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Perspective

Plasmalogen deficiency and neuropathology in Alzheimer's disease: Causation or coincidence?

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Abstract Causation of Alzheimer's disease (AD) is not well understood. It is necessary to look beyond neuropathology to identify the underlying causes of AD and many other common neurological diseases. Lipid abnormalities are well documented in the preclinical phases of many neurological diseases including AD. Here, we use AD as an example to examine the role of lipid abnormalities as an underlying cause of neurodegeneration. Role of lipids, particularly phospholipids, in the optimal function of the nervous system, impact of the aberrations of phospholipid metabolism on β-amyloid deposition and cholinergic neuronal function, epidemiological evidence on the association of phospholipids with AD, and preliminary data on the possible modulation of risk factors of AD by phospholipids are examined. Implications of these findings on diagnosis and prevention are also discussed.

Keywords: Phospholipids; Neurodegeneration; Alzheimer's disease; Cholinergic hypothesis; Amyloid hypothesis; Biochemical abnormalities

1. Introduction

The number of persons living beyond age 80 years has increased dramatically over the past decades and is projected to continue to rise [1]. As a result of these demographic changes, the cognitive, motor, and emotional impairments resulting from age-associated neurodegeneration has grown into a public health and societal crisis. For example, the economic cost of senile dementia of the Alzheimer's type (commonly referred to as simply Alzheimer's disease [AD] or late-onset AD [LOAD]) in the United States alone surpassed $227 billion in 2018 and is projected to rise to $1.1 trillion by the year 2050 [2].

Indeed, clinical symptomology in combination with specific neuropathologies is the very basis of neurological disease nomenclature. The definition of AD is reduced cognition in persons with, neuritic plaque, and neurofibrillary tangle neuropathology [3]. The disease process can be described as having a biochemical, cellular, and clinical phase. It is also an undeniable fact that it is the neurodegeneration of specific neuronal cell types beyond a certain threshold that is the cause of specific clinical symptoms. It is the underlying cause(s) of this neurodegeneration that continues to elude clinical and preclinical researchers.

The failure of β-amyloid–lowering therapies to demonstrate clinical benefit in AD [4] is a strong cautionary tale regarding the assumption that neuropathology (Aβ) is the primary causative mechanism responsible for neurodegeneration and subsequent impaired clinical function (decreased cognition). These findings suggest that there are pathophysiological or biochemical events "upstream" of neuropathologies that are causative to both neuropathology and reduced clinical function and which represent early preclinical stages of neurodegenerative diseases [4]. Indeed, the prevalence of dementia of persons living until age 90 years is over 38% [5]. Such a large disease penetrance suggests that there is a common, generalized mechanism underlying neurodegeneration

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and that the environmental and genetic variance in the human population merely tinkers with the timing of the inevitable.

We postulate that such a common and generalized mechanism can be found with aberrant lipid metabolism and that changes in lipid metabolism precede the neuropathological and clinical symptomology associated with neurodegeneration. Using AD as a representative neurodegenerative disease, this article explores the hypothesis that AD neuropathology, neurodegeneration, and symptomology have a common root cause.

2. Role of lipids in the brain

Lipids play critical structural and physiological roles in the brain [6]. Brain has the second-highest lipid content after the adipose tissue with lipids consisting about 50% of the dry weight of the brain [7]. However, phospholipids predominate in the brain in contrast to triglyceride dominance in the adipose tissue [7]. Mono- and polyunsaturated fatty acids such as oleic acid, arachidonic acid, and docosahexaenoic acid are abundant in the brain tissue with phospholipids acting as reservoirs of these fatty acids [8]. Phospholipids are one of the major constituents in cellular membranes and are critically important for their function [9]. Cholesterol and phospholipids bearing saturated fatty acids are concentrated in lipid rafts in the cellular membranes, which are involved in cellular signaling [10]. Membrane fluidity, which is maintained by phospholipids consisting of unsaturated fatty acids, is vital for membrane-associated functions including amyloid precursor protein (APP) processing and plays a substantial role in AD pathogenesis [11]. Vesicular fusion, neurotransmitter release, and synaptic function are influenced by the composition of phospholipids in cellular membranes [12]. These functions are critical for optimal neuronal functions and are compromised in AD. Among phospholipids, plasmalogens play a disproportionately larger role in maintaining optimal brain function, and as described in the following, appear to be the missing link between biochemical and functional abnormalities seen in AD and its pathological hallmarks such as Aβ accumulation.

3. Role of plasmalogens, an important phospholipid, in the brain

Plasmalogens are glycerophospholipids vital for brain function [12]. Although choline plasmalogens also exist in nature, the predominant species in the human body is plasmalogen ethanolamines (PlsEtn). More than half of the ethanolamine phospholipids in the brain are PlsEtn [13]. Plasmalogen levels in the brain increase up to about 30-40 years of age and then dramatically decline by about 70 years of age [14], which incidentally coincides with a period of life where the AD incidence increases exponentially [15]. Plasmalogens are major structural components of lipoproteins, myelin, synaptic membranes, and cellular membranes. Plasmalogens possess unique physicochemical characteristics that help modulate membrane fluidity, lipid packing in lipoproteins, and interaction with neural receptors and ion channels [13]. These lipids are also required for synaptogenesis, myelination, and ion transport [16,17]. Given the dramatic decline of brain plasmalogen levels with aging [14] and the critical roles played by these lipids in the central nervous system, it is not surprising that serum and brain plasmalogen deficits are closely associated with disease progression for aging-related neurodegenerative disorders such as AD and Parkinson's disease (PD) [12,16,18–20].

4. Plasmalogen synthesis, degradation, and possible causes of plasmalogen deficiency in AD

Critical steps in plasmalogen synthesis take place in peroxisomes. Fatty acyl reductase (FAR1), an enzyme located in the outer peroxisomal membrane, reduces fatty acid to a fatty alcohol and is considered to be the rate-limiting step of plasmalogen synthesis by some researchers [21]. Other steps of peroxisomal plasmalogen synthesis (catalyzed by enzymes glycerone phosphate O-acyltransferase and alkylglycerone phosphate synthase) are equally critical for plasmalogen synthesis [22]. Congenital deficiency of alkylglycerone phosphate synthase causes rhizomelic chondrodysplasia punctata (RCDP), which is a severe debilitating disease characterized by very low levels of tissue and blood plasmalogens [23].

A fatty alcohol (16:0, 18:0 or 18:1) is connected to a glycerol backbone at the sn-1 position of plasmalogens through a vinyl-ether bond. Polyunsaturated fatty acids docosahexaenoic acid (DHA, 22:6, n-3), eicosapentaenoic acid (EPA, 20:5, n-3), arachidonic acid (20:4, n-6), and monounsaturated fatty acid oleic acid (18:1, n-9) are the major constituent fatty acids at the sn-2 position of plasmalogens [22]. These fatty acids are connected through an acyl bond to the glycerol backbone. Polyunsaturated fatty acid–containing fatty acids (at the sn-2 position) are commonly found in gray matter of the brain whereas oleic acid is a major constituent of plasmalogens in the white matter [24]. A phosphoethanolamine group is bound to the glycerol backbone at the sn-3 position. Structurally, phosphatidylethanolamine (PtdEtn) molecules are similar to plasmalogens except for the vinyl-ether bond at sn-1. In PtdEtn, an acyl bond replaces the vinyl-ether bond at sn-1, altering the geometry of the molecule considerably. PtdEtn and PlsEtn appear to be in equilibrium with each other with the equilibrium shifting toward PtdEtn during PlsEtn deficiency [25], potentially replacing membrane PlsEtn with PtdEtn altering the membrane structure due to change in geometry.

Oxidative cleavage of the vinyl-ether bond by cytochrome C in the presence of H_2O_2 as demonstrated by Jenkins et al [26], is a potential mechanism for plasmalogen degradation. Negatively charged lipids such as cardiolipin and phosphatidylserine promote oxidative cleavage of the vinyl-ether bond by cytochrome C [26]. Elevation of

peroxisomal H_2O_2 with aging, diseases and chronic exposure to xenobiotics compromises peroxisomal function [27]. Reduced peroxisomal function coupled with higher levels of H_2O_2 potentially causes permanent plasmalogen deficiency that lead to membrane changes, signaling abnormalities, neurotransmission deficits, and lowering antioxidant defenses [22]. Oxidative stress associated with inflammation potentially exacerbates plasmalogen degradation by attacking the vinyl-ether bond, further reducing the anti-inflammatory and antioxidative capacity of the tissues initiating an irrevocable vicious cycle that progress to pathological abnormalities [24].

Lipid peroxidation products derived from plasmalogens increase from about 70 years of age indicating oxidative degradation of plasmalogens increasing with age [28]. Consequently, Jenkins et al proposed cytochrome c–mediated degradation of plasmalogens due to increased oxidative stress as a potential mechanism responsible for the decrease in plasmalogens and resultant cognitive abnormalities in patients with AD [26]. Elevated levels of plasmalogen peroxides relative to plasmalogens in aging brains and in AD-affected brains [28] provide further evidence on the significance of the maintenance of plasmalogens in intact state in the brain.

Severity-dependent increase in very-long-chain saturated fatty acids (C24:0 and C26:0), which are metabolized by peroxisomes, in AD-affected brains compared with controls further indicate peroxisomal dysfunction in AD [29]. Loss of plasmalogens in AD might also be related to oxidative stress because the vinyl-ether bond is susceptible to reactive oxygen species. Thus, deficiency of plasmalogens in AD brains might be a consequence of reduced synthesis (due to peroxisomal dysfunction) or enhanced degradation due to cytochrome C activity, oxidative stress, inflammation, and homeostatic conversion to other phospholipids to compensate for membrane deficits as described in the next section.

5. Convergence of cholinergic and amyloid hypotheses of AD through brain phospholipids

The cholinergic hypothesis of AD postulates that the loss of basal forebrain neurons which utilize acetylcholine as a neurotransmitter contributes to cognitive decline in AD [30]. Moreover, the autocannibalism theory of AD [31] is based on the tendency of these cholinergic neurons to use choline phospholipids both as a membrane constituent and as a source of free choline to synthesize acetylcholine. When choline levels in the extracellular fluid are too low to sustain acetylcholine synthesis, cholinergic neurons are thought to cannibalize their membrane choline phospholipids to generate choline for acetylcholine synthesis [31]. Consequently, when choline phospholipids cannot be regenerated through the canonical pathway that requires choline, ethanolamine phospholipids are used to generate choline phospholipids through the PtdEtn N-methyltransferase pathway, depleting ethanolamine phospholipids as well [32]. As mentioned previously, more than half of ethanolamine phospholipids in the brain are PlsEtn. Replenishment of PlsEtn becomes particularly problematic when peroxisomal function gets compromised because of aging [22], since critical steps of plasmalogen synthesis take place in the peroxisomes [16]. As a result of these phenomena, integrity of the neuronal membranes gets compromised leading to the loss of neuronal viability [18,31].

Interestingly, phospholipids are linked not only to cholinergic hypotheses of AD causation, and autocannibalism theory, but also to amyloid pathology. For example, enrichment of membranes with polyunsaturated fatty acids [11] and plasmalogens have been shown to increase soluble APPα (sAPPα) secretion [19]. This effect is thought to be due to increased membrane fluidity brought about by conformational changes in the membranes [11]. Conversely, peroxisomal dysfunction, which result in reduced plasmalogen biosynthesis, may be triggered by increased Aβ levels as observed in AD, initiating a vicious cycle [33,34].

6. ApoE isoforms and phospholipids

Emerging evidence also suggests a link between phospholipids and apoE lipoproteins. The *APOE ε4* allele is the second strongest risk factor after aging for AD [35]. *APOE* codes for apolipoprotein E (apoE), which is a major carrier of cholesterol in the circulation as well as in the brain [36]. ApoE ε4 isoform modulates AD pathogenesis potentially through its effects on cholesterol metabolism and Aβ deposition [37,38]. Types of lipids in the surface of the apoE-bearing high-density lipoprotein (HDL)-like particles govern cellular cholesterol efflux mediated by scavenger receptor class B type I [39]. Cholesterol efflux capacity of HDL-like particles is proportional to their phospholipid content [40]. Moreover, the single amino acid difference coded by *APOE ε2, ε3* and *ε4* alleles affects the structures of apoE isoforms and influence their ability to bind lipids, receptors, and Aβ [37]. Aβ accumulation in human brain follows the pattern of *APOE ε4* > *APOE ε3* > *APOE ε2* [41] and Aβ clearance is promoted by lipidation of apoE [38]. In addition to preventing Aβ oligomer formation and inducing Aβ degradation, lipidated apoE promotes Aβ efflux via blood-brain barrier and might enhance peripheral clearance through apoE-containing HDL particles [38].

These data strongly suggest a potential role for disrupted phospholipid metabolism in AD pathogenesis.

7. Epidemiological evidence supporting the association of phospholipids with AD

The notion that derangement of phospholipid metabolism as an upstream event in AD pathophysiology is supported by epidemiological evidence as well. We have demonstrated earlier that deficiency of PlsEtn is associated with cognitive impairment and AD [18], and that lower baseline PlsEtn levels are associated with a higher rate of cognitive decline

in patients with AD [20]. Dramatic reductions of white matter PlsEtn in AD brains and correlation of gray matter PlsEtn with Clinical Dementia Rating have also been demonstrated [42]. A recent report indicated that changes in serum levels of 10 phospholipids including phosphatidyl inositols were predictive of phenoconversion to either amnestic MCI or early AD in 2–3-year interval [43]. In addition to phospholipids, other lipids such as ceramides and sulfatides have also been found to be perturbed in preclinical stages of AD [44].

8. Association of plasmalogens with AD and cognitive dysfunction

In recent studies, we focused on the associations of PlsEtn on AD and obtained preliminary data supporting the hypothesis that specific phospholipid species are linked to AD pathogenesis. We studied five independent populations comprising 400 clinically demented and 350 nondemented subjects and observed significant reductions in serum PlsEtn levels in dementia of the Alzheimer's type (DAT) subjects, compared with nondemented controls. The severity of this decrease correlated with the severity of dementia. This effect was seen both in mono- and di-unsaturated PlsEtn species (found mostly in white matter) and polyunsaturated PlsEtn species (found mostly in gray matter). However, corresponding PtdEtn species were not affected even in severely demented subjects highlighting the specific nature of PlsEtn association with dementia. In the same study, a linear regression model predicted that serum PlsEtn levels decrease years before clinical symptoms [18].

In a subsequent study, we explored whether AD patients with depleted PlsEtn levels show greater decline in Alzheimer Disease Assessment Scale–Cognitive (ADAS-COG) scores compared with age-matched controls. We observed that over a period of one year, there was a significantly greater ADAS-COG decline in AD patients having mild, moderate or severe depletion of baseline serum PlsEtn (75%, 50% or 25% serum levels respectively compared to cognitively normal controls). There was no ADAS-COG decline in patients with AD having normal PlsEtn levels [20].

We then examined the cross-sectional association of serum PlsEtn levels and *APOE* genotype with odds of being diagnosed with AD, in older persons (n = 1255). In stratified analyses, the association of PlsEtn levels with the odds of AD differed by *APOE* genotype (*P*'s < .001). In *APOE ε3/ε3* persons, AD OR was 0.08 and in *ε3/ε4* persons AD OR was 0.11 for higher plasmalogen levels (*P*'s < .01). By contrast, no effect was observed in *APOE ε2/ε3* persons. These results imply that higher serum PlsEtn levels are associated with lower odds of AD irrespective of the *APOE* allele status, which is a significant finding suggestive of possible modulation of *APOE* allele effects by serum PlsEtn levels [45].

We also examined PlsEtn levels, amyloid, tangles, *APOE* genotype, and mRNA transcripts for enzyme, and transport proteins involved in PlsEtn synthesis in the temporal cortex of 100 subjects with premortem cognitive assessments. After adjusting for demographic variables (age, education, gender), the key variables associated with cognition were: brain DHA-PlsEtn levels (Coef: 0.620, P = 8.7E-09, r^2 = 0.329, n = 99), tangles (Coef: −0.552, P = 4.0E-06, r^2 = 0.267, n = 99), amyloid (Coef: −0.394, P = 1.1E-04, r^2 = 0.150, n = 99), *APOE ε4* (Coef: −0.369, P = 4.2E-03, r^2 = 0.131, n = 91), dihydroxyacetone phosphate alkyltransferase (DHAPAT, Coef: 0.307, P = 2.7E-02, r^2 = 0.109, n = 94). Neither amyloid nor *APOE ε4* was associated with cognition in combined models of these variables where brain DHA-PlsEtn level, tangle density and age showed independent associations with cognition. However, *APOE ε4* was the dominant variable associated with amyloid (Coef: 1.185, P = 5.3E-07, r^2 = 0.297, n = 91). Overall, 46% of the variance in cognition was associated with DHA-PlsEtn, tangles, DHAPAT, and age. Amyloid (Coef: −0.025 P = 1.4E-03, r^2 = 0.084, n = 99), *APOE ε4* (Coef: −0.044, P = 2.1E-02, r^2 = 0.081, n = 92), and tangles (Coef: −0.044, P = 1.1E-04, r^2 = 0.153, n = 100) were associated with DHA-PlsEtn levels. Therefore, of the variables investigated, temporal cortex DHA-PlsEtn level exhibited the strongest association with cognition. Only tangles and age were associated with cognition independent of brain DHA-PlsEtn levels [46].

The association between cognition, *APOE* genotype, premortem serum PlsEtn/PtdEtn index [ratios of serum PlsEtn and PtdEtn species bearing DHA (22:6), eicosapentaenoic acid (EPA, 20:5) and adrenic acid (22:4)], and postmortem (1.6 ± 1.6 years after premortem assay) brain amyloid and tangle density and cognition in elderly persons (n = 1743) was investigated. In participants who became deceased (n = 847), higher premortem PlsEtn and PtdEtn index (coef = 0.195, P < .001) and an *APOE ε2ε3* genotype (relative to ε3ε3; coef = 0.320, P = .002) were associated with higher cognition and a *APOE ε3ε4/ε4ε4* genotype (relative to ε3ε3; coef = −0.446, P < .001) was associated with lower cognition. Higher brain tangle density (Coef = −0.365, P < .001) was observed to be associated with lower cognition and higher PlsEtn/PtdEtn index with higher cognition (Coef = 0.180, P < .001). *APOE* genotype and postmortem brain amyloid density was not associated with cognition after adjusting for tangles and PlsEtn/PtdEtn index levels [47].

9. Correction of plasmalogen deficiency using alkyl glycerols

Critical steps in plasmalogen synthesis take place in peroxisomes [16]. The decline in peroxisomal function in aging as described previously is therefore considered responsible for plasmalogen depletion [22]. Plasmalogen replacement may therefore slow disease progression in AD [22]. However, dietary sources for plasmalogens are rare [22]. Because peroxisome function is impaired in AD, plasmalogen precursors that bypass the peroxisomal steps of plasmalogen synthesis are required to achieve plasmalogen replacement

[48]. Plasmalogen precursors must also be designed to resist oxidative degradation of the vinyl-ether bond of plasmalogens [48]. 1-0-Alkylglycerols such as chimyl and batyl alcohol can enter the plasmalogen synthetic pathway bypassing the peroxisomal steps [13,48]. These ether lipids are also more stable than vinyl-ether bond containing plasmalogens. However, doses as high as 4 g/kg of 1-0-alkylglycerol were given orally over 4 months to mice with genetic mutations that inactivate peroxisomal function; brain plasmalogen levels were only restored to 2.9% of wild-type mice [48]. A study was conducted using a series of 1-acyl 2-acyl and 1-0-alkyl 2-acyl glycerol compounds bearing various fatty acids at sn-1, sn-2, and sn-3 positions to determine the efficiency of bioconversion of these molecules to plasmalogen [49]. These compounds were tested using a plasmalogen-deficient cell line (NRel-4) which lack the critical peroxisomal enzyme DHAPAT and the results demonstrated the ability of alkyl compounds to restore PlsEtn in sn-1-specific manner [49]. Unlike sn-1 fatty acids, sn-2 fatty acids in these molecules undergo remodeling in vivo elevating all major PlsEtn species with various sn-2 configurations. Molecules without an ether bond at sn-1 (acyl species) did not increase plasmalogen levels in NRel-4 cells [49]. When a synthetic ether lipid plasmalogen precursor with DHA at sn-2 and lipoic acid at sn-3 was given orally to rabbits at a dose of 200 mg/kg, a time-dependent increase in the bioconversion of the precursor to circulating PlsEtn was observed [50]. Deacylation at sn-2 released DHA, with maximal plasma DHA levels at 6 hours. The greatest incorporation was into PlsEtn 16:0/22:6, 18:0/22:6, and 18:1/22:6 [50].

Similar results were observed with an oral dose of 100 mg/kg of ^{13}C labeled precursor. Deacylation of the precursor at sn-2 released [$^{13}C_3$] DHA, with plasma [$^{13}C_3$] DHA levels constituting 40% of the plasma free DHA pool at 6 hours. Fully labeled form of the precursor (glycerol-palmitic acid-DHA) as well as DHA-only labeling (at sn-2) was observed for PlsEtn species with 16:0 at sn-1. Only DHA was labeled (at sn-2) in PlsEtn species with 18:0 and 18:1 at sn-1, which demonstrate remodeling at sn-2 and conservation of sn-1 configuration. Dose-dependent increases in plasma and tissue levels of PlsEtn and elevated levels in plasma and tissues after repeated dosing were also observed. Results of these experiments provide proof of the principle that ether lipid precursors having unsaturated fatty acids at sn-2 are absorbed and metabolized to PlsEtn bypassing the peroxisomal pathway [50].

These results were further confirmed by oral supplementation of stable-isotope labeled 1-0 alkyl acyl plasmalogen precursor to plasmalogen-deficient PEX7-/- mice [51]. Incorporation of the label at 4% (relative to the unlabeled plasmalogen) was observed in the brain when these plasmalogen precursors were given orally at a dose of 100 mg/kg/day for 3 days [51]. This dose achieved comparable plasmalogen restoration as that produced by a much higher dose of 4 g/kg of the alkyl glycerol-based compounds [50,51].

10. Supplementation of ether-lipid precursor in animal models of disease

A 1-alkyl-2-acyl-glycerol plasmalogen precursor was then tested for therapeutic efficacy in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse and monkey models of PD. Parkinsonian MPTP moneys were treated with L-Dopa to induce dyskinesia (LID) and given the vehicle, DHA or DHA-containing plasmalogen precursor in a crossover manner with washout periods in between. Significant reductions of LID were seen in MPTP monkeys treated with DHA (100 mg/Kg) or plasmalogen precursor (50 mg/kg) [52]. However, plasmalogen precursor elicited a beneficial response earlier than the DHA treatment. LID scores were inversely correlated with serum DHA-PlsEtn/total PlsEtn ratios levels in DHA and plasmalogen precursor treated monkeys.

In another study, pretreatment with a plasmalogen precursor (10, 50, and 200 mg/kg or with vehicle daily for 10 days) followed by MPTP administration on day 5 prevented MPTP-induced striatal dopamine loss in precursor-treated (10 and 50 mg/kg doses) MPTP mice [53]. MPTP treatment reduced Dopamine Transporter and vesicular monoamine transporter 2 ligand binding. These reductions were prevented by precursor treatment, indicating neuroprotection.

11. Mechanistic studies using human brain samples

Human brains affected by AD were found to be deficient in plasmalogen choline and had lower γ-secretase activity ex vivo. Incubation of membrane preparations from these postmortem human brains with plasmalogen choline enhanced their γ-secretase activity [33]. These data provide direct evidence on the effect of ex vivo plasmalogen supplementation on AD-related pathological process. Enhancement of cholesterol biosynthesis and attenuation of reverse cholesterol transport because of plasmalogen deficiency [54], and the influence of cholesterol on the generation of Aβ [55] further highlight the possible consequences of plasmalogen deficiency on the AD pathological processes.

Despite overwhelming evidence on the association of PlsEtn with neurodegeneration and AD [18,20,42,56,57], exact mechanisms of this association or direct effects of PlsEtn on AD-related mechanisms and pathology such as vesicular release [58], membrane composition [59], brain volume [60] and cell loss [61] are not yet known. However, studies described earlier such as prevention of striatal dopamine loss and improved ligand binding of DAT and vesicular monoamine transporter-2 provide preliminary evidence on mechanisms of action.

Nevertheless, an accurate understanding of the mechanisms of action and any modulatory effects on specific pathological processes is crucial to identify the time in the pathological cascade that PlsEtn augmentation will be effective. Experimental and epidemiological data to date indicate that intervention with PlsEtn will be effective potentially early in the disease process. Longitudinal and mechanistic information will help select the appropriate patient

subgroups, the best phase (preclinical/prodromal/clinical) to start treatment and to determine the specific molecules that can be used for diagnosis or as surrogate markers of efficacy. Improvement of bioavailability, enhanced blood-brain barrier penetration, and knowledge about configuration of fatty acids in PlsEtn needed for restoration of plasmalogens for specific tissues will also be needed to improve efficacy.

12. Human intervention studies

A multicenter, randomized, double-blind, placebo-controlled trial was conducted to evaluate the memory-improving efficacy of scallop-derived plasmalogens in patients with mild AD and MCI. They did not observe a significant elevation of plasma levels of plasmalogens in the treated group relative to the baseline. Although red blood cell plasmalogen levels were elevated in the treated group, similar elevations were also seen in the placebo group. Comparatively, lower dose of plasmalogens (1 mg twice daily) and the labile nature of the vinyl-ether bond might have limited the absorption of the intact molecule and might have contributed to the lack of response in terms of plasmalogen levels in the blood as well as the cognitive function (Mini Mental State Exam-Japanese version or Welchsler Memory Scale-Revised), except for female patients with mild AD given plasmalogen supplementation who showed a significant improvement in Welchsler Memory Scale-Revised compared with the placebo group. The authors suggested that studies with longer duration and observation of converters from MCI to AD might be needed to evaluate the efficacy of plasmalogen supplementation because the study duration of the study might be too short [62]. The dose of plasmalogens used in this study (2 mg/day, which amounts to about 0.03 mg/kg) is an inadequate dosage in our view for elevating tissue levels. Besides, reported instability of plasmalogens in acidic environments [63] questions the stability of preformed plasmalogens in the gastric juice during digestion which might reduce plasmalogen bioavailability.

13. Cause or consequence?

To understand whether the association of plasmalogen deficiency with AD is a cause or consequence, we need to eliminate the possibility of spurious associations and reverse causality. If we follow Bradford Hill criteria to establish causation, the first step of establishing causation is the demonstration of the strength of the association of exposure and disease and consistence of this association [64]. As described in previous sections, the association of plasmalogen deficiency in the brain with AD has been amply demonstrated with large effect sizes and consistency in epidemiological studies. Although plasmalogen deficiency is consistently demonstrated in AD, deficiency of plasmalogens is seen in other neurodegenerative diseases as well, lowering the specificity of its relationship to AD.

Preliminary results of a longitudinal study presented by us at the Alzheimer's Association International Conference in 2015 indicated that age at the baseline (OR = 1.52, $P < .001$), ApoE ε3ε4/ε4ε4 genotype (relative to ε3ε3; OR = 1.30, $P = .006$), and a decrease in plasmalogen index (plasmalogen to phosphatidyl ratio) from the baseline (OR = 1.45, $P = .002$) were associated with a higher odds of being a converter (from NCI to MCI/AD or MCI to AD) and a higher plasmalogen index at the baseline (OR = 0.63, $P < .001$) were associated with a lower odds of being a converter [46], providing indirect evidence for temporality and the presence of a biological gradient between the exposure and the effect. Data presented in sections 4, 9, and 10 support biological plausibility, coherence, and experimental evidence on the causation of neurodegenerative and to a lesser extent AD pathology, by plasmalogen deficiency. Brites et al. demonstrated that plasmalogen levels significantly increased in Pex7 knockout (KO) mice exhibiting plasmalogen deficiency fed alkyl glycerol precursor compared with control fed KO mice. Alkyl glycerol precursor arrested the progression of the pathology in testis, adipose tissue and the Harderian gland in KO mice. Furthermore, nerve conduction in peripheral nerves was also improved by precursor treatment demonstrating that when given before the occurrence pathology, the alkyl glycerol precursor ameliorated the pathology observed in Pex7 KO mice [48], providing experimental evidence on the causal association of plasmalogens and neurodegeneration. Severe neurological diseases caused by plasmalogen deficiency such as RCDP is analogous situation that demonstrate neurodegenerative effects of plasmalogen deficiency. Myelination deficits, enlarged ventricles, and subarachnoidal spaces, and cerebellar atrophy are the main neurological abnormalities described in RCDP [65]. Cerebellar atrophy is considered to be due to loss of Purkinje cells [65]. Therefore, plasmalogen deficiency appears to meet Bradford Hill criteria for causal association with neurodegeneration to a considerable extent, but not for AD causation. Therefore, we believe that plasmalogen deficiency increases the susceptibility to neurodegeneration, and because AD is a multifactorial disease, other risk factors trigger the disease process in a brain made susceptible by plasmalogen deficiency. The AD pathological process in turn might also exacerbate plasmalogen deficiency [24], initiating a vicious cycle.

14. Conclusions

Although sporadic, there appear to be strong evidence supporting possible causative association between neuropathology and relative plasmalogen deficiency. Nevertheless, it appears that rather than being directly causative, these phospholipid abnormalities possibly interact with other pathophysiological mechanisms to precipitate neurodegeneration in AD.

The role of fatty acids such as DHA and EPA in AD pathogenesis is also clarified to a certain extent by the foregoing data. The association of composite indices comprising of various ratios of PlsEtn and PtdEtn species bearing polyunsaturated fatty acids at sn-2 position (DHA, adrenic acid, and EPA) with cognitive scores and AD diagnosis appear to confirm early

studies on the association of these fatty acids with AD and might explain why interventional studies using these fatty acid preparations (vs. phospholipids bearing these fatty acids) failed [66,67]. It seems more likely that rather than the specific fatty acid such as DHA or EPA, the specific molecular configuration of the phospholipids these fatty acids are associated with is more important than fatty acids itself.

15. Next steps and future directions

Most of the data described here come from cross-sectional studies which limit their applicability unless verified longitudinally. Therefore, phospholipid species linked with neurodegeneration need to be further validated using longitudinal studies to conclusively identify biochemical systems associated with preclinical AD and neurodegeneration. Specific metabolites thus identified can be optimized to serve as biomarkers to identify individuals in the preclinical phase of the disease. Community-based trials that monitor older individuals longitudinally over a period of several years will provide better external validity for validation and optimization of these biomarkers [68]. Long-term interventional trials with biological precursors of these molecules should be carried out to investigate the preventive/disease modifying ability of these biological precursors.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional sources such as PubMed, meeting abstracts, and presentations. While causation of Alzheimer's disease (AD) and related neurological diseases is not known and therapies based on lowering β-amyloid have failed, emerging data are suggestive of a link between neuropathology and phospholipid abnormalities in the brain.
2. Interpretation: Collective evidence from literature and our own data steered us toward an integrated hypothesis that link neurodegeneration, amyloid pathology, and phospholipid abnormalities to AD pathogenesis, which could possibly be exploited for early diagnosis and prevention.
3. Future directions: This article provides a framework for a testable new paradigm on AD causation and AD risk reduction. Longitudinal studies that look into lipid abnormalities in the preclinical stages of AD would help confirm this hypothesis. A similar approach should be taken for other intractable neurodegenerative diseases which appear to have similar lipid abnormalities in the preclinical stages.

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