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Alzheimer's Disease—A Dysfunction in Cholesterol and Lipid Metabolism

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SUMMARY

1. Strong etiological association exists between dysfunctional metabolism of brain lipids, age-related changes in the cerebral vasculature and neurodegenerative features characteristic of Alzheimer's disease (AD) brain.

2. In this short review, recent experimental evidence for these associations is further discussed below.

KEY WORDS: Alzheimer; amyloid; cholesterol; lipids; oxidation; statins.

INTRODUCTION

Abundant data suggest that (1) cumulative oxidative modification of biomolecules, including lipids, plays an important role in aging, and free radical damage to brain lipids is involved in neuronal death in neurodegenerative disorders; (2) the 'γ-secretase complex' of presenilin, nicastrin and beta-amyloid precursor protein (βAPP), which gives rise to amyloid beta (Aβ) peptides 40 to 42 amino acids in length (Aβ40-42), are integral, amphipathic components of cholesterol-rich lipid raft domains located in neural membranes; (3) cholesterol, itself an important modulator of the biophysical state of biological membranes, modulates the rate of cleavage of the βAPP and thus regulates the production of Aβ peptides, and a causal relationship exists between cholesterol-mediated Aβ-peptide generation, aggregation, and the neuropathological lesions that characterize AD brain; (4) abundant epidemiological studies repeatedly link cardiovascular factors, such

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as high blood pressure (hypertension) and high blood cholesterol, to an increased risk for the development and progression of AD; (5) the apolipoprotein E type 4 (apoE4) allele, encoding the major cholesterol transporter in blood plasma and the brain, represents a strong genetic risk factor for both familial and sporadic AD; and (6) in epidemiological studies, statins, which lower serum cholesterol, and other lipid lowering agents, have been reported to have beneficial effects in reducing the risk for AD. Recent data pertaining to these associations are further summarized below.

Lipid Peroxidation in AD

Over the last few years several laboratories have presented evidence indicating that brains with AD are subject to a pervasive level of oxidative stress. One important set of data comes from *in vitro* models and suggests that the amyloid peptide, the main constituent of senile plaques, causes extensive degeneration and death of neurons by mechanisms that involve reactive oxygen intermediates and free radicals. In AD brain there are increases in the products of lipid peroxidation, decreases in polyunsaturated fatty acids (normally enriched in brain tissues), decreases in membrane fluidity and increases in hydroxynonenal, a toxic free radical second messenger and neurotoxic aldehyde of polyunsaturated fatty acid oxidation. Another independent and equally important body of evidence demonstrates that numerous markers of oxidative stress are accentuated around areas of amyloid deposition in human brains and in AD transgenic mice (Pappolla *et al*., 1992, 1998). Markers of lipid peroxidation, such as isoprostanes and hydroxynonenal, are also increased in AD CSF and brain. Immunocytochemical studies reveal the accumulation of lipid peroxidation products within the neurofibrillary tangles and senile plaques whose overabundance characterize AD brain. Oxidative stress may be further enhanced by toxic metal ions (such as Al^{3+}, Fe^{2+} and Zn^{2+}) that associate with and accumulate within these same lesions. In AD brain, and as a consequence of protein, nucleic acid and lipid oxidation, inflammatory response markers are up-regulated and include cyclooxygenases (in particular the inducible COX-2), cytokines, proteases, acute phase reactants and other components of the complement cascade (Colangelo *et al.*, 2002; Lukiw, 2004). Interestingly, COX-2 up-regulation in AD brain may have a direct bearing on γ-secretase activity and increased generation of neurotoxic Aβ peptides (Qin *et al*., 2003; unpublished observations). Combined with epidemiologic studies showing that non-steroidal anti-inflammatory drugs (NSAIDs) and related anti-inflammatory agents may reduce the risk of developing AD (McGeer *et al*., 1996; Launer, 2003) these findings suggest that inflammation may participate in AD progression and that inflammatory cascades fuelled by oxidative stress are important factors that contribute to neurodegeneration in AD brain (Bazan *et al.*, 2002).

The Presenilin-Nicastrin-βAPP 'γ-Secretase Complex'

Presenilin, βAPP and the nicastrin "docking molecule," organized into the high molecular weight 'γ-secretase complex,' contain both peripheral and transmembrane domains intimately associated with the lipid bilayers of neuronal

lysosomal, Golgi, endoplasmic reticular and plasma membranes (Schenk, 2000; Puglielli *et al*., 2003; Pasternak *et al*., 2004). βAPP, a type-1 integral membrane glycoprotein, along with nicastrin and presenilin together generate ragged Aβ peptides 37–43 amino acids in length, the most neurotoxic of which appears to be Aβ40-42 peptides. Aβ peptides are synthesized intracellularly before secretion from neuronal cells (Turner *et al*., 1996). As major components of the amyloid plaques progressively deposited in AD brains, Aβ40-42 peptides are thereby 'secreted' into the extracellular space by the sequential cleavage by β-secretase (BACE-1) followed by γ-secretase cleavage at positions 40 or 42 of βAPP (the β pathway; Puglielli *et al.,* 2003). The unusual γ-secretase cleavage within the hydrophobic trans-membrane domain suggests that pathological events which disorganize the structure of the lipid bilayer associated with the γ-secretase complex may accelerate or contribute to Aβ40-42 peptide generation. Presenilins (PS1 and PS2) have been suggested to possess either inherent γ-secretase activity or act as an essential cofactor for γ-secretase activity. Interestingly, in transgenic animals, over-expression of nicastrin, mutant βAPP or mutant presenilin (PS1 or PS2) increases Aβ42 peptide production (Flood *et al.*, 2002; Murphy *et al*., 2003; Jankowsky *et al*., 2004). BACE-1 activities are also enhanced by cellular targeting into cholesterol-rich microdomains, and these are dispersed by statins, which may be one mechanistic basis for the effective lowering of Aβ42 peptide generation by statin activities (Sidera *et al*., 2004).

Cholesterol and Aβ Generation in AD Brain

Representing only about 3% of total body mass, the CNS contains about 25% of total body cholesterol, the highest content of any organ. In the CNS, unesterified cholesterol is predominantly present in two compartments that include neural cell plasma membranes and the myelin sheath of axons (Dietschy and Turley, 2001; Gibson *et al*., 2003). As a major regulator of membrane fluidity, cholesterol is an intrinsic modulator of the biophysical state of the lipid bilayer, modifying the rate of cleavage of βAPP, and thereby regulating cellular production of Aβ peptides (Flood *et al.*, 2002; Zatta *et al*., 2002; Gibson *et al*., 2003). The enzymatic conversion of CNS cholesterol to 24S-hydroxycholesterol, which readily crosses the blood-brain barrier, is the major pathway for brain cholesterol transport, elimination and maintenance of brain cholesterol homeostasis (Lutjohann and von Bergmann, 2003). 24S-hydroxycholesterol induces neurotoxic effects and increased concentrations have been detected in the CNS of AD patients (Kolsch *et al*., 2003). Interestingly, polymorphisms in the CYP46 gene (which encodes cholesterol 24S-hydroxylase) influence both Aβ peptide load in the brain and the genetic risk for late-onset sporadic AD (Kolsch *et al*., 2003; Wolozin, 2003). It has been suggested that increases in the membrane distribution of cholesterol (in contrast to total cholesterol content) may provide an enriched environment for Aβ production and release in the brain (Gibson *et al*., 2003; Kolsch *et al*., 2003). In addition, Aβ peptide induction of membrane-associated oxidative stress may contribute to altered ceramide and cholesterol metabolism that in turn trigger AD-type neurodegeneration and brain disease (Cutler *et al*., 2004).

Cardiovascular Disease, Cholesterol and AD

Abundant epidemiological studies repeatedly link cardiovascular disease and blood-borne factors, such as hyperglycemia, elevated triglycerides, hypertension and high blood plasma cholesterol, to a significantly increased risk for the development of AD (Aliev *et al*., 2003; Ravona-Springer *et al*., 2003; Fitzpatrick *et al*., 2004). Amyloid accumulation in the cerebral vasculature may cause obstruction of regional cerebral blood flow and disturbances in the function of the blood-brain barrier and this appears to ultimately manifest as cognitive impairment (Aliev *et al*., 2003; Chaney *et al*., 2003). Peripheral sources of Aβ peptides, cholesterol and inflammatory lipids, by transversing a damaged or leaky blood brain barrier, may further contribute to Aβ deposition in the AD brain (Aliev *et al*., 2003; Chaney *et al*., 2003). Due to a reduction in brain perfusion and altered hemodynamic properties, damaged, diseased, or dysfunctional cerebral vasculature may promote premature neuronal death as observed in multiple infarct dementia, in cerebral vascular dementia and in AD brain (Cacabelos *et al*., 2003; Aliev *et al*., 2003; Ravona-Springer *et al*., 2003; Fitzpatrick *et al*., 2004).

As previously mentioned, there is increasing evidence from laboratory and clinical studies that cholesterol may play a role in AD (Bodovitz and Klein, 1996; Mizuno *et al*., 1998; Simons *et al*., 1998; Frears *et al*., 1999; Rohe *et al*., 1999; Grant, 1999; Refolo *et al*., 2000; Sparks *et al*., 2000; Wolozin *et al*., 2000; Jick *et al*., 2000). This evidence includes *in vitro* studies indicating that cellular cholesterol levels modulate Aβ production (Frears *et al*., 1999; Simons *et al*., 1998; Mizuno *et al*., 1998; Sparks *et al.*, 2000), animal studies demonstrating that cholesterol levels modulate Aβ accumulation in the brain (Sparks, 1997; Refolo *et al*., 2000) and observational, retrospective clinical studies reporting that patients taking statins have a reduced risk of developing AD (Wood *et al*., 1999; Jick *et al*., 2000). Taken together, the data support the hypothesis that Alzheimer's disease may be a condition in which cholesterol homeostasis is altered. A number of studies regarding cholesterol and AD, however, have provided contradictory information (Mason *et al*., 1992; Zlokovic *et al*., 2000). Examining the frontal cortex of AD patients with apoE4 genotypes, one study reported that the cholesterol content was significantly increased compared to non-demented controls (Mason *et al*., 1992). Data from another study, however, found that the cholesterol content in the CNS of AD patients was significantly lower than that measured in control cases (Zlokovic *et al.,* 2000). In addition, there are variable data and no consensus among studies regarding whether the levels of serum and CNS cholesterol are elevated or decreased in AD patients (Grant, 1999). There are several possible explanations for these discrepancies. First, negative studies correlating the clinical diagnosis of AD with cholesterolemia did not have neuropathological confirmation of the diagnosis and may have included some cases of non-AD dementia, weakening the association between cholesterol and AD. Second, experimental data suggest that the extent of amyloid deposition may be influenced by events that regulate removal of amyloid peptides from the brain (Notkola *et al*., 1998), and therefore, better clearance mechanisms in certain individuals may preclude clinically significant accumulations of amyloid, despite increased cholesterol-mediated amyloidogenesis. Third, population-based studies of patients who subsequently developed AD show a

significant decrease in plasma cholesterol levels preceding development of cognitive symptoms (Newschaffer *et al.*, 1992), potentially obscuring the past effects of higher cholesterol levels earlier in life. Finally, subjects with the highest levels of plasma cholesterol generally die at younger ages from cardiovascular events and are lost from the sample of elderly subjects, by virtue of introducing a "survivorship effect" into the sample (Mirra *et al.*, 1991). In this regard, a recent study has shown that cholesterol may be an *early* risk factor for the development of amyloid deposition in the brain (Pappolla *et al.*, 2003). High cholesterol can be a source of oxidative stress and inflammation. Interestingly, NSAIDs and other inhibitors of inducible COX-2 activity not only alter the balance of vasoactive eicosanoids, such as thromboxane and prostacyclin, but may also suppress the actions of related inflammatory and/or angiogenic mediators implicated in the progression of brain arterial wall damage triggered by ischemia and atherosclerosis (Bazan and Flower, 2002; Weir *et al.*, 2003; Fitzpatrick *et al.*, 2004 Lukiw, 2004).

The Apolipoprotein E Type 4 (ApoE4) Allele

Apolipoprotein E (ApoE) is a major Aβ peptide, lipid and cholesterol transporter in the blood plasma and in the brain (Puglielli *et al.*, 2003; Petanceska *et al.*, 2003). The Apo epsilon 4 (ApoE4) variant allele represents the strongest known genetic risk factor for sporadic AD (Strittmatter *et al.*, 1993; Bassett and Montine, 2003; Puglielli *et al.*, 2003). Variations in total plasma cholesterol can lead to up-regulation of ApoE transcription in the brain. Moreover, cholesterol loading of glial cells increases both cellular and secreted ApoE levels, and long-term treatment of glial cells with statins results in a decrease in the cellular and/or secreted ApoE, suggesting that cholesterol metabolism may increase the risk for AD due, in part, to cholesterol's effect on ApoE expression in the brain (Petanceska *et al.*, 2003).

Statins and AD

Statins are a highly effective and well-tolerated class of plasma lipid-altering agents that have been shown to reduce the development and risk of cerebral and cardiovascular pathologies (reviewed by Edwards and Moore, 2003; Miller and Chacko, 2004). Statins (for example, lovastatin, pravastatin, simvastatin, atorvastatin; essentially 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and related pharmacological compounds) that inhibit the cholesterol biosynthesis pathway are the most widely used oral cholesterol-lowering drugs. In addition, statins also possess cell- and tissue-specific anti-inflammatory and immuno-modulation properties (Jick *et al.*, 2000; Stuve *et al.*, 2003; Miller and Chacko, 2004; Wilson *et al.*, 2004). Both *in vitro* and *in vivo* experiments have suggested a significant inter-relationship between elevated serum cholesterol, cholesterol metabolism, and the enhanced processing of βAPP into neurotoxic Aβ40-42 peptides (Refolo *et al.*, 2000; Pappolla *et al.*, 2003; Petanceska *et al.*, 2003). In preliminary clinical trials, statins have been shown to increase serum βAPP/Aβ ratios, while reducing cholesterol and blood plasma lipids in AD patients (Jick *et al.*, 2000; Wolozin *et al.*, 2000; Baskin *et al.*, 2003). However, there are studies that raise controversy, and in one recent prospective,

randomized, 36-week clinical treatment trial, no significant change in the level of Aβ peptides was found after statin therapy in hypercholesterolemic patients (Hoglund *et al.*, 2004). Another recent study showed that in female Tg2576 (transgenic, amyloid overexpressing) mice, lovastatin indeed lowered plasma cholesterol concentrations, but actually enhanced the rate of Aβ production and senile plaque deposition in the brain (Park *et al.*, 2003). These preliminary results should therefore be interpreted with caution, and further careful experimentation is required. Larger, well controlled human clinical trials using statins as a potential treatment for AD are now in progress. Hypothetically, endogenous bioactive docosanoids and/or combinatorial pharmacological strategies directed at anti-oxidative, anti-inflammatory and anti-cholesterol mechanisms may rescue both normal lipid and brain function (Marcheselli *et al.*, 2003).

SUMMARY

Strong molecular genetic, neurochemical, and epidemiological evidence suggests that dietary fat, and in particular cholesterol transport through, and association with the cerebral vasculature, in conjunction with cholesterol metabolism in neural cells, are causally involved in the pathological events that lead to AD. One of the pathological hallmarks of AD still remains the progressive deposition of amyloid plaques composed of highly condensed Aβ peptides and related pro-inflammatory protein and lipid components. Aβ40-42 peptides induced by elevated cholesterol and/or plasma lipids may trigger other Aβ peptides (such as Aβ37-40) to aggregate. Aggregation of these neurotoxic Aβ peptides, produced by the sequential proteolytic cleavage of βAPP, first by BACE-1 and then by γ-secretase, appears to occur in cholesterol and lipid-enriched raft domains of the cytoplasmic or plasma membranes of neural cells. Interestingly, BACE-1 activity significantly increases with age in mouse, monkey, and human brain, and via increased generation and accumulation of Aβ peptides, may potentially predispose aging humans to AD (Fukumoto *et al.*, 2004). Cholesterol and related lipids appear to fundamentally affect the normal rate of Aβ peptide generation and assembly into dense, insoluble, and potentially neurotoxic amyloid plaques. High dietary intake of cholesterol and lipids may influence the ability of Aβ-binding proteins to enhance Aβ clearance from the brain, and variation in the dietary intake of cholesterol and fat among human populations may help explain global variations in both the incidence and prevalence of AD.

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