted out of the brain. In AD, altered plasma and CSF levels of brain derived 24S-hydroxycholesterol have been described (Lütjohann et al., 2000; Papassotiropoulos et al., 2002). Moreover, recent studies indicate an association between the Cyp46-gene and Alzheimer's disease (Wolozin et al., 2004; Kölsch et al., 2002). However, further studies are necessary to enlighten the role of 24S-hydroxycholesterol in brain cholesterol homeostasis and AD.

Statins, cholesterol and Alzheimer's disease: evidence from clinical studies

Two retrospective, epidemiological trials indicate that statins significantly decrease the incidence of AD: Long-term treatment of patients suffering from

coronary heart disease (CHD) with lovastatin and pravastatin (Wolozin et al., 2000) or simvastatin and pravastatin (Jick et al., 2000) dramatically lowered the risk of developing AD up to 70% compared to control subjects receiving other antiatherosclerotic medication. These findings are supported by a subsequent retrospective cohort studies (Hajjar et al., 2002; Rockwood et al., 2002; Zamrini et al., 2004). After covariate adjustments, patients on statins were less likely to have dementia. At follow-up, patients on statins showed an improvement on their Mini-Mental Status Examination score compared to a decline in controls (Hajjar et al., 2002). In a cohort study of lipid lowering agents (LLA) use and a case-control study of dementia in relation to LLA use, Rockwood et al. reported that the use of statins and other LLAs reduced the risk of Alzheimer disease in subjects younger than 80 years, an effect that persisted after adjustment for sex, educational level, and self-rated health (odds ratio, 0.26; 95% confidence interval, 0.08–0.88). There was no significant effect in subjects 80 years and older (Rockwood et al., 2002). Zamrini et al. (2004) reported that statin users had a 39% lower risk of AD relative to non-statin users in a nested case-control study including 309 AD patients.

Prospective studies were initiated to evaluate the ability of statins to prevent AD. First evidence that statins in clinically relevant dosages indeed affect cerebral cholesterol metabolism came from a case-control study on elderly non-demented subjects (Fassbender et al., 2002). However, the reported change of cholesterol, lathosterol and 24S-hydroxysterol levels in the cerebrospinal fluid (CSF) were not associated with altered intrathecal secretion of Aβ (Fassbender et al., 2002). Statin induced changes in cholesterol, lathosterol and 24S-hydroxycholesterol levels were recently confirmed in blood samples from AD patients (Vega et al., 2003; Locatelli et al., 2002). The effects of lovastatin on Aβ serum levels *in vivo* were investigated during a double-blind, randomised, placebo-controlled study including human subjects who had elevated low-density lipoprotein cholesterol levels (Friedhoff et al., 2001). Serum Aβ levels were measured before and after up to 3 months of treatment. Serum Aβ concentrations were dose-dependently decreased after 3 months of treatment and analysis of variance indicated that treatment was statistically significant (Friedhoff et al., 2001). In a 26-week randomised, placebo controlled, double-blind study, effects of simvastatin on cholesterol metabolites and Aβ levels in the cerebrospinal fluid of 24 AD patients and 20 controls were tested (Simons et al., 2002). Overall, simvastatin did not significantly alter cerebrospinal fluid (CSF) levels of $A\beta_{40}$ and $A\beta_{42}$. In post hoc analysis, however, simvastatin significantly decreased $A\beta_{40}$ levels in the CSF of a small cohort of patients with mild AD. The reduction of Aβ correlated with the reduction of 24S-hydroxycholesterol. These changes were not observed in more severely affected AD patients (Simons et al., 2002). Besides the small sample sizes, the reported changes in the above mentioned prospective studies were generally small. Hence, data have to be interpreted with caution. Recently, Hoglund and colleagues tested the effect of statin treatment on Aβ metabolism in humans. Twenty AD patients were treated with 20 mg/d atorvastatin and nineteen AD patients with 40 mg/d simvastatin for six weeks, respectively. The plasma levels of $A\beta_{1-40}$, $A\beta_{1-42}$, and total Aβ were stable in individual patients during

the treatment period. No significant change in the level of $A\beta_{1-40}$, $A\beta_{1-42}$, and total Aβ was found (Hoglund et al., 2004).

Three large randomised controlled trials of statins for prevention of cardiovascular disease failed to show cognitive benefits of treatment: In the Heart Protection Study 20,536 participants aged from 40–80 and in the Cholesterol Reduction in Seniors Program Pilot Trial (CRISP) 431 participants aged from 70–82 were exposed to simvastatin or placebo for 6 years or 3 months, respectively (Breitner et al., 2004). The PROSPER Trial involved 5,804 participants, aged from 70–82 who were at risk for cardiovascular disease. Patients were administered pravastatin or placebo for three years (Shepherd et al., 2002). The reasons for the failure of pravastatin to prevent AD in this study are unclear (Wolozin et al., 2004). Although pravastatin permeates the brain much less than simvastatin, the retrospective epidemiological studies suggest that pravastatin reduces the incidence of AD to the same extent as statins that are more lipophilic, such as simvastatin or lovastatin. It was suggested that subtle bias in patient selection, such as the use of subjects with high cholesterol, might have altered the risk of AD or that the study was not properly designed to detect dementia (Wolozin et al., 2004).

The potential of statins for prevention of AD is currently investigated in three large cohort studies using prospective designs: These include the Cache County Study that examined the association of statin use at baseline (183 participants) with incidence of AD (102 cases among 3308 participants) over three years using multivariable discrete-time survival methods, the Cardiovascular Health Cognition Study (CHS) determined exposure to statins annually among 2,798 participants and the Adult Changes in Thought Study (ACT) investigates the association of antecedent statin use in 168 cases of incident AD among 2,356 participants. John C. Breitner recently summarized the preliminary outcome of these studies at the 9th conference on Alzheimer's Disease and Related Disorders: Each study examined similar associations of other lipid-lowering agents (LLAs) and AD, and of statin and LLA use with all-cause dementia.

Table 3a. Published clinical trails on statins – retrospective studies

Reference	Participants	Main outcome measures	Outcome
(Wolozin et al., 2000)	57.104 CHD patients	Diagnosis of probable AD	Statin use: 60–73% lower prevalence rate of AD
(Jick et al., 2000)	284 cases with dementia, 1.080 control cases	Relative risk estimates of dementia	Statin use: 37–70% lower risk of dementia
(Hajjar et al., 2002)	655 patients with dementia, or hypercholesterolaemia	Relative risk estimates of dementia	Statin use: 77–84% lower risk of dementia
(Rockwood et al., 2002)	336 incident cases of AD	Relative risk estimates of dementia	Use of statins and other LLAs reduced the risk of AD in subjects younger than 80 years
(Zamrini et al., 2004)	309 incident cases of AD	Odds ratio for association between AD and statin use	Statin use: 39% lower risk of AD relative to non-statin users

Table 3b. Published clinical trails on statins – prospective studies

Reference	Participants	Main outcome measures	Outcome
(Friedhoff et al., 2001)	15 control subjects, 20 subject assigned to 10–60 mg lovastatin	Changes in serum Aβ levels	Subjects assignee to 40 mg or 60 mg lovastatin showed significant decreased Aβ serum levels after 12 wks of treatment
(Fassbender et al., 2002)	50 patients assigned to 10–20 mg atorvastatin, 10–40 mg simvastatin, pravastatin, or lovastain, 40 mg fluvastatin or 0.4–0.6 mg cerivastatin; 50 subjects with normocholesterolaemia (control); 50 non treated subjects with hypercholesterolaemia	Changes in cholesterol metabolites levels and Aβ1–42 levels in CSF samples	Statin use: Decreased cholesterol metabolites, no changes in Aβ levels
(Locatelli et al., 2002)	18 patients assigned to 80 mg Simvastatin	Changes in 24OH-cholesterol levels in serum samples	Statin use: Decreased cholesterol metabolites in serum after 24 wks of treatment
(Simons et al., 2002)	44 AD patients (20 assigned to placebo, 24 assigned to 40–80 mg simvastatin)	Changes in cholesterol metabolites levels and Aβ1–42 levels in CSF samples	Statin use: no changes in cholesterol or Aβ levels, decreased lathosterol and 24OH-cholesterol levels. Post hoc analysis: significant effect on Aβ levels in patients with mild AD after 26 wks of treatment
(Vega et al., 2003)	61 AD patients assigned to 40 mg Lovastatin	Changes in cholesterol metabolites levels in serum samples	Statin use: Decreased cholesterol metabolites in serum after 6 wks of treatment

However, preliminary data, emphasise that the inverse association of statin use and AD, which is evident in cross-sectional studies, does not appear to be sustained in above mentioned prospective study designs (Breitner et al., 2004). Large-scale clinical trials are needed to resolve the questions raised by these studies. Trials currently underway include (http://www.clinicaltrials.gov):

A. The Cholesterol Lowering Agent to Slow Progression (CLASP) of Alzheimer's Disease Study, sponsored by the National Institute on Aging (NIA). CLASP is investigating the safety and effectiveness of simvastatin to slow the progression of AD.
B. The Effect of Short-Term Statin and NSAID Treatment on CSF Beta-Amyloid. The study determines whether short-term use of ibuprofen and lovastatin affects Aβ levels in people at risk for developing AD. This study is sponsored by the National Institute of Mental Health (NIMH).
C. The LEADe Study, sponsored by Pfizer, Inc. This study is comparing the efficacy and safety of atorvastatin in combination with donepezil in patients with mild to moderate AD.

The available prospective studies focussing on cognitive or anti-amyloid benefits for statins are at variance. Recently, data from a small study examining the effect of statin use on hippocampal volume in elderly people with mild cognitive impairment were also rather negative: Neither hippocampal volume nor white matter was affected by statins treatment, indicating that statin use over a time period of two and four years were unable to halt hippocampal loss in AD (Doraiswamy et al., 2004). In addition, case reports raised the possibility that statins, in rare cases, may be associated with cognitive impairment (Wagstaff et al., 2003) and it was discussed that patients with AD may be particularly susceptible to adverse effects of statin treatment (Algotsson and Winblad, 2004).

Conclusion

Experimental *in vitro* and *in vivo* findings as well as retrospective epidemiological data link cholesterol homeostasis with AD and identified statins as possible pharmacological tools to treat dementia. In contrast, prospective studies evaluated so far do not fulfil the expectations of statins as possible drugs against AD. Whether dosage and duration of treatment period are factors that account for the discrepancy of the prospective studies reported so far needs further clarification.

Acknowledgements

This study was supported by the Hanna Bragard Foundation, the Alzheimer Forschung Initiative (AFI 02806), the Hirnliga and National Institute of Health grant 1P0AG-18357.

References

Algotsson A, Winblad B (2004) Patients with Alzheimer's disease may be particularly susceptible to adverse effects of statins. Dement Geriatr Cogn Disord 17: 109–116

Bellosta S, Ferri N, Bernini F, Paoletti R, Corsini A (2000) Non-lipid-related effects of statins. Ann Med 32: 164–176

Björkhem I, Lütjohann D, Diczfalusy U, Stahle L, Ahlborg G, Wahren J (1998) Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. J Lipid Res 39: 1594–1600

Bodovitz S, Klein WL (1996) Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. J Biol Chem 271: 4436–4440

Bossy-Wetzel E, Schwarzenbacher R, Lipton SA (2004) Molecular pathways to neurodegeneration. Nat Med 10 (Suppl): S2–S9

Botti RE, Triscari J, Pan HY, Zayat J (1991) Concentrations of pravastatin and lovastatin in cerebrospinal fluid in healthy subjects. Clin Neuropharmacol 14: 256–261

Breitner JCS, Zandi PP, Li G, Rea TG, Psaty BM (2004) Can statins prevent AD, or are they just prescribed less often to those with cognitive disorders? Neurobiol Aging 25[S2]: 5

Buxbaum JD, Geoghagen NS, Friedhoff LT (2001) Cholesterol depletion with physiological concentrations of a statin decreases the formation of the Alzheimer amyloid Abeta peptide. J Alzheimers Dis 3: 221–229

Cole SL, Grudzien A, Manhart I, Kelly B, Oakley H, Vassar R (2004) A tale of two statins: the effects of lovastatin and simvastatin on app metabolism. Neurobiol Aging 25[S2]: 77

Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F (1999) New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 84: 413–428

Dietschy JM, Turley SD (2002) Control of cholesterol turnover in the mouse. J Biol Chem 277: 3801–3804

Dietschy JM, Turley SD (2004) Thematic review series: Brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 45: 1375–1397

Doraiswamy PM, Steffens D, McQuoid D (2004) Statin use and hippocampal volume in subjects at risk for Alzheimer's disease. Neurobiol Aging 45[S2]: 13

Eckert GP, Kirsch C, Müller WE (2001) Differential effects of lovastatin treatment on brain cholesterol levels in normal and apoE-deficient mice. Neuroreport 12: 883–887

Eckert GP, Igbavboa U, Müller WE, Wood WG (2003a) Lipid rafts of purified mouse brain synaptosomes prepared with or without detergent reveal different lipid and protein domains. Brain Res 962: 144–150

Eckert GP, Kirsch C, Leutz S, Wood WG, Müller WE (2003b) Cholesterol modulates amyloid beta-peptide's membrane interactions. Pharmacopsychiatry 36 (Suppl 2): S136–S143

Eckert GP, Keller JH, Weber CC, Franke C, Peters I, Karas M, Schubert-Zsilavecz M, Müller WE (2004) Brain availability and effects on lipid homeostasis of statins. Neurobiol Aging 25[S2]: 579

Farnier M, Davignon J (1998) Current and future treatment of hyperlipidemia: the role of statins. Am J Cardiol 82: 3J–10J

Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K, Hartmann T (2001) Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. Proc Natl Acad Sci USA 98: 5856–5861

Fassbender K, Stroick M, Bertsch T, Ragoschke A, Kuehl S, Walter S, Walter J, Brechtel K, Muehlhauser F, von Bergmann K, Lutjohann D (2002) Effects of statins on human cerebral cholesterol metabolism and secretion of Alzheimer amyloid peptide. Neurology 59: 1257–1258

Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM (1999) The role of cholesterol in the biosynthesis of beta-amyloid. Neuroreport 10: 1699–1705

Friedhoff LT, Cullen EI, Geoghagen NS, Buxbaum JD (2001) Treatment with controlled-release lovastatin decreases serum concentrations of human beta-amyloid (A beta) peptide. Int J Neuropsychopharmacol 4: 127–130

Gong JS, Sawamura N, Zou K, Sakai J, Yanagisawa K, Michikawa M (2002) Amyloid beta-protein affects cholesterol metabolism in cultured neurons: implications for pivotal role of cholesterol in the amyloid cascade. J Neurosci Res 70: 438–446

Hajjar I, Schumpert J, Hirth V, Wieland D, Eleazer GP (2002) The impact of the use of statins on the prevalence of dementia and the progression of cognitive impairment. J Gerontol A Biol Sci Med Sci 57: M414–M418

Hamelin BA, Turgeon J (1998) Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. Trends Pharmacol Sci 19: 26–37

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297: 353–356

Hoglund K, Wiklund O, Vanderstichele H, Eikenberg O, Vanmechelen E, Blennow K (2004) Plasma levels of beta-amyloid(1–40), beta-amyloid(1–42), and total beta-amyloid remain unaffected in adult patients with hypercholesterolemia after treatment with statins. Arch Neurol 61: 333–337

Howland DS, Trusko SP, Savage MJ, Reaume AG, Lang DM, Hirsch JD, Maeda N, Siman R, Greenberg BD, Scott RW, Flood DG (1998) Modulation of secreted beta-amyloid precursor protein and amyloid beta-peptide in brain by cholesterol. J Biol Chem 273: 16576–16582

Igbavboa U, Pidcock JM, Johnson LN, Malo TM, Studniski AE, Yu S, Sun GY, Wood WG (2003) Cholesterol distribution in the Golgi complex of astrocytes is differentially altered by fresh and aged amyloid beta-peptide1–42. J Biol Chem 278: 17150–17157

Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA (2000) Statins and the risk of dementia. Lancet 356: 1627–1631

Johnson-Anuna LN, Eckert GP, Keller JH, Igbavboa U, Franke C, Fechner T, Schubert-Zsilavecz M, Karas M, Muller WE, Wood WG (2005) Chronic administration of statins alters multiple gene expression patterns in mouse cerebral cortex. J Pharmacol Exp Ther 312: 786–793

Kabouridis PS, Janzen J, Magee AL, Ley SC (2000) Cholesterol depletion disrupts lipid rafts and modulates the activity of multiple signaling pathways in T lymphocytes. Eur J Immunol 30: 954–963

Kirsch C, Eckert GP, Müller WE (2002) Cholesterol attenuates the membrane perturbing properties of β-amyloid peptides. Amyloid 9: 149–159

Kirsch C, Eckert GP, Koudinov AR, Muller WE (2003a) Brain cholesterol, statins and Alzheimer's disease. Pharmacopsychiatry 36 (Suppl 2): S113–S119

Kirsch C, Eckert GP, Müller WE (2003b) Statins affect cholesterol micro-domains in brain plasma membranes. Biochem Pharmacol 65: 843–856

Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F (2001) Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10. Proc Natl Acad Sci USA 98: 5815–5820

Kölsch H, Lutjohann D, Ludwig M, Schulte A, Ptok U, Jessen F, von Bergmann K, Rao ML, Maier W, Heun R (2002) Polymorphism in the cholesterol 24S-hydroxylase gene is associated with Alzheimer's disease. Mol Psychiatry 7: 899–902

Koudinov AR, Koudinova NV (2001) Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J 15: 1858–1860

Li L, Cao D, Wan H, Chen J (2004) Enhancement of learning and memory by statins beyond Alzheimer's disease. Neurobiol Aging 25[S2]: 82

Liscum L, Munn NJ (1999) Intracellular cholesterol transport. Biochim Biophys Acta 1438: 19–37

Locatelli S, Lütjohann D, Schmidt HH, Otto C, Beisiegel U, von Bergmann K (2002) 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. Arch Neurol 59: 213–216

Lousberg TR, Denham AM, Rasmussen JR (2001) A comparison of clinical outcome studies among cholesterol-lowering agents. Ann Pharmacother 35: 1599–1607

Lütjohann D, Papassotiropoulos A, Bjorkhem I, Locatelli S, Bagli M, Oehring RD, Schlegel U, Jessen F, Rao ML, von Bergmann K, Heun R (2000) Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. J Lipid Res 41: 195–198

Meske V, Albert F, Richter D, Schwarze J, Ohm TG (2003) Blockade of HMG-CoA reductase activity causes changes in microtubule-stabilizing protein tau via suppression of geranylgeranylpyrophosphate formation: implications for Alzheimer's disease. Eur J Neurosci 17: 93–102

Michikawa M (2003) The role of cholesterol in pathogenesis of Alzheimer's disease: dual metabolic interaction between amyloid beta-protein and cholesterol. Mol Neurobiol 27: 1–12

Ohm TG, Treiber-Held S, Distl R, Glockner F, Schonheit B, Tamanai M, Meske V (2003) Cholesterol and tau protein-findings in Alzheimer's and Niemann Pick C's disease. Pharmacopsychiatry 36 (Suppl 2): S120–S126

Papassotiropoulos A, Lutjohann D, Bagli M, Locatelli S, Jessen F, Buschfort R, Ptok U, Bjorkhem I, von Bergmann K, Heun R (2002) 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia. J Psychiatr Res 36: 27–32

Park IH, Hwang EM, Hong HS, Boo JH, Oh SS, Lee J, Jung MW, Bang OY, Kim SU, Mook-Jung I (2003) Lovastatin enhances Abeta production and senile plaque deposition in female Tg2576 mice. Neurobiol Aging 24: 637–643

Parvathy S, Ehrlich M, Pedrini S, Diaz N, Refolo L, Buxbaum JD, Bogush A, Petanceska S, Gandy S (2004) Atorvastatin-induced activation of Alzheimer's alpha secretase is resistant to standard inhibitors of protein phosphorylation-regulated ectodomain shedding. J Neurochem 90: 1005–1010

Petanceska SS, DeRosa S, Olm V, Diaz N, Sharma A, Thomas-Bryant T, Duff K, Pappolla M, Refolo LM (2002) Statin therapy for Alzheimer's disease: will it work? J Mol Neurosci 19: 155–161

Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW (1991) Aggregation-related toxicity of synthetic beta-amyloid protein in hippocampal cultures. Eur J Pharmacol 207: 367–368

Pradier L, Canton T, Bouaiche Z, Benoit P, Benavides J (2004) The squalene synthase inhibitor RPR 107393A reduces Aβ peptide level in an APP transgenic mouse model. Neurobiol Aging 25[25]: 568

Refolo LM, Pappolla MA, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurti K, Duff K (2000) Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. Neurobiol Dis 7: 321–331

Refolo LM, Pappolla MA, LaFrancois J, Malester B, Schmidt SD, Thomas-Bryant T, Tint GS, Wang R, Mercken M, Petanceska SS, Duff KE (2001) A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. Neurobiol Dis 8: 890–899

Rockwood K, Kirkland S, Hogan DB, MacKnight C, Merry H, Verreault R, Wolfson C, McDowell I (2002) Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. Arch Neurol 59: 223–227

Saheki A, Terasaki T, Tamai I, Tsuji A (1994) In vivo and in vitro blood-brain barrier transport of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Pharm Res 11: 305–311

Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, Ford I, Gaw A, Hyland M, Jukema JW, Kamper AM, Macfarlane PW, Meinders AE, Norrie J, Packard CJ, Perry IJ, Stott DJ, Sweeney BJ, Twomey C, Westendorp RG (2002) Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet 360: 1623–1630

Simons K, Ehehalt R (2002) Cholesterol, lipid rafts, and disease. J Clin Invest 110: 597–603

Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K (1998) Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc Natl Acad Sci USA 95: 6460–6464

Simons M, Schwarzler F, Lutjohann D, von Bergmann K, Beyreuther K, Dichgans J, Wormstall H, Hartmann T, Schulz JB (2002) Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: a 26-week randomized, placebo-controlled, double-blind trial. Ann Neurol 52: 346–350

Sparks DL, Scheff SW, Hunsaker JC, Liu H, Landers T, Gross DR (1994) Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. Exp Neurol 126: 88–94

Vega GL, Weiner MF, Lipton AM, von Bergmann K, Lütjohann D, Moore C, Svetlik D (2003) Reduction in levels of 24S-hydroxycholesterol by statin treatment in patients with Alzheimer disease. Arch Neurol 60: 510–515

Wagstaff LR, Mitton MW, Arvik BM, Doraiswamy PM (2003) Statin-associated memory loss: analysis of 60 case reports and review of the literature. Pharmacotherapy 23: 871–880

Wahrle S, Das P, Nyborg AC, McLendon C, Shoji M, Kawarabayashi T, Younkin LH, Younkin SG, Golde TE (2002) Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains. Neurobiol Dis 9: 11–23

Wolozin B (2001) A fluid connection: cholesterol and Abeta. Proc Natl Acad Sci USA 98: 5371–5373

Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G (2000) Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arch Neurol 57: 1439–1443

Wolozin B, Brown J, III, Theisler C, Silberman S (2004) The cellular biochemistry of cholesterol and statins: insights into the pathophysiology and therapy of Alzheimer's disease. CNS Drug Rev 10: 127–146

Wood WG, Schroeder F, Igbavboa U, Avdulov NA, Chochina SV (2002) Brain membrane cholesterol domains, aging and amyloid beta-peptides. Neurobiol Aging 23: 685

Wood WG, Eckert GP, Igbavboa U, Müller WE (2003) Amyloid beta-protein interactions with membranes and cholesterol: causes or casualties of Alzheimer's disease. Biochim Biophys Acta 1610: 281–290

Wood WG, Igbavboa U, Eckert GP, Johnson-Anuna LN, Franke C, Müller WE (2004) Is hypercholesterolemia a risk factor for Alzheimer's disease? Mol Neurobiol (in press)

Yankner BA, Duffy LK, Kirschner DA (1990) Neurotrophic and neurotoxic effects of amyloid-beta protein: reversal by tachykinin neuropeptides. Science 250: 279–282

Yao ZX, Papadopoulos V (2002) Function of β-amyloid in cholesterol transport: a lead to neurotoxicity. FASEB J 16: 1677–1679

Zacco A, Togo J, Spence K, Ellis A, Lloyd D, Furlong S, Piser T (2003) 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors protect cortical neurons from excitotoxicity. J Neurosci 23: 11104–11111

Zamrini E, McGwin G, Roseman JM (2004) Association between statin use and Alzheimer's disease. Neuroepidemiology 23: 94–98

Authors' address: G. P. Eckert, Department of Pharmacology, Biocenter Niederursel, University of Frankfurt, Marie-Curie-Strasse 9, D-60439 Frankfurt, Germany, e-mail: G.P.Eckert@em.uni-frankfurt.de