Increased Lysophosphatidylethanolamine and Diacylglycerol levels in Alzheimer’s Disease Plasma

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Abstract

Background: Previous studies have demonstrated deficits in biosynthesis of plasmalogens and sphingolipids in Alzheimer’s Disease (AD).

Objective: We undertook an analysis of alternate biomarkers of lipid synthesis, metabolism, and remodeling “Lands” cycle in the plasma of control and cognitively impaired patients based upon their Mini-Mental State Examination (MMSE) scores.

Methods: Plasma levels of 600 lipids were measured utilizing a high-resolution mass spectrometric shotgun analysis platform.

Results: The lyso metabolites of phosphatidylethanolamines and phosphatidylglycerols, along with diacylglycerols were all elevated in the plasma of patients with cognitive impairment. There were no significant differences in the plasma levels of fatty acids metabolized by peroxisomes.

Conclusion: The increased levels of lysolipids and diacylglycerols in the plasma of all patient groups suggest that lipid remodeling is dysfunctional early in the AD disease process. It remains to be determined if this involves increased phospholipase A2 activity, decreased acyltransferase activity, or decreased cellular uptake of lysophospholipids for subsequent acylation.

INTRODUCTION

The search for robust blood-based biomarkers for AD has involved both proteomics [1] and lipidomics [2] approaches. Plasma lipidomics findings include decrements in peroxisomal-dependent biosynthesis of plasmalogens in AD, altered ceramide levels in Mild Cognitive Impairment (MCI) and AD, and decreases in sphingomyelins in AD [2]. The major goals of current lipidomics research efforts are: i) to identify biomarkers of early-stage disease processes such that preclinical therapeutic interventions can be initialized, ii) to monitor disease progression and the efficacy of therapeutic interventions, and iii) to increase our understanding of upstream disease pathophysiology in efforts to define new therapeutic targets that might halt neurodegeneration.

Since several alterations in the levels of circulating plasmalogens have been reported in AD plasma [3,4], we decided to evaluate glycerophospholipid metabolites to more fully characterize potential early alterations in lipid dynamics. This evaluation involved monitoring lysoglycerophospholipids as biomarkers of lipid remodeling via deacylation/reacylation at sn-2 of the glycerol backbone [2,5-7].

Previous studies have demonstrated decreases in the ability of peroxisomes to synthesize plasmalogens in AD liver and brain [8,9]. We therefore investigated if peroxisomal-dependent lipid metabolism might also be altered in AD. To this end, we measured the branched chain fatty acid, phytanic acid and the very-long-chain fatty acid (VLCFA), hexacosanoic acid (26:0), both of which are dependent upon peroxisomes for their metabolism [10].

MATERIALS AND METHODS

Study sample

Plasma samples were obtained from cognitively healthy controls and AD patients (Table 1) followed at the Oregon Health and Science University Aging and Alzheimer’s disease center. Alzheimer’s disease was diagnosed by NINDS-
ADRSA criteria and patients were stratified by clinical severity measured by the Mini-Mental State Examination (MMSE) score. The clinical studies were approved by the OHSU Institutional Review Board.

**Laboratory methods**


Extracts were dried by centrifugal vacuum evaporation prior to dissolution in isopropanol: methanol: chloroform (4:2:1) containing 7.5 mM ammonium acetate. Shotgun lipidomics were performed utilizing high-resolution (140,000 at 200 amu) data acquisition, with sub-ppm mass accuracy on an orbitrap mass spectrometer (Thermo Q Exactive) with successive switching between polarity modes [11]. Washes between samples with hexane/ethyl acetate (3:2) were used to minimize ghost effects. In negative ion ESI, the anions of ethanolamine plasmalogens, phosphatidylethanolamines, lysosphatidylglycerols, cerebrosides, sterol sulfates, and fatty acids were quantitated and lipid identities validated by MS/MS. In positive ion ESI, the cations of choline, serine, phosphatidylserines, lysosphatidylethanolamines, lysosphatidylglycerols, lysosphatidylcholines, phosphorylcholines, lysosphatidylcholines, and the ammonium functions are altered in AD.

Statistical analysis

Data were analyzed by 1-way ANOVA, followed by the Tukey-Kramer test to determine differences between groups.

**RESULTS**

Sample storage effects

Since the majority of the plasma samples used in this study were acquired and stored since 2000-2001, we included 4 samples that had been stored no longer than 4 months to assess potential storage artifacts. While most lipids were stable with storage at -80°C, we found 3- to 5-fold elevations of lysosphatidylcholines and 6- to 10-fold elevations of lysosphatidylinositols with long-term sample storage.

Fatty acids metabolized by peroxisomes

Neither phytanic acid or docosanoic (26:0) acid levels were altered in cognitively impaired patients suggesting that peroxisomal metabolism of branched chain fatty acids and VLCFA is unaltered in AD (Figure 1).

Lysoglycerophospholipids

LPE 16:0 and 18:1 were both increased in the plasma of cognitively impaired patients (Figure 2). Similarly, diacylglycerols like DAG 36:4 and lysosphatidylglycerols like LPG 18:1 were elevated in cognitively impaired patients (Figure 3). In contrast, to these phospholipase A2 (PLA2) metabolic products, phospholipase D products like alkenyl-acylglycerol 34:2 were not altered in the plasma of cognitively impaired patients (Figure 4).

**DISCUSSION**

Lipidomics studies have revealed a number of alterations in both peroxisome-dependent synthesis of plasmalogens and in glycerophospholipid and sphingolipid metabolism [2]. Our evaluation of the branched chain fatty acid, phytanic acid and the VLCFA docosanoic acid suggest that the peroxisomal deficit in plasmalogen synthesis is specific and that not all peroxisomal functions are altered in AD.

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Our evaluation of glycerophospholipid metabolites demonstrated increases in lysosphatidylethanolamines (LPE), lysosphatidylglycerols (LPG), and diacylglycerols (DAG). Increased DAG levels [13] and increased MAG and DAG lipases [14] have been reported in AD brain, suggesting altered lipid metabolism and signaling are present in the AD brain. Our data are the first to report altered DAG levels in the plasma of cognitively impaired patients.

The augmented levels of LPE and LPG that we monitored in the plasma of cognitively impaired patients are likely products of augmented PLA2 activity which is involved in the dynamic regulation of fatty acid substitutions at sn-2 of the glycerol backbone [2,15]. However, PLA2 biochemistry is extremely...
complex with over 26 PLA2 genes and multiple enzyme isoforms [16] and increased levels of different isoforms in AD cortex and CSF [2]. However, plasma lipoprotein-associated PLA2 is unaltered in both MCI and AD patients [17], suggesting that alternate isoforms of circulating PLA2 or tissue PLA2 are involved in augmenting circulating levels of these lipid metabolites. Other factors that might contribute to elevated lysoglycerophospholipids would be an uncoupling of the highly regulated deacylation-reacylation enzymes [2,15,18] and/or dysregulation of cellular uptake of lysoglycerophospholipids and subsequent intracellular acylation [19].

The implications for these findings of altered glycerophospholipid metabolism are twofold. First, alterations in the levels of these complex lipid families will have structural effects on cellular membrane and organelle function [2]. Second, lysoglycerophospholipids are involved in multiple cell signaling functions [20], thereby potentially contributing to altered neurotransmission in AD.

**Limitations and future research**

This is a robust pilot study except for the small N in the more severely cognitively impaired groups. Further validation of our findings will require augmenting these cohorts. Further investigation of the potential role of changes in DAG and LPE metabolism on cardiovascular health, a risk factor for AD, is warranted.

**CONCLUSION**

In summary, our data demonstrate significant increases in lysoglycerophospholipids, lysoglycerolipids, and DAG in the plasma of cognitively impaired patients indicating that these lipid changes occur early in the disease process. The next steps will be to define if these changes in the lipidome involve dysfunctional lipid remodeling and/or altered cellular uptake of lysoglycerolipids for acylation. These data will help to define AD lipid pathophysiology in more detail and increase our understanding of the biochemical processes that ultimately result in the cognitive decline associated with AD.
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