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Deregulation of sphingolipid metabolism in Alzheimer's disease

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Abstract

Abnormal sphingolipid metabolism has been previously reported in Alzheimer's disease (AD). To extend these findings, several sphingolipids and sphingolipid hydrolases were analyzed in brain samples from AD patients and age-matched normal individuals. We found a pattern of elevated acid sphingomyelinase (ASM) and acid ceramidase (AC) expression in AD, leading to a reduction in sphingomyelin and elevation of ceramide. More sphingosine also was found in the AD brains, although sphingosine-1-phosphate (S1P) levels were reduced. Notably, significant correlations were observed between the brain ASM and S1P levels and the levels of amyloid beta (Aβ) peptide and hyperphosphorylated tau protein. Based on these findings, neuronal cell cultures were treated with Aβ oligomers, which were found to activate ASM, increase ceramide, and induce apoptosis. Pre-treatment of the neurons with purified, recombinant AC prevented the cells from undergoing Aβ-induced apoptosis. We propose that ASM activation is an important pathological event leading to AD, perhaps due to Aβ deposition. The downstream consequences of ASM activation are elevated ceramide, activation of ceramidases, and production of sphingosine. The reduced levels of S1P in the AD brain, together with elevated ceramide, likely contribute to the disease pathogenesis.

Keywords: Alzheimer's disease; Human brain; Neurons; Sphingomyelinases; Ceramidases; Sphingomyelin; Ceramide; Sphingosine-1-phosphate

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in adults. It affects ∼10% of the population over 65 years of age, and approaches 50% by age 85. There are about 15 million individuals with AD worldwide. AD is characterized clinically by progressive loss of memory, pathologically by the presence of neuritic plaques and neurofibrillary tangles, and biochemically by the accumulation of amyloid beta (Aβ) peptides and hyperphosphorylated tau proteins (Morishima-Kawashima and Ihara, 2002; Yankner, 1996; Yankner et al., 2007).

Numerous hypotheses have been put forth to describe the molecular mechanisms leading to AD. The five most common hypotheses include: excessive Aβ production, tau protein abnormalities, genetic predisposition (including mutations or polymorphisms in the presenilin 1 and 2, Aβ peptide precursor [APP], and/or apolipoprotein E genes), oxidative stress, and lipid alterations (phospholipids and neutral lipids) (Farooqui et al., 2007; Hartmann et al., 2007; Yankner et al., 2007).

A large body of evidence supports the fact that Aβ plays an important role in AD. *In vitro*, Aβ has been shown to induce apoptosis via the sphingomyelin/ceramide pathway in various brain cells, including human and rat primary neurons (Jana and Pahan, 2004; Ju et al., 2005; Malaplate-Armand et al., 2006), rat oligodendrocytes (Cheng et al., 2003; Lee et al., 2004; Malaplate-Armand et al., 2006; Zeng et al., 2005), rat astrocytes and glial cells (Ayasolla et al., 2004), and murine neuroblastoma cells (Satoi et al., 2005). Calcium-dependent phospholipase A (cPLA) (Malaplate-Armand et

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al., 2006), inducible nitric oxide synthase (iNOS) (Ayasolla et al., 2004; Zeng et al., 2005), the p75 neurotrophin receptor (p75NTR) (Costantini et al., 2005), and NADPH oxidase (Jana and Pahan, 2004) have each been shown to be involved in the Aβ-related activation of the sphingomyelin/ceramide pathway. Tumor necrosis factor-alpha (TNF-α) (Ayasolla et al., 2004; Zeng et al., 2005) and interleukin-6 (Fiebich et al., 1995) also are involved in Aβ-induced apoptosis. *In vivo*, Alessenko and colleagues found that the activation of the sphingomyelin/ceramide pathway lies downstream of the oxidative stress that follows Aβ administration (Alessenko et al., 2004).

Ceramide is the core constituent of most sphingolipids. It can be produced by hydrolysis of sphingomyelin via sphingomyelinases, or synthesized *de novo* from fatty acyl CoA and sphingosine. Sphingomyelin degradation is the probable source of most ceramide in cells (Goni and Alonso, 2002). Ceramide is an important second messenger molecule that regulates diverse cellular processes including cell growth, differentiation, and apoptosis. Ceramide levels also increase in response to aging and various age-related stress factors (e.g., oxidative stress), and are directly involved in apoptotic signaling in various cell types, including neurons (Costantini et al., 2005; Cutler et al., 2004; Kolesnick and Kronke, 1998; Perez et al., 2005). Furthermore, ceramide stabilizes the APP-cleaving enzyme 1 (BACE1), promoting Aβ biogenesis (Patil et al., 2007; Puglielli et al., 2003), and reduction of ceramide levels leads to reduced secretion of APP and Aβ in human neuroblastoma cells (Tamboli et al., 2005). Thus, it has been suggested that ceramide and Aβ may synergize to induce neuronal death in AD.

Several studies have examined the lipid abnormalities in AD brain. For example, the total phospholipid and sulfatide content in AD was decreased as compared to normal (Cheng et al., 2003; Gottfries et al., 1996; Han et al., 2002; Pettegrew et al., 2001; Soderberg et al., 1992), while the ceramide and cholesterol levels were elevated (Cutler et al., 2004; Han et al., 2002). Satoi et al. found that the ceramide levels in the cerebrospinal fluid (CSF) also were increased in patients with AD (Satoi et al., 2005), and we recently reported that the level and activity of acid ceramidase (AC) was elevated as well, perhaps in response to the elevated ceramide (Huang et al., 2004). Herein we investigated the levels of sphingomyelin and several other sphingolipid metabolites in the brains of AD patients, as well as the levels and activities of several lipid-related enzymes. We report for the first time activation of acid sphingomyelinase (ASM), elevation of sphingosine, and reduction of sphingosine-1-phosphate (S1P). The elevated ASM and reduced S1P levels in AD were highly correlated with the levels of Aβ and hyperphosphorylated tau protein. We also found that treatment of neuronal cell cultures with Aβ mimicked these sphingolipid changes and induced apoptosis, which was prevented by pre-treatment with recombinant human AC (rhAC). The therapeutic implications of these findings and the role of ceramide and its metabolites in AD are discussed.

2. Materials and methods

2.1. Chemicals and reagents

Sphingomyelin and ceramide were from Matreya (Pleasant Gap, PA, USA). Sphingosine and S1P were from Avanti Polar Lipids (Alabaster, AL, USA). Bodipy-C12-sphingomyelin, Amplex Red, naphthalene-2,3-dialdehyde (NDA), and a human Aβ42 ELISA (HS) kit were from Invitrogen (Carlsbad, CA, USA). Aβ (40-1) and Aβ (1-42) were obtained from the American Peptide Company (Sunnyvale, CA, USA). Bodipy-C12-ceramide was a gift from Professor Shimon Gatt (Hebrew University-Hadassah School of Medicine, Jerusalem, Israel). High performance liquid chromatography (HPLC)-grade solvents and cell culture materials were from Fisher Scientific (Pittsburgh, PA, USA). A protein assay kit was purchased from Bio-Rad (Hercules, CA, USA). All other biochemical reagents were from the Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Human brain tissue

All of the human brain tissues were obtained from the Harvard Brain Tissue Resource Center (Belmont, MA). Postmortem tissues from nine AD patients (mean age 73.2 ± 10.1 years) and six control individuals (mean age 73.6 ± 8.2 years) were included in this study. The average postmortem delay before tissue collection was 4.7 ± 3.2 h for AD and 5.0 ± 1.2 h for controls. All of the AD brain samples were analyzed by histopathology to confirm the diagnosis of AD. This was further confirmed by determining the levels of Aβ and hyperphosphorylated tau proteins (see below). The use of frozen human brain tissue was in accordance with the National Institutes of Health guidelines and approved by our Institutional Review Boards.

2.3. Cell culture

Neuronal progenitor cells were isolated from the adult rat hippocampus and cultured in neurobasal A medium consisting of 2% B27, 0.5 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 ng/ml FGF at 37 °C in a humidified 5% CO_2 atmosphere (Chen et al., 2007). The media was routinely changed every 2–3 days. When the cells reached ~80% confluency, they were differentiated by replacing FGF with 5 μM retinoic acid and 10% fetal calf serum. The neuronal cultures were used for experiments after 5–7 days of growth in the differentiation medium. At this stage, ~80% of the cells expressed the neuronal markers βIII-tubulin and microtubule-associated protein 2, and less than 5% expressed the astroglial marker GFAP or the oligodendrocyte marker O4. For Aβ treatment of the neuronal cells, stocks of soluble Aβ in distilled water were incubated at room temperature for 5 days and allowed to form oligomers before addition to the cell media. The presence of Aβ oligomers was confirmed by SDS-PAGE and immunoblot analysis.