Cholesterol Level and Statin Use in Alzheimer Disease

II. Review of Human Trials and Recommendations

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Substantial evidence has accumulated in support of the hypothesis that elevated cholesterol levels increase the risk of developing Alzheimer disease (AD). As a result, much work has investigated the potential use of lipid-lowering agents, particularly statins, as preventive or therapeutic agents for AD. Although epidemiology and preclinical statin research (described in part I of this review) have generally supported an adverse role of high cholesterol levels regarding AD, human studies of statins (reviewed herein) show highly variable outcomes, making it difficult to draw firm conclusions. We identify several confounding factors among the human studies, including differing blood-brain barrier permeabilities among statins, the stage in AD at which statins were administered, and the drugs’ pleiotropic metabolic effects, all of which contribute to the substantial variability observed to date. We recommend that future human studies of this important therapeutic topic (1) take the blood-brain barrier permeabilities of statins into account when analyzing results, (2) include specific analyses of the effects on low- and high-density lipoprotein cholesterol, and, most important, (3) conduct statin treatment trials solely in patients with mild AD, who have the best chance for disease modification.

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STUDY SELECTION

We performed an unbiased search of the PubMed database for relevant studies in the English language, without regard to publication date. Additional studies were identified by citations in the resultant studies and also by the recommendation of the coauthors or consultants (identified in the "Acknowledgments" section). We included all articles that provided well-controlled studies and clearly interpretable conclusions about this topic. Studies of the effects of statins in human subjects were required to include at least 50 persons, and human studies examining LLAs were required to specify the drugs being investigated.

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OBSERVATIONAL STUDIES

A number of observational studies in human subjects bolster the hypothesis that statins may be able to mitigate the course of AD or reduce the probability of developing it (Table 1). A case-control study by Jick and colleagues found that people older than 50 years who were taking statins had a lower risk of developing dementia. An examination of patients older than 60 years demonstrated that patients taking lovastatin and/or pravastatin had a lower AD risk compared with the general population or patients taking other medications for hypertension or heart disease. In a study of postmenopausal women younger than 80 years with heart disease, subjects taking statins had better scores on the modified Mini-Mental State Examination and were less likely to be cognitively impaired. Wolozin and colleagues found that simvastatin treatment significantly reduced the risk of dementia and Parkinson disease. A study of elderly Mexican Americans reported that statin treatment was associated with a reduced risk of dementia and cognitive impairment without frank dementia. Similarly, participants in the Rotterdam epidemiological study were less likely to develop AD if they were taking statins, an effect that was not dependent on the apolipoprotein E (ApoE) genotype.

Other studies have examined the effects of cholesterol-lowering treatment on subjects who had already been given a diagnosis of AD. A neuropathological assessment by Li et al concluded that, although statin users did not have lower scores on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) instrument (a quantitative scale of amyloid plaque pathological features) than nonusers, they had lower Braak stages (a quantitative scale of neurofibrillary tangle and neuritic pathological features). Furthermore, study subjects using statins were less likely to have “typical AD-type neuropathology,” which the authors defined as a Braak stage of at least IV (maximum score, VI) and a CERAD score of at least moderate (maximum score, severe).

Not all observational studies conclude that statins are beneficial in the setting of AD (Table 1). When a large cohort (4895 for prevalent dementia and 3308 for incident dementia) of elderly people was surveyed for overall dementia or AD and the use of statins (an aggregate of lovastatin, simvastatin, cerivastatin sodium, atorvastatin calcium, pravastatin, and fluvastatin sodium) in 1995 to 1997 and again in 1998 to 2000, statin use at the initial visit and at follow-up visits did not correlate with the risk of developing any dementia or AD during the period between the 2 visits, although statin use was less common among those who already had dementia at the initial visit. Another study found that statin treatment of patients 65 years or older did not affect their risk of developing AD or other forms of dementia, and this result did not change when more lipophilic (lovastatin, simvastatin, or cerivastatin) and less lipophilic (atorvastatin, pravastatin, or fluvastatin) drugs were analyzed separately. Wolozin et al showed a protective effect with simvastatin, failed to find such an effect with lovastatin. Arvanitakis et al reported that, although statin users were less likely to be demented at the time of death, statin use did not affect AD risk or cognitive ability.

RANDOMIZED CONTROLLED TRIALS

As with the human observational studies described in the preceding subsections, the results of randomized controlled trials in AD patients have been contradictory (Table 2). Administering a controlled-release form of lovastatin to non-demented subjects has been shown to decrease serum β-amyloid (Aβ) levels in a dose-dependent manner (Figure 1). Sparks et al observed differences between atorvastatin users and nonusers on the cognitive subscale of the Alzheimer Disease Assessment Scale at 6 months and on the Geriatric Depression Scale at 12 months. However, large double-blind randomized controlled trials of pravastatin and simvastatin with mean follow-up ranging from 3.2 to 5.0 years were unable to demonstrate a protective effect, and the study by Sparks et al did not find an effect on the Mini-Mental State Examination, Neuropsychiatric Inventory, or Alzheimer Disease Cooperative Study—Activities of Daily Living Scale. Most recently, a randomized controlled trial found that cognitive decline in patients who already had mild to moderate AD was unaltered by a 72-week administration of atorvastatin. Overall, we interpret these various studies to indicate that statins have not yet been shown to influence the course of AD in controlled trials.

EFFECTS OF NONSTATIN LLAs

Some observational studies have examined the effects of nonstatin LLA compounds (Table 1). Yaffe et al examined a combination of niacin, cholestyramine resin, and other nonspecified compounds and did not find these to be effective at preventing cognitive impairment in postmenopausal women without dementia. Fibrates (a group of amphipathic carboxylic acids used for hypercholesterolemia treatment) were also reported to be ineffective for dementia or AD protection (although Masse et al found them to be efficacious), as were cholestyramine and nicotinic acid and a combination of niacin, bile acid sequestrants, and probucol.

DIFFERING BBB PERMEABILITIES AMONG THE STATINS

The substantial variability in outcomes of the diverse human studies reviewed in the preceding paragraphs makes it difficult to ascertain whether statins could have a beneficial role in preventing or treating AD (Tables 1 and 2). One possible reason for the inconsistency is that, to potentially affect the course of AD, any prospective treatment must be able to pass through the BBB and enter the brain. In this respect, all statins are not created equal; some have a much greater capacity to cross the BBB than others, but this is not always commented on by authors in interpreting their studies.

Differences in BBB permeability among the various statins (Table 3) were recognized while investigating why some statins, when prescribed as a treatment for hypercholesterolemia, had a higher incidence of neurological adverse effects than others. Lovastatin, but not fluvastatin, efficiently crossed a monolayer of bovine brain microvessel endothelial cells (a model for the BBB) and was detected in the brain extracts of rats receiving the compound. In
Table 1. Human Observational Studies of the Efficacy of LLAs for the Treatment and Prevention of AD and Dementia

<table>
<thead>
<tr>
<th>Source</th>
<th>LLAs Used</th>
<th>Study Size</th>
<th>Disease Severity</th>
<th>Length of Follow-up</th>
<th>Outcome Measures</th>
<th>Effectiveness</th>
<th>BBB Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arvanitakis et al, 2008</td>
<td>SIM, LOV, ATOR, PRA, and FLU analyzed together and in more- and less-lipophilic groups</td>
<td>929 Clinical evaluations of living patients; 262 postmortem neuropathological examinations</td>
<td>Incident</td>
<td>≤12 y</td>
<td>Risk of developing AD; composite of cognitive test scores; neuropathology</td>
<td>Yes for amyloid pathological changes; no for risk of AD, cognitive ability, and global AD pathological changes</td>
<td>Mixed</td>
</tr>
<tr>
<td>Cramer et al, 2008</td>
<td>ATOR, CER, FLU, LOV, PRA, ROS, and SIM analyzed together</td>
<td>1021 Subjects</td>
<td>Incident</td>
<td>5 y</td>
<td>Risk of developing dementia or cognitive impairment without dementia</td>
<td>Yes</td>
<td>Mixed</td>
</tr>
<tr>
<td>Haag et al, 2009</td>
<td>ATOR, CER, FLU, LOV, PRA, and SIM analyzed together and in lipophilic and hydrophilic groups; SIM and PRA also analyzed separately</td>
<td>6992 Subjects</td>
<td>Incident</td>
<td>Varied; mean, 12.2 y</td>
<td>Risk of developing AD</td>
<td>Yes for all statins, lipophilic statins, and hydrophilic statins; no for other LLAs</td>
<td>Mixed</td>
</tr>
<tr>
<td>Jick et al, 2000</td>
<td>ATOR, CER, FLU, PRA, and SIM analyzed together</td>
<td>1364 Subjects</td>
<td>Incident</td>
<td>6 y</td>
<td>Risk of developing dementia</td>
<td>Yes for statins; no for other LLAs</td>
<td>Mixed</td>
</tr>
<tr>
<td>Li et al, 2007</td>
<td>SIM, LOV, PRA, and ATOR analyzed together</td>
<td>110 Subjects</td>
<td>Incident</td>
<td>≤12 y</td>
<td>Incidence of dementia; Braak stages; CERAD scores</td>
<td>No for incidence of dementia and CERAD scores; yes for Braak stages and MMSE scores</td>
<td>Mixed</td>
</tr>
<tr>
<td>Masse et al, 2005</td>
<td>Unspecified statins</td>
<td>342 Subjects</td>
<td>Mild to moderate</td>
<td>Varied; mean, 34.8 mo</td>
<td>Rate of decline in MMSE scores</td>
<td>Yes for all LLAs together and fibrates; no for statins and other LLAs</td>
<td>Mixed</td>
</tr>
<tr>
<td>Rea et al, 2005</td>
<td>LOV, SIM, CER, ATOR, PRA, and FLU analyzed together and in more- and less-lipophilic groups</td>
<td>2798 Subjects</td>
<td>Incident</td>
<td>Varied; median, 5 y</td>
<td>Risk of dementia, AD, and/or vascular dementia</td>
<td>No</td>
<td>Mixed</td>
</tr>
<tr>
<td>Wolozin et al, 2000</td>
<td>lov, PRA, LOV + PRA, and SIM analyzed separately</td>
<td>60,349 Subjects</td>
<td>Unspecified</td>
<td>NA (retrospective)</td>
<td>Prevalence of AD</td>
<td>Yes for LOV, PRA, and LOV + PRA; no for others</td>
<td>Yes for LOV and SIM; no for PRA; mixed for LOV + PRA</td>
</tr>
<tr>
<td>Wolozin et al, 2007</td>
<td>ATOR, LOV, and SIM analyzed separately</td>
<td>1,290,071 Subjects</td>
<td>Incident</td>
<td>NA (retrospective)</td>
<td>Risk of developing dementia</td>
<td>Yes for LOV; no for SIM and ATOR</td>
<td>Yes for LOV and SIM; disputed for ATOR</td>
</tr>
<tr>
<td>Yaffe et al, 2002</td>
<td>SIM, ATOR, LOV, PRA, and FLU analyzed together</td>
<td>1,037 Subjects</td>
<td>Incident</td>
<td>4 y</td>
<td>MMSE-m scores</td>
<td>Yes for statins; no for other LLAs</td>
<td>Mixed</td>
</tr>
<tr>
<td>Zandi et al, 2005</td>
<td>LOV, SIM, CER, ATOR, PRA, and FLU analyzed together</td>
<td>4,895 (Unspecified severity); 3,308 (incident)</td>
<td>Unspecified</td>
<td>3 y incident</td>
<td>Likelihood of preexisting dementia or AD; risk of developing dementia or AD</td>
<td>Yes for statins for preexisting dementia or AD; no for statins for risk of developing dementia or AD; no for other LLAs</td>
<td>Mixed</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; ATOR, atorvastatin calcium; BBB, blood-brain barrier; CER, cerivastatin sodium; CERAD, Consortium to Establish a Registry for Alzheimer Disease; FLU, fluvastatin sodium; LLAs, agents to lower lipid levels; LOV, lovastatin; NA, not available; PRA, pravastatin sodium; ROS, rosuvastatin calcium; SIM, simvastatin; MMSE-m, modified Mini-Mental State Examination; 
* Cohort studies and case-control studies are included. Study size represents the number of participants who completed the study, unless otherwise noted.
another study, 19 lovastatin and simvastatin had much higher BBB permeability coefficients than did pravastatin (Figure 2).

Like pravastatin, rosuvastatin calcium has a limited ability to cross the BBB. 20 The evidence on atorvastatin and cerivastatin is mixed; some researchers reported that they cross the BBB effectively (ie, Kandiah and Feldman 21 for atorvastatin and Knopp 22 for cerivastatin), whereas others did not (ie, Knopp 22 for atorvastatin and Kandiah and Feldman 21 for cerivastatin).

What is responsible for this disparity in BBB permeability among the statins? One key determinant is lipophilicity. Lovastatin and simvastatin are much more lipophilic than pravastatin, 23 whereas fluvastatin falls somewhere in between. 24 Also, fluvastatin is negatively charged and so would be repelled by the anionic microdomains in the plasma membranes of BBB endothelial cells. 18 Different transport mechanisms may also play a role; simvastatin and lovastatin appear to cross the BBB via passive diffusion, whereas pravastatin relies on an active transport system, for which it may have a low affinity. 25

This variation in the biochemical properties of statins provides an important clue as to why some studies may have shown a beneficial effect of statin treatment on dementia and AD whereas others have not. However, because several investigators 4,8 reported that they cross the BBB effectively (ie, Kandiah and Feldman 21 for atorvastatin and Knopp 22 for cerivastatin), whereas others did not (ie, Knopp 22 for atorvastatin and Kandiah and Feldman 21 for cerivastatin).
The data on the variability of BBB penetration across statins (Table 3) provide a potentially important explanation for the inconsistency in outcome. Some studies focus on a statin that does not cross the BBB well, or they aggregate data from penentrant and nonpenentrant drugs (Tables 1 and 2). With substantial variation in BBB permeability among statins, it becomes difficult to reconcile the conflicting findings in the literature. Also, some studies base their conclusions for statins as a whole on the analysis of a single drug.

Various methodological issues are also likely to contribute to the inconsistency. In their review, Kandiah and Feldman identified as particularly important (1) the distinction between preexisting and incident AD, (2) the time of statin use vs the time of cognitive assessment, (3) the dose of the statin, (4) the duration of statin use, (5) the duration of observation, (6) the confounding of AD with other forms of dementia (a major concern), (7) patient adherence to the statin prescription regimen, and (8) whether vascular risk factors were controlled.

In accord with that review, for example, discussed survival bias as one of the difficulties in studying the relationship between cholesterol levels and AD. Statin dosage and brain penetration were alluded to by Meske et al as potential confounding factors in various epidemiological studies; that is, negative results may be due to statins not reaching a therapeutic concentration in the brain. The importance of dosage was stressed in an elegant study conducted by Ostrowski et al, which found, that although simvastatin and lovastatin can affect Rho and Rab family proteins, applying a physiologically relevant concentration of the statin would influence some members of these protein families but not others. Those authors postulate that Rab- and Rho-dependent effects overlap at high statin doses, whereas Rho-dependent effects dominate at lower doses.

Indication bias (in which a drug is prescribed to treat a condition that is associated with the variable of interest) and cessation bias (in which some of the observed protective effects of a drug may be due to patients stopping the drug therapy after being diagnosed as having a condition) have also been suggested as problems that may occur in statin studies. Another potential pitfall is the question of whether those who are in generally good health are more likely to be prescribed statins, resulting in a misleading apparent effect of these drugs on disease prevention or treatment. This was addressed by Rockwood et al, who found that there was no association between self-reported health and LLA use. On the contrary, there was an association between LLA use and other vascular risk factors, indicating that LLAs are not more likely to be used by healthier people.

The period during which a study was conducted may be a particularly important factor. After finding protective effects for lovastatin and pravastatin but not simvastatin, Wolozin et al pointed out that simvastatin was (at the time) a relatively new drug, so prescribing patterns may have been different than for the other 2 compounds studied. In another study, Wolozin and colleagues chose not to assess fluvastatin because the number of people taking it increased drastically during the course of the study, which they believed introduced a confounding variable. The time in the patient’s life at which measurements are taken may also be critical. Yaffe et al mentioned that dementia might alter a patient’s diet or metabolism, thus having an effect on lipid levels, and part I of this article emphasized that studies conducted in middle vs old age reach different conclusions about the relationship between cholesterol levels and dementia risk.

The statistical power of a study is a major issue to consider. Li et al expressed concern that the “crude, semiquantitative” CERAD scale may not have very high power to detect relationships between statin use and AD pathological characteristic. Arvanitakis et al indicated that their study was limited in statistical power, which could account for the observed lack of correlation between statin use and overall AD brain pathological features or tangle immunoreactivity. Difficulties can arise when only a small percentage of even a large initial cohort develop AD during the course of the study, making it problematic to detect the potential effects of therapeutic interventions on outcome.

Another confounding factor is the fact that an AD patient’s ApoE genotype may affect not only AD risk but also the effectiveness of statins as an AD prevention or treatment. In one study, people with the ApoE4 allele saw less benefit (in terms of cholesterol levels) from statin treatment than those with the E2 or E3 alleles. Although a detailed discussion of the potential effects of ApoE is not within the purpose of this review, some trials of statins have taken ApoE genotype into account, and the mechanism by which the ApoE genotype might influence AD risk continues to be the subject of intense research (eg, Morris et al and Belinson et al).

### PLEIOTROPIC EFFECTS OF STATINS

Statins have other effects on physiology and metabolism besides lowering cholesterol levels, some of which could independently affect AD risk. For example, statins can alter the expression of genes related to cell growth, signaling, trafficking, and apoptosis. Here, too, we see differences among the statins. In the study by Johnson-Anuna et al, 38 genes were affected by simvastatin, 26 by lovastatin, and only 21 by pravastatin.

Rosuvastatin, atorvastatin, and simvastatin have all been found to increase the expression and activity of endothelial nitric oxide synthase in mice. Furthermore, treatment of normocholesterolemic, spontaneously hypertensive rats with a physiologically relevant dose of atorvastatin mediated a host of beneficial effects. It lowered blood pressure, enhanced carbachol-induced vasodilation, inhibited angiotensin II–induced vasoconstriction, decreased the production of superoxide in blood vessel walls, lowered messenger RNA and protein expression of angiotensin type 1 receptor, and reduced messenger...
RNA expression of the nicotinamide adenine dinucleotide phosphate oxidase subunit p22phox. Although the mechanisms behind these effects are largely unknown, the authors speculated that they may involve down-stream isoprenoids, some of which are important for post-translational modification of other proteins. In any event, the broad spectrum of actions of some or most statins means that any effect they have on AD incidence or course (or on preclinical measures, such as degree of Aβ patho-logical features) may not necessarily relate to their cho-les terol level–lowering effects per se.

One potential effect of statin treatment deserves spe-cial mention. Inhibition of hydroxymethylglutaryl–CoA reductase activity interferes with the isoprenyla-tion of other proteins, impairing their functions and causing a wide variety of downstream effects. Isoprenylation is important in protein trafficking and signalling and in cytoskeletal structure. If statins are added to cells in the presence of mevalonate, cholesterol synthesis will still be blocked, but isoprenylation can continue unimpeded. This gives researchers a way to separately analyze the cholesterol- and isoprenoid-dependent ef-fects of statins. Using this strategy, Cole et al36 con-cluded that low isoprenoid levels inhibit the passage of amyloid precursor protein through the secretory pathway, leading various processing products to accumulate intracellularly. Low cholesterol levels, meanwhile, may inhibit receptor-mediated endocytosis of amyloid precursor protein. The authors hypothesized that the fol-lowing 2 largely separate pools of Aβ exist: an intracel-lular pool that is mainly affected by isoprenoids and a secreted pool that is mainly influenced by cholesterol (Figure 3).

In summary, the diverse potential roles for choles-terol in aspects of AD pathogenesis have led to numer-ous studies evaluating LLAs such as statins for potential therapeutic effects. Based on all available studies, stat-ins appear to hold greater promise than other classes of LLAs. However, individual statins differ in ways that could affect their efficacy with respect to AD. The conflation of statins with other LLAs, the disparities among statins in their respective BBB permeabilities, and the differences in biochemical effects among these drugs are all likely to contribute to the unfortunate variability in study outcomes observed to date.

RECOMMENDATIONS FOR FUTURE RESEARCH

Most of the laboratory studies discussed in part I of this review4 converge on a model suggesting that increased cho-les terol levels promote Aβ formation and accumulation in the brain. However, the human epidemiological studies (also in part I) are difficult to bring together with similar con-viction. Throughout this second part of our review, we have found reconciling the many human LLA studies difficult because of (1) the different biochemical properties of the statins and other LLAs used in the studies, (2) the myriad points of cholesterol level measurements (see part I) and
that have precluded a clear answer to the overarching question of whether statins, such widely prescribed and generally safe drugs in our society, represent viable therapeutic or preventive agents for the most common cause of progressive cognitive failure in older humans.

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