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Lysophosphatidic acid induces increased BACE1 expression and Aβ formation

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ABSTRACT

The abnormal production and accumulation of β-amyloid peptide (Aβ), which is produced from amyloid precursor protein (APP) by the sequential actions of β-secretase and γ-secretase, are thought to be the initial causative events in the development of Alzheimer's disease (AD). Accumulating evidence suggests that vascular factors play an important role in the pathogenesis of AD. Specifically, studies have suggested that one vascular factor in particular, oxidized low density lipoprotein (oxLDL), may play an important role in regulating Aβ formation in AD. However, the mechanism by which oxLDL modulates Aβ formation remains elusive. In this study, we report several new findings that provide biochemical evidence suggesting that the cardiovascular risk factor oxLDL may contribute to Alzheimer's disease by increasing Aβ production. First, we found that lysophosphatidic acid (LPA), the most bioactive component of oxLDL induces increased production of Aβ. Second, our data strongly indicate that LPA induces increased Aβ production via upregulating β-secretase expression. Third, our data strongly support the notion that different isoforms of protein kinase C (PKC) may play different roles in regulating APP processing. Specifically, most PKC members, such as PKCα, PKCβ, and PKCε, are implicated in regulating α-secretase-mediated APP processing; however, PKCδ, a member of the novel PKC subfamily, is involved in LPA-induced upregulation of β-secretase expression and Aβ production. These findings may contribute to a better understanding of the mechanisms by which the cardiovascular risk factor oxLDL is involved in Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is characterized clinically by progressive dementia, including loss of learning and memory. Pathologically, it is characterized by degeneration of neurons and abnormal protein structures, including intracellular deposition of neurofibrillary tangles (NFT) that are composed of the microtubule-associated protein TAU, and extracellular deposits of various types of β amyloid (Aβ), which is a mix of peptides that are 39 to 43 amino acids in length. Several lines of evidence support the hypothesis that progressive accumulation of Aβ is an early and critical event in the pathogenesis of AD [1]. Studies have revealed that the accumulation of Aβ initiates a series of downstream neurotoxic events, including synaptic failure [2] and hyperphosphorylation of TAU, which results in neuronal dysfunction and death [3]. Aβ is proteolytically derived from a large amyloid precursor protein (APP) by β-secretase, which produces the N-terminus of Aβ, and γ-secretase, which produces the C-terminus of Aβ [4]. Thus, it is clear that β- and γ-secretases are the key enzymes in the production of Aβ. Augmented expression of any of these two secretases or APP itself would result in increased production of Aβ. β-Secretase (also known as β-site APP cleaving enzyme 1 [BACE1]) has been identified as a type I membrane aspartyl protease [5–8]. γ-Secretase is a complex composed of at least four subunits, namely, presenilin (PS1 or PS2), nicastrin, Aph-1, and Pen-2, of which presenilin may be the putative catalytic subunit [9].

Recent progress in AD etiology studies suggests that vascular factors also play an important role in the pathogenesis of AD [10–12]. Specifically, it has been reported that one vascular factor in particular, oxidized low density lipoprotein (oxLDL), may play an important role in neuronal cell death in AD [13]. In this regard, post-mortem analyses revealed that the overall level of oxidative damage to proteins and lipids is elevated in AD [10,14], and specifically, cerebrospinal fluid lipoproteins are more vulnerable to oxidation in AD and are neurotoxic when oxidized [15]. In this regard, it is also notable that a recent study revealed a positive correlation between the cerebrospinal fluid (CSF) levels of Aβ and oxLDL in AD patients, suggesting that oxLDL may contribute to AD by manipulating Aβ production [16]. Studies have further shown that lysophosphatidic acid (LPA), the major bioactive component of oxLDL, can disrupt blood–brain barrier function and cause AD-related cellular events; for review, see [17]. Interestingly, in an effort to study the pathogenetic effect of this bioactive component

Abbreviations: AD, Alzheimer's disease; NFT, neurofibrillary tangles; Aβ, β amyloid; APP, amyloid precursor protein; BACE1, β-site APP cleaving enzyme 1; oxLDL, oxidized low-density lipoprotein; CSF, cerebrospinal fluid; LPA, lysophosphatidic acid; CREB, cAMP response element-binding protein; APPsw, Swedish mutant APP; PS1wt, wild type presenilin 1; CM, conditioned medium; SRF, serum response element; CRE, cAMP-responsive element; PTX, pertussis toxin; wt, wild type; DN, dominant negative; lysoPLD, lysophospholipase D

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of oxLDL, we found that LPA can enhance Aβ production in a cultured cell system. Furthermore, our data also demonstrate that LPA induces increased expression of β-secretase (BACE1), suggesting that LPA causes an increase in Aβ production by up-regulating β-secretase expression. This hypothesis is further supported by our finding that LPA induces the activation of the MAPK signaling pathway, which is known to mediate HNE- and LPA-induced gene expression [18,19]. In addition, we also found that LPA markedly induces activation (phosphorylation) of the transcription factor cAMP response element-binding protein (CREB) and induces CREB binding activity. Notably, CREB is one of the possible transcription regulators of the BACE1 gene [20]. Thus, our novel finding that components of oxLDL cause increased production of Aβ opens a new avenue of investigation into the mechanisms by which oxLDL contributes to the development of AD.

2. Materials and methods

2.1. General reagents

Aβ40 and Aβ42 were purchased from American Peptide (Sunnyvale, CA, USA). Monoclonal antibody 6E10 against Aβ and polyclonal antibodies against Aph1α and Pen- 2 were purchased from COVANCE (Dedham, MA, USA). Polyclonal antibody anti-nicastrin (NCT) was purchased from Sigma (St. Louis, MO, USA). Polyclonal antibodies Anti-PS1N raised against residues 27–50 of PS1 and C15 raised against the C-terminal 15 residues of human APP have been described previously [21,22]. The polyclonal antibodies against phospho-MEK, phospho-ERK, phospho-p90RSK, phospho-CREB, phospho-ATF2, phospho-PKCα (Ser657), and phospho-PKCδ (Tyr311) were from Cell Signaling Technology (Danvers, MA, USA). Polyclonal antibodies against proteins of PKCα and PKCδ and monoclonal antibody against β-actin were from BD Biosciences (San Jose, CA, USA). Anti-BACE1 antibody was from Abcam (Cambridge, MA, USA). LPA (1-oleoyl-2-hydroxy-sn-glycero- 3-phosphate) was from Avanti Polar Lipids (Alabaster, AL, USA). Pertussis toxin (PTX), U0126, and GF109203X were from Biomol International (Plymouth Meeting, PA, USA). Protein-A agarose beads and ECL-Plus Western blotting reagents were purchased from Amersham Biosciences (Piscataway, NJ, USA). Phosphate buffer was from Sigma (St. Louis, MO, USA). TRIzol reagent was from Invitrogen (Carlsbad, CA, USA). dCTP-^{32}P was from MP Biochemicals (Solon, OH, USA), and DNA labeling kit was from GE Health Care (Piscataway, NJ, USA).

2.2. Cell culture and treatment

The mouse neuroblastoma N2a cell line (WT-7) stably expressing wild type presenilin 1 (PS1wt) and Swedish mutant APP (APPsw) were kindly provided by Drs. Sangram S. Sisodia and Seong-Hun Kim (University of Chicago) and were maintained in DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% L-glutamine as described previously [23]. For treatment with LPA, cells were starved for 24 h in serum-free DMEM and then treated with LPA in fresh serum-free medium at different concentrations or for a different length of time as indicated in the each related experiment. Cells were treated with PTX overnight and kinase inhibitors for 30 min prior to addition of LPA to examine their effects on the LPA-induced response.

2.3. Immunoprecipitation and Western blot analysis

Immunoprecipitation and Western blot analysis were carried out as described previously [21]. Secreted Aβ was immunoprecipitated from conditioned medium (CM) using a monoclonal Aβ-specific antibody 6E10 and Protein A beads. The immunoprecipitated Aβ peptides were analyzed by 13% urea (8 M) SDS–PAGE followed by Western blotting using 6E10. For detection of other proteins, cells were lysed in Western blotting lysis buffer (50 mM Tris–HCl, pH 6.8, 8 M urea, 5% β-mercaptoethanol, 2% SDS, and protease inhibitor) and separated by Tris-glycine SDS–PAGE. After being transferred to a polyvinylidene fluoride membrane (Immobilon-P, Millipore, Billerica, MA, USA), the blots were probed with specific antibodies, and the immunoreactive bands were visualized using the ECL-Plus reagent.

2.4. Northern blot analysis

Total RNA of the cells was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and subjected to electrophoresis in formaldehyde-agarose gels. RNA was transferred onto nylon membranes (Amersham Biosciences) and hybridized with radiolabeled cDNA probes as described previously [24]. A [α-^{32}P] dCTP-labeled 685-bp fragment of mouse BACE1 cDNA was used as a probe to detect mouse BACE mRNA.

2.5. Electrophoretic mobility shift assays

Oligonucleotides AGACCATGTAGT**TGAGGTCA**ATGAAGGGGT, AACGG GCC**GGGGGGTGGT**GGCACACGCCTT, GGTGTTAACCAATG**CTGATTCAG**GAA AGTG, and TGCACTGGCT**CCTGCTATGG**GTGGGCTCGG containing the putative CRE, Sp-1, AP-1, and SRE sites of the mouse BACE1 promoter, respectively, were radiolabeled using [γ-^{32}P] dCTP. Electrophoretic mobility shift assays (EMSAs) were performed as described previously [24]. All results presented in this study are representative of at least three experiments.

2.6. Data analysis

All Western blots shown are representative of a minimum of three independent experiments. For statistical analysis, the density of each band was quantified using the Gel Digitizing Software UN-SCAN-IT (Silk Scientific, Orem, UT, USA). The ratios of protein levels between treated samples and controls are expressed as mean ± SEM, $n = 3$. Comparisons between multiple groups were performed by using a one-way ANOVA with Dunnett posthoc t tests. A single comparison analysis was made using two-tailed unpaired Student t tests. For ANOVA or t tests, p values of ≤ 0.05 or ≤ 0.01 were considered to be statistically significant. Single and double asterisks indicate significant differences between control and treatment at $p < 0.05$ and 0.01, respectively.

3. Results

3.1. LPA, a bioactive component of oxLDL, induced an increase in Aβ production

A recent study has shown a positive correlation between CSF levels of Aβ and oxLDL in AD patients, suggesting oxLDL may have effect on Aβ production [16]. Studies have further shown that LPA, the major bioactive component of oxLDL, can disrupt blood–brain barrier function and cause AD-related cellular events [17]. These observations prompted us to determine the effect of LPA on the production of Aβ. N2a neuroblastoma cells, which stably express PS1wt and myc-tagged APPsw, have been used in many previous studies for investigating the mechanism of Aβ production [21,23,25]. These cells were starved in a serum-free medium for 24 h and then treated with LPA. The secreted Aβ was immunoprecipitated from CM with 6E10 (COVANCE), a monoclonal antibody that recognizes residues 1–17 of Aβ [26], and analyzed by urea (8 M) SDS–PAGE (13%) followed by Western blot using 6E10. As shown in Fig. 1, LPA remarkably induced Aβ production in a dose-dependent manner (top panel), and a peak level of Aβ was reached at 25 μM LPA.