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Elevated plasma triglyceride levels precede amyloid deposition in Alzheimer's disease mouse models with abundant Aβ in plasma

Braydon L. Burgess,[a] Sean A. McIsaac,[a] Kathryn E. Naus,[a] Jeniffer Y. Chan,[a] Gavin H.K. Tansley,[a] Jing Yang,[b] Fudan Miao,[c] Colin J.D. Ross,[c] Miranda van Eck,[d] Michael R. Hayden,[c] William van Nostrand,[e] Peter St. George-Hyslop,[b] David Westaway,[b] and Cheryl L. Wellington[a,*]

[a]*Department of Pathology and Laboratory Medicine, University of British Columbia, 980 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4*
[b]*Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ontario, Canada*
[c]*Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada*
[d]*Division of Biopharmaceutics, Gorlaeus Laboratories, Leiden/Amsterdam Center for Drug Research, Leiden, The Netherlands*
[e]*Department of Medicine, Stony Brook University, Stony Brook, New York, USA*

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Dietary or pharmacological manipulation of plasma lipids markedly influences amyloid deposition in animal models of Alzheimer's Disease (AD). However, it is not known whether baseline plasma lipids in AD models differ from wild-type littermates throughout the natural history of disease. To address this question, we measured plasma total cholesterol and triglyceride levels over time in three transgenic AD mouse models in the absence of dietary or pharmacological treatments. Total cholesterol levels were not significantly different between transgenic and wild-type mice during the development of AD neuropathology in all models tested. In contrast, elevated very-low-density lipoprotein (VLDL) triglyceride levels preceded amyloid deposition in two AD models with abundant plasma Aβ. Elevated triglycerides were not accompanied by increased inflammatory markers nor decreased lipase activity, but were associated with a significant 30% increase in VLDL-triglyceride secretion rate. Our results suggest that the presence of Aβ in plasma may affect peripheral lipid metabolism early in AD pathogenesis.

Keywords: Triglyceride; Cholesterol; Very low density lipoprotein; Amyloid; Aβ; Alzheimer's Disease

Introduction

Increasing evidence suggests that cholesterol plays a key role in the pathogenesis of Alzheimer's disease (AD), the most common cause of senile dementia (Casserly and Topol, 2004; Michikawa, 2003; Puglielli et al., 2003; Raffaï and Weisgraber, 2003; Wolozin, 2004). The first association between cholesterol metabolism and AD emerged through studies of apolipoprotein E (apoE), which is the major cholesterol carrier protein in the brain and the only validated genetic risk factor for late-onset AD (Mahley and Rall, 2000; Strittmatter and Roses, 1995). ApoE exists as the three major alleles apoE2, apoE3, and apoE4, and inheritance of apoE4 increases the risk of developing AD at an earlier age, whereas inheritance of apoE2 delays the age of onset of AD (Corder et al., 1993, 1994). Additionally, many groups have demonstrated that intracellular cholesterol levels have a major influence on amyloid precursor protein (APP) metabolism. High levels of intracellular cholesterol facilitate processing of APP by β- and γ-secretase, thereby enhancing the release of Aβ in vitro and in vivo (Burns et al., 2003; Refolo et al., 2000; Shie et al., 2002; Wahrle et al., 2002), whereas low intracellular cholesterol favors the nonamyloidogenic processing of APP by α-secretase, leading to decreased Aβ formation (Bodovitz and Klein, 1996; Buxbaum et al., 2001; Ehehalt et al., 2003; Fassbender et al., 2001; Kojro et al., 2001; Simons et al., 1998).

These observations have prompted considerable interest in determining whether pharmacological manipulation of lipid levels may provide therapeutic benefit for AD. Initial retrospective studies suggested that the prevalence of AD could be reduced by up to 70% by statins, drugs that inhibit HMG Co-A reductase, which catalyses the rate-limiting step in cholesterol biosynthesis (Jick et al., 2000; Rockwood, 2002; Wolozin et al., 2000). Furthermore, inhibition of cholesterol biosynthesis by statins or other compounds decreases amyloid burden in guinea pigs and in transgenic murine models of AD (Fassbender et al., 2001; Refolo et al., 2001). In humans, statin treatment reduces the levels of 24-hydroxycholesterol, the major cholesterol metabolite of the brain (Hoglund et al., 2005; Locatelli et al., 2002; Simons et al., 2002; Vega et al., 2003). However, the efficacy of statins to affect Aβ

* Corresponding author. Fax: +1 604 875 3120.
E-mail address: cheryl@cmmt.ubc.ca (C.L. Wellington).
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levels, the prevalence or incidence of AD, or cognitive function remains to be fully elucidated. Although several studies suggest that statins may have a neuroprotective effect especially in subjects with mild AD (Buxbaum et al., 2002; Hajjar et al., 2002; Jick et al., 2000; Rockwood, 2002; Rodriguez et al., 2002; Simons et al., 2002; Sjogren et al., 2003; Sparks et al., 2005; Yaffe et al., 2002; Wolozin et al., 2000), other studies do not support an association between statin use, cognitive decline, or dementia risk (Heart Protection Study Collaborative Group, 2002; Li et al., 2004; Santanello et al., 1997; Shepherd et al., 2002; Zandi et al., 2005). Because statins reduce plasma low density lipoprotein (LDL) levels, modestly increase high density lipoprotein (HDL) levels, affect isoprenoid signaling pathways, and lead to diminished inflammation, additional investigations will be needed to clarify the role of statins, and their mechanism of action, in the pathogenesis of AD.

Although LDL and HDL levels are well-established risk factors for cardiovascular disease, the relationship of plasma lipid levels to AD risk is far from clear (Hoglund et al., 2005; Michikawa, 2003). In animal models of AD, diet-induced hypercholesterolemia significantly exacerbates AD neuropathology (Refolo et al., 2000; Schmechel and Sullivan, 2002; Shie et al., 2002). However, there are conflicting reports about whether plasma cholesterol levels are elevated in AD patients and whether plasma lipid levels could be considered a risk factor or a biomarker for AD. Several groups have reported that increased midlife total cholesterol (TC) levels are associated with a 2–3-fold increase in AD risk later in life (Kivipelto et al., 2002; Notkola et al., 1998; Pappolla et al., 2003; Whitmer et al., 2005), and that AD risk is further increased by elevated systolic blood pressure in hypercholesterolemic subjects (Kivipelto et al., 2001). Elevated TC and triglyceride (TG) levels have also been reported in subjects with probable/possible AD (Sabbagh et al., 2004; Suryadevara et al., 2003). However, other studies have found no significant associations between plasma TC or HDL levels in AD subjects compared to controls (Adunsky et al., 2002; Merched et al., 2000; Reitz et al., 2004; Romas et al., 1999; Yoshitake et al., 1995; Zaldy et al., 2003).

Although it is clear that dietary or pharmacological alterations in plasma lipid metabolism can influence the development of AD neuropathology in animal models, it is not yet known how the baseline lipid profiles in untreated AD transgenic models compare to nontransgenic littermate controls during the natural history of disease. Because the liver catabolizes most of the Aβ found in the peripheral circulation (Ghiso et al., 2004; Hone et al., 2003), it is possible that uptake of peripheral Aβ by hepatocytes could lead to changes in plasma lipid homeostasis even in the absence of confounding factors such as a high fat diet or lipid-altering drugs. Furthermore, changes in lipid homeostasis that precede the onset of amyloid deposition may be of interest for the development of biomarkers for AD. Investigating this matter is difficult in humans, because of the genetic heterogeneity of subjects, as well as variability in dietary habits. In contrast, animal models have several advantages that make them well suited to addressing this question, including control over dietary lipid intake and control over genetic background, which has marked effects on plasma lipid levels and susceptibility to atherosclerosis (Allayee et al., 2003; Paigen et al., 1985, 1987). Additionally, the ability to assess plasma lipids longitudinally allows any alterations to be placed within the temporal context of pathological changes including circulating and brain Aβ levels and amyloid deposition.

We therefore sought to elucidate the relationship between the natural history of plasma lipids relative to amyloid deposition and Aβ levels in three independent murine models of AD compared to nontransgenic littermate controls (Chishti et al., 2001; Davis et al., 2004; Jankowsky et al., 2003), specifically in the absence of additional experimental manipulation of lipid levels by diet or drug treatments. Cohorts of sex- and age-matched APP-transgenic mice and controls on a standard low-fat chow diet were assessed for plasma TC and TG levels, plasma and brain Aβ levels, and extent of amyloid deposition over time. Here we report that plasma cholesterol levels are not significantly altered in any of the murine models tested. In contrast, we observed significantly increased plasma VLDL-TG levels in models with abundant plasma Aβ (Tg-CRND8 and APP/PS1 mice), prior to the onset of amyloid deposition in vivo. This ability of peripheral Aβ to affect plasma TG metabolism is unlikely to be due to systemic inflammatory processes, as no differences in plasma IL-6 or CRP levels were detected at any age tested. We observed no differences in lipoprotein lipase (LPL) or hepatic lipase (HL) activities in Tg-CRND8 mice, suggesting that impaired lipolysis could not account for the elevated TG levels. Plasma free fatty acids and urinary ketone levels were also indistinguishable between groups. Instead, elevated plasma TG levels in Tg-CRND8 mice were associated with a 1.3-fold increase in the rate of VLDL-TG secretion. Our results show that alterations in plasma TG but not TC levels can be observed prior to amyloid deposition in mice with high levels of circulating Aβ and suggest that brain-derived peripheral Aβ can influence plasma lipid metabolism early in the pathogenesis of AD.

Materials and methods

Mouse models

The Tg-CRND8 mouse model expresses the human APP650 cDNA containing the Swedish (KM670/671NL) and Indiana (V717F) mutations (Chishti et al., 2001). The APP transgene was introduced into a cosmid-based expression vector derived from the Syrian hamster prion promoter, which directs high levels of expression in neurons and low level expression in astrocytes (Chishti et al., 2001). The level of transgenic APP expression in the brains of Tg-CRND8 mice is estimated to be approximately 5-fold over murine APP levels (Chishti et al., 2001). The Tg-CRND8 mice used in this study are on a congenic 129SvEv/Tac genetic background, and are reported here to develop parenchymal Thioflavine-S-positive amyloid deposits beginning at approximately 12–15 weeks of age (Table 1).

The APP/PS1 (line 85) mouse model coexpresses two transgenes that are each expressed from the mouse prion promoter. One transgene is a chimeric mouse/human APP650 cDNA containing the Swedish (KM670/671NL) Familial AD (FAD) mutation, and the other is the human presenilin 1 (PS1) gene containing the DeltaE9 (deletion of exon 9) FAD mutation (Jankowsky et al., 2003). Both transgenes are inserted at a single locus and are inherited together. The level of transgenic APP expression in the APP/PS1 mice is estimated to be approximately 2–4-fold over murine APP levels (Jankowsky et al., 2003). The APP/PS1 mice are maintained on a mixed F1 C3H/H3J × C57Bl/6 genetic background and develop parenchymal Thioflavine-S-positive amyloid deposits at approximately 36–40 weeks of age