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Amyloid β 1–42 oligomer inhibits myelin sheet formation in vitro

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Abstract

Accumulating evidence indicates that white matter degeneration contributes to the neural disconnections that underlie Alzheimer's disease pathophysiology. Although this white matter degeneration is partly attributable to axonopathy associated with neuronal degeneration, amyloid β ($A\beta$) protein-mediated damage to oligodendrocytes could be another mechanism. To test this hypothesis, we studied effects of soluble $A\beta$ in oligomeric form on survival and differentiation of cells of the oligodendroglial lineage using highly purified oligodendroglial cultures from rats at different developmental stages. $A\beta$ oligomer at 10 μ M or higher reduced survival of mature oligodendrocytes, whereas oligodendroglial progenitor cells (OPCs) were relatively resistant to the $A\beta$ oligomer-mediated cytotoxicity. Further study revealed that $A\beta$ oligomer even at 1 μ M accelerated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formazan exocytosis in mature oligodendrocytes, and, more significantly, inhibited myelin sheet formation after induction of in vitro differentiation of OPCs. These results imply a novel pathogenetic mechanism underlying $A\beta$ oligomer-mediated white matter degeneration, which could impair myelin maintenance and remyelination by adult OPCs, resulting in accumulating damage to myelinating axons thereby contributing to neural disconnections.

Keywords

Alzheimer's disease; Amyloid β ; Lipid; Myelin; Oligodendroglia; White matter degeneration

1. Introduction

As an essential component of extended neural networks in the brain, white matter is also critical for many higher order cognitive processes including attention, executive functioning, nonverbal/visual-spatial processing, and generalized processing speed (Gunning-Dixon and Raz, 2000), all of which are invariably impaired in Alzheimer's disease (AD). Although the

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Disclosure statement

There are no actual or potential conflicts of interest. Usage of animals was performed in conformity with the protocol approved by University of California, Davis Institutional Animal Care and Use Committee.

AD degenerative process is believed to affect mostly neurons and their associated constituents in the gray matter, recent neurobiological and imaging studies find evidence of diffuse white matter pathology from AD in both animal models and human subjects (Cho et al., 2008; Kavcic et al., 2008; Nakata et al., 2009; Stokin et al., 2005; Zhou et al., 2008). In humans, diffusion tensor imaging, a new noninvasive magnetic resonance imaging technique highly sensitive to white matter microstructural changes, has revealed white matter abnormalities particularly in the posterior regions of not only AD patients but also those with mild cognitive impairment (MCI), an early stage of clinical AD (Chua et al., 2008). Postmortem studies of AD white matter find loss and/or abnormalities of myelin, loss of glial cells (especially oligodendrocytes), and axonopathy (Barber et al., 1999; Stokin et al., 2005). Although amyloid β ($A\beta$) protein aggregation is posited to be the pathologic first step of the AD process (Haass and Selkoe, 2007), $A\beta$ protein can also be found in relatively high concentration in cerebral white matter (Roher et al., 2002). However, it remains to be clarified whether $A\beta$ protein oligomers also play a direct role in development of the diffuse white matter pathology of AD.

The myelination of cerebral white matter develops in a strictly reproducible time- and region-dependent manner. In humans, cerebral myelination continues until the end of the fifth decade in the prefrontal and other association areas (Baumann and Pham-Dinh, 2001), and recently these late-myelinating neocortical regions have been shown to be most affected by AD (Bartzokis, 2004). Moreover, recent histological and microarray studies have indicated continuing or even increased brain myelinogenic activity in association with advancing age or cognitive decline (Kadish et al., 2009; Peters and Sethares, 2003). In fact, oligodendroglial progenitor cells (OPCs) remain in the adult brain (Nishiyama et al., 2009; Polito and Reynolds, 2005), suggesting active myelin maintenance possibly to preserve plasticity of brain circuits. It is therefore reasonable to hypothesize that, if myelin maintenance and de novo myelination in the adult brain are compromised by $A\beta$ proteins, damage to the circuitry caused by the degeneration process or other factors would accumulate, further enhancing the progressive neuronal network degeneration in AD.

Most prior studies on the effects of $A\beta$ oligomer on oligodendrocytes have focused on relatively acute cytotoxicity paradigms (Chen et al., 2006; Jantaratnotai et al., 2003; Lee et al., 2004; Zeng et al., 2005). Given the chronic progressive nature of AD, however, more delayed but significant pathological mechanisms could be more relevant to the white matter degeneration in AD. Using an in vitro model, we demonstrate inhibition of myelin sheet formation by $A\beta$ oligomer, which could occur at physiologically relevant concentrations of $A\beta$ proteins in white matter (Roher et al., 2002).

2. Methods

2.1. Reagents and chemicals

All reagents and culture media used in this study were purchased from Sigma (St. Louis, MO) and Invitrogen (Carlsbad, CA), respectively, unless otherwise noted. Human recombinant fibroblast growth factor 2 and platelet-derived growth factor A homodimer (PDGF_{AA}) were from R&D Systems (Minneapolis, MN). Rat anti myelin basic protein (MBP) antibody and rabbit anti proteolipid protein (PLP) antibody were from Novus Biologicals (Littleton, CO). Mouse antiglyceraldehyde-3-phosphate dehydrogenase (GAPDH) anti-body was from Chemicon (Temecula, CA).

2.2. Preparation of unaggregated and oligomeric $A\beta$ solutions and $A\beta$ fibrils

Solutions of seedless, unaggregated $A\beta$ 1–42 and $A\beta$ 1–42 oligomer were prepared according to established protocols (Maezawa et al., 2008). Our preparation of oligomer followed the

procedure described by Lambert et al. (1998) except that the A β 1–42 peptide was diluted with Opti-MEM culture medium instead of the F12 medium originally described, before incubation at 4 °C for 24 hours to generate oligomers. This preparation of A β 1–42 oligomer has been extensively characterized in our laboratory (Maezawa et al., 2006, 2008). To ensure consistency of quality, a random sample from each batch prepared from a new lot of synthetic A β 1–42 peptide was imaged using electron microscopy and atomic force microscopy to characterize the size and shape of the aggregates (Hong et al., 2007; Maezawa et al., 2006, 2008). The biological activity of each batch was confirmed by determining A β 1–42 oligomer's neurotoxic activity, synaptic binding activity, and ability to rapidly induce exocytosis of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formazan, as previously described (Hong et al., 2007). Consistent with previous observations (Klein, 2002), by atomic force microscopy our preparations showed a heterogeneous population of globular oligomers up to 4 nm in *z*-height; while by gel electrophoresis they consisted of species ranging from trimer to 24-mer. In addition, once the oligomeric assemblies were constituted in culture medium for cell treatment, the solution was used immediately and the unused solution was discarded. In this study, 7 independent batches of A β 1–42 oligomer preparation were used in total, and consistent results were obtained from at least 2 different batches in each experimental group.

2.3. Immunopanning purified rat A2B5⁺ OPC cultures

Highly enriched primary oligodendroglial cultures were prepared as reported elsewhere (Horiuchi et al., 2010; Itoh et al., 2002). Briefly, brains were dissected from 0 to 2-day-old Lewis rats, and dissociated cell suspension was obtained by digestion with trypsin. Usage of animals was performed in conformity with the protocol approved by University of California, Davis Institutional Animal Care and Use Committee. The cells were resuspended in minimum essential medium alpha containing 5% v/v fetal bovine serum and 5% v/v calf serum, and plated onto a 10-cm culture dish. One day after plating, attached cells (designated as passage 0) were transferred to the medium (GM), a 3:7 mixture (v/v) of B104 neuroblastoma-conditioned medium and the N1 medium (high glucose Dulbecco's Modified Eagle's Medium supplemented with 6 mM L-glutamine, 10 ng/mL biotin, 5 μ g/mL insulin, 50 μ g/mL apo-transferrin, 30 nM sodium selenite, 20 nM progesterone, and 100 μ M putrescine as final concentrations). Cultures were fed with fresh GM every other day for approximately 5 days, at which time the proliferating glial cells were almost confluent. Then OPCs were isolated by serial immunopanning. Mixed glial cells were suspended in the N1 medium containing 0.1% (w/v) bovine serum albumin (BSA), and plated and incubated on the negative immunopanning plates coated with RAN-2 antibody (ATCC, Manassas, VA) for 30 minutes at 37 °C to exclude RAN-2-positive population. Following 2 rounds of this negative selection, nonadherent cells were transferred to the A2B5 positive panning plates. Purified cultures consisted of more than 95% of OPCs which were positive for A2B5, but negative for both O4 and glial fibrillary acidic protein. To induce in vitro differentiation of OPCs, the culture medium was switched from GM to differentiation medium (DM), a 1:1 mixture (v/v) of high glucose Dulbecco's Modified Eagle's Medium and Ham's F-12 medium supplemented with 4.5 mM L-glutamine, 10 ng/mL biotin, 12.5 μ g/mL insulin, 50 μ g/mL transferrin, 24 nM sodium selenite, 10 nM progesterone, 67 μ M putrescine, 0.4 μ g/mL 3,5,3',5'-tetraiodothyronine, 100 U/mL penicillin, and 100 μ g/mL streptomycin as final concentrations.

2.4. Immunocytochemistry, and cholesterol and F-actin staining

Cells cultured on round coverslips were incubated with supernatants from O4 and/or H8H9 hybridomas for 30 minutes at room temperature, and then, fixed with 4% paraformaldehyde for 15 minutes. For subsequent double staining for MBP or PLP, the fixed cells were permeabilized by 100% methanol for 20 minutes at –20 °C. After incubation with rat anti-

MBP (1:5) or rabbit anti-PLP antibody (1:100) for 30 minutes, cells were incubated with fluorophore-conjugated secondary antibodies (1:100) and 4,6-diamidino-2-phenylindole (DAPI; 0.5 μ g/mL) in phosphate-buffered saline (PBS) at room temperature for 30 minutes. For visualization of F-actin, the fixed cells were permeabilized with 0.1% Triton-X 100 for 5 minutes, and then incubated with Texas Red-X phalloidin (5 U/mL; Invitrogen) together with secondary antibodies against O4 antibody and 4,6-diamidino-2-phenylindole (DAPI) at room temperature for 20 minutes.

For double staining for cholesterol and galactocerebroside, live cells were first incubated with supernatant of H8H9 hybridoma for labeling galactocerebroside at room temperature for 30 minutes, washed 3 times with PBS, and fixed with 4% paraformaldehyde for 15 minutes. After quenching paraformaldehyde with 1.5 mg/mL glycine in PBS for 10 minutes, cells were incubated with 0.05 mg/mL filipin and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody (1:200) in PBS supplemented with 10% fetal bovine serum at room temperature for 1 hour in dark.

2.5. Immunoblots

Protein lysates were prepared as described previously (Horiuchi et al., 2006). Twenty micrograms of protein from each sample was size-fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, transferred onto a nitrocellulose membrane (Schleicher & Schnell, Keene, NH) and probed with primary antibodies for MBP (1:1000) and PLP (1:1000) for 1 hour. Full range recombinant Rainbow Molecular Weight Markers (Amersham Biosciences, Piscataway, NJ) was used as a reference for molecular sizes. Immunoreactive signals were detected by enhanced chemiluminescence according to the manufacture's protocol (Amersham Biosciences). Equal protein loading was confirmed by subsequent probing with the mouse monoclonal antibody against GAPDH in each experiment.

2.6. MTT assay and time-lapse imaging

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/mL; Sigma) was added to the culture medium and incubated at 37 °C for 75 minutes. At the end of the incubation, produced formazan was dissolved in dimethyl sulfoxide, and quantified by a microplate reader at 560 nm. In parallel experiments, formation of MTT formazan in live cells was monitored by time-lapse imaging from 5 to 75 minutes after addition of MTT. Time-lapse images of selected fields were taken at 10-minute intervals for 70 minutes by the Nikon Perfect focus system (Nikon, Tokyo, Japan) equipped with an environmental chamber.

2.7. LDH assay

Lactate dehydrogenase (LDH) released from dead cells into the medium was measured using cytotox 96 nonradioactive cytotoxicity assay kit (Promega, Madison, WI). Fifty μ L of culture supernatant was mixed with the same volume of substrate solution, and incubated for 30 minutes at room temperature. LDH-catalyzed conversion of a tetrazolium salt into a formazan product was stopped by adding 50 μ L of 1 M acetic acid, and then, the formazan product was quantified by a microplate reader at 490 nm.

2.8. Quantitative RT-PCR

Total ribonucleic acid (RNA) was isolated by RNeasy RNA extraction kit (Qiagen, Valencia, CA), and reverse transcription (RT)-reaction was done as reported previously (Itoh et al., 2003). Quantitative polymerase chain reaction (PCR) was performed by MX3005P (Stratagene, La Jolla, CA) using TaqMan, Assay-on-Demand assay kits (assay

nos.: Rn00690431_m1 and Rn00456892_m1 for rat MBP and PLP, respectively; Applied Biosystems, Carlsbad, CA). Plasmids containing PCR-amplified complementary DNA (cDNA) of the target genes were serially diluted and used for the concentration standards. For standardization, GAPDH cDNA levels were quantified with TaqMan Rodent GAPDH Control Reagents according to manufacturer's instruction, and the absolute cDNA amounts were expressed as ratios to GAPDH cDNA.

2.9. Statistical analysis

Unpaired 2-tailed Student *t* test was employed for comparisons of 2 experimental groups, and analysis of variance (ANOVA) followed by the Bonferroni/Dunn posthoc test for the datasets from more than 2 experimental groups. Results were considered significant, when *p* < 0.05.

3. Results

3.1. A β 1–42 oligomer at 10 μ M was cytotoxic only to mature oligodendrocytes, but not to OPCs

We first examined effects of A β 1–42 oligomer on viability of both OPCs and mature oligodendrocytes. The standard MTT assay, the result of which generally correlates well with the number of viable oligodendrocytes (Itoh et al., 2000), demonstrated a significant decrease in MTT reduction in mature oligodendrocytes treated with A β 1–42 oligomer at 0.1 μ M or higher. In contrast, decreased MTT reduction was not observed in OPCs treated with A β 1–42 oligomer at 1 μ M (Fig. 1A). The standard LDH release assay, a cell death assay which measures leakage of cytosolic LDH through the breached plasma membrane, revealed that actual death of mature oligodendrocytes occurred only when they were exposed to A β 1–42 oligomer at 10 μ M for 48 hours (Fig. 1B). Decreased MTT reduction in mature oligodendrocytes treated with 1 μ M or lower concentrations of A β 1–42 oligomer without accompanying actual cell death could be due to A β -induced enhanced exocytosis of MTT formazan-containing vesicles as reported in the various other cell types (Abe and Saito, 1998; Liu et al., 1998, 2004). To test this possibility, we employed time-lapse imaging and observed that, in the presence of 1 μ M A β 1–42 oligomer, needle-like MTT formazan crystal formation surrounding the processes and cell somas of mature oligodendrocytes became detectable after about 30 minutes of exposure to MTT. This needle-like crystal formation on the cell surface was consistent with the observation by Liu et al. (1997) when MTT-formazan containing granules were exocytosed at the cell surface. Formazan crystal formation was complete around 55 minutes after MTT addition in the presence of 1 μ M A β 1–42 oligomer, whereas formazan granules accumulated but remained in the cell soma during a 75-minute incubation period in control mature oligodendrocytes and in those treated with the synthetic peptide containing the reverse amino acid sequence of A β 1–42 (A β 42–1) (Fig. 2A and B). In contrast, needle-like MTT formazan crystal formation was infrequent in OPCs treated with A β 1–42 oligomer (Fig. 2C and D). These results indicate that A β 1–42 oligomer at 1 μ M or less accelerates exocytosis of formazan granules in mature oligodendrocytes forming myelin membranes, whereas OPCs which have not yet produced myelin components are insensitive to this effect of A β 1–42 oligomer.

3.2. A β 1–42 oligomer inhibited myelin sheet formation at 1 μ M in vitro

We next induced in vitro differentiation of OPCs in the presense of A β peptides, and examined their effects on differentiation of the oligodendroglial lineage. As demonstrated in Fig. 3, a large area of the control cultures was covered with MBP-positive myelin sheets at 4 days after induction of differentiation, whereas, in the sister cultures treated with 1 μ M A β 1–42 oligomer, the membranous sheet formation was markedly inhibited with accumulation of MBP immunoreactivity in the proximity to the cell soma (Fig. 3A–D). These observations

were quantified by measuring areas of MBP-positive myelin sheets in at least 3 randomly captured images of each condition. MBP-positive areas were significantly decreased in the A β 1–42 oligomer-treated cultures, but not in the unaggregated A β 1–42-treated cultures, compared with the control cultures (Fig. 3E). Nevertheless, a quantitative immunocytochemical analysis revealed that numbers of MBP-expressing mature oligodendrocytes were not reduced by A β 1–42 oligomer (Fig. 3F). Double staining with antibodies for PLP, one of other myelin proteins, and O4 antigen, a surface marker of oligodendroglial lineage cells, further confirmed that distribution of PLP protein was also disturbed due to the compromised myelin sheet formation by A β 1–42 oligomer, although O4-positive ramified processes were still formed even in the presence of A β 1–42 oligomer (Fig. 4).

3.3. A β 1–42 oligomer at 1 μ M did not inhibit induction of myelin genes during in vitro differentiation

In vitro differentiation of purified OPCs in the primary cultures progresses after removal of mitogens even in the absence of neurons. To determine whether A β 1–42 oligomer inhibits this intrinsic differentiation process, we quantified the messenger ribonucleic acid (mRNA) and protein levels of MBP and PLP, 2 major myelin proteins, after induction of in vitro differentiation in the presence or absence of A β 1–42 oligomer. No significant inhibition of these myelin-associated genes was found in the A β 1–42 oligomer-treated cultures (Fig. 5), indicating that, in support of the quantitative immunocytochemical results (Fig. 3F), the inhibition of myelin sheet formation by A β 1–42 oligomer is not a consequence of its inhibitory effect on progression of the intrinsic differentiation program.

3.4. A β 1–42 oligomer altered cholesterol distribution

A β oligomer is known to alter cholesterol homeostasis (Liu et al., 1998). Moreover, cholesterol is a major lipid component of the myelin membrane, and myelination is substantially affected by inhibitors of cholesterol synthesis such as statins (Maier et al., 2009; Miron et al., 2009). We therefore examined whether A β 1–42 oligomer altered distribution of free cholesterol on the myelin membrane by staining with filipin that binds specifically to free or unesterified cholesterol. In the control cultures, the myelin membrane sheets were evenly labeled with filipin. In the presence of A β 1–42 oligomer, however, we observed that cholesterol was concentrated in the plasma membrane, particularly in the bleb formation of the processes (Fig. 6A–F). We further tested whether addition of exogenous cholesterol might cause an effect on myelin sheet formation mimicking that of A β 1–42 oligomer, because free cholesterol is known to enhance MTT formazan exocytosis as well (Liu et al., 1998). In contrast to A β 1–42 oligomer, exogenous cholesterol at 5 μ M had a minimum inhibitory effect on myelin sheet formation (Fig. 6G and H).

3.5. A β 1–42 oligomer inhibited distribution of F-actin in the distal portions of processes

Myelin sheet formation is tightly associated with cytoarchitectural reorganization in differentiating oligodendrocytes, particularly extensive outgrowth and arborization of processes. Recent studies indicate that A β oligomer induces the disassembly of synaptic actin filaments in neurons through inhibition or aberrant activation of p21-activated kinases (PAKs), resulting in a loss of dendritic spines (Ma et al., 2008; Zhao et al., 2006). In differentiating oligodendrocytes, F-actin microfilaments are enriched at the leading edge of the processes and newly generated branches, and presumably guide the local reorganization of microtubules for the elongation and branching of oligodendrocyte processes (Song et al., 2001; Thomas et al., 2002). We therefore examined whether A β 1–42 oligomer altered F-actin distribution in the processes of differentiating immature oligodendrocytes. As shown in Fig. 7, 1 μ M A β 1–42 oligomer caused accumulation of phalloidin-labeled F-actin in the proximity to the cell soma, whereas F-actin microfilaments distributed even in the distal

portions of oligodendrocyte processes in control cultures and the cultures treated with A β 42–1 or A β 1–42 monomer. We quantified the F-actin-abundant territories of oligodendrocyte processes in control and A β 1–42 oligomer-treated cultures. This quantitative analysis revealed that the F-actin-abundant territories were significantly smaller in the cultures treated with A β 1–42 oligomer compared with those in control cultures (Fig. 7).

4. Discussion

The in vitro model of oligodendroglial differentiation provides the best system to understand the molecular basis of oligodendroglial differentiation and myelin membrane production under a highly pure and defined condition (i.e., no effects of serum components and no indirect effects of other cellular populations such as microglia) (Raff et al., 1983). With this model, we found 2 significant effects of A β 1–42 oligomer at submicromolar concentrations on cells of the oligodendroglial lineage.

First, A β 1–42 oligomer accelerates MTT formazan exocytosis in mature myelin-producing oligodendrocytes, an effect to which OPCs appear relatively resistant. In the primary cultures of neurons, accelerated MTT formazan exocytosis by A β peptides is closely associated with their neurotoxic effects (Hong et al., 2007; Liu and Piasecki, 2001). Our results indicate that this association holds true for the oligodendroglial lineage, because myelin-producing mature oligodendrocytes were more susceptible to A β 1–42 oligomer-mediated cytotoxicity than OPCs at 10 μ M. Such relatively high concentrations (10 μ M or higher) of A β peptides have already been known to induce ceramide-mediated apoptosis in oligodendrocytes via activation of neutral sphingomyelinase but not acidic sphingomyelinase (Lee et al., 2004). Accumulation of ceramide activates the c-JUN NH₂ terminal kinase pathway resulting in increased expression of a proapoptotic protein DP5/Hrk (Chen et al., 2006), and simultaneously enhances tumor necrosis factor- α -mediated induction of inducible nitric oxide synthase (iNOS) (Zeng et al., 2005). However, our results demonstrated that, more notably, A β 1–42 oligomer-accelerated MTT formazan exocytosis occurs in myelin-producing mature oligodendrocytes at submicromolar concentrations despite no detectable cell death. Our result urges caution when evaluating the effect of A β peptides on cell survival by the MTT assay which have been used as a standard method in many previous studies on the oligodendroglial lineage. Although the biomolecular mechanisms underlying MTT formazan exocytosis are not yet fully understood (Diaz et al., 2007), Schubert and his colleagues demonstrated that A β peptides inhibited cellular cholesterol esterification and altered the distribution of free cholesterol in neurons, indicating that alteration in cholesterol homeostasis by A β peptides is involved in this phenomenon (Liu et al., 1998). Unlike the plasma membrane of most cell types, myelin is characterized by a high content of lipids, in particular, enriched in cholesterol and galactosylceramide. Differences in cholesterol homeostasis between myelin-producing mature oligodendrocytes and OPCs might contribute to higher sensitivity of mature oligodendrocytes to A β oligomer-accelerated MTT formazan exocytosis and A β 1–42 oligomer-mediated cytotoxicity compared with OPCs. Importantly, we show that this process appears to occur at A β 1–42 oligomer concentrations previously found in AD brains (Roher et al., 2002), suggesting potential relevance for disease pathology.

Second, we found that A β 1–42 oligomer inhibits myelin sheet formation in vitro at 1 μ M. However, our quantitative biochemical analysis of the myelin proteins indicated that the intrinsic differentiation process progressed in the presence of 1 μ M A β 1–42 oligomer on a schedule indistinguishable from that of controls. Because of the distinct lipid and protein composition as mentioned above, the myelin membrane can be considered one of lipid rafts; the glycosphingolipid-enriched membrane microdomains which provide cellular polarity

and the platforms essential for signaling and interaction with other cells and matrixes (Debruin and Harauz, 2007; Gielen et al., 2006). Synthesis of these membrane microdomains requires specific sorting and transport of domain-specific proteins (de Vries et al., 1998; Maier et al., 2008). Cholesterol homeostasis is critically involved in these sorting and transport machineries. Recently, Maier et al. (2009) demonstrated that inhibition of cholesterol synthesis by lovastatin, one of statins which are well known inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, perturbed myelin sheet formation by inhibiting MBP mRNA transport into oligodendrocyte processes and by inhibiting translocation of PLP protein to the membrane surface. They have further indicated that, the inhibition of PLP protein translocation is due to reduced cholesterol synthesis, whereas the inhibition of MBP mRNA transport is mediated by impaired isoprenylation. In contrast to their results from the inhibition of cholesterol synthesis, A β 1–42 oligomer did not reduce MBP protein levels, indicating that translation of MBP mRNA was not affected by A β 1–42 oligomer at 1 μ M despite the significant inhibitory effect on myelin sheet formation. Therefore, A β 1–42 oligomer inhibits myelin sheet formation by a mechanism distinct from inhibition of the sorting and transport machineries for MBP and PLP, and from direct inhibition of the mevalonate pathway. Given the evidence that myelin proteins and cholesterol are colocalized with actin filaments in differentiating oligodendrocytes (Lintner and Dyer, 2000; Taguchi et al., 2005), reduced distribution of F-actin in distal portion of processes by A β 1–42 oligomer could alter cholesterol distribution, which was demonstrated by filipin labeling, and eventually result in inhibition of myelin sheet formation. Further research is necessary to test directly whether A β 1–42 oligomer impairs myelin maintenance through dysfunction of actin-mediated cytoskeletal reorganization essential for myelin formation.

There have been few studies on physiological and pathological concentrations of A β protein in the central nervous system (CNS) tissues of AD patients. A simple calculation based on the quantitative biochemical study by Roher et al. (2002) from the white matter tissues of AD patients indicates that A β 1–42 peptide levels are likely to be in a submicromolar range consistent with concentrations tested in this study. Our results indicate that pathologically relevant concentrations of A β 1–42 oligomer can inhibit the myelination process without inducing acute cell death, which may underlie some of the myelinopathy observed in AD.

Several studies using mouse models of AD have demonstrated evidence for white matter abnormalities in parallel with deposition of A β peptides (Desai et al., 2009; Harms et al., 2006; Sun et al., 2005; Wirths et al., 2007). A part of this white matter degeneration is attributable to axonopathy associated with neuronal degeneration (Stokin et al., 2005; Wirths et al., 2007). Given the mutual dependence of neurons and oligodendrocytes as a functional unit of the brain circuitry, our results support the hypothesis that oligodendrocytes could be another cellular injury mechanism contributing to A β protein-mediated white matter degeneration. Consistent with this hypothesis, Desai et al. (2009) recently provided the first ultrastructural evidence of myelination abnormalities in 3xTg-AD mice at ages that precede overt amyloid or tau pathology.

We believe that our in vitro findings support and extend our understanding of current in vivo studies of white matter microstructure in AD (Chua et al., 2008; Lee et al., 2009). Our data suggest that the white matter pathology of AD likely results both from direct neurotoxicity and degeneration (Stokin et al., 2005) as well as impaired maintenance of myelin integrity that may hasten axonal degeneration. Moreover, these data suggest that the AD process may also render axonal myelin more susceptible to injury from other processes such as cerebrovascular disease (Lee et al., 2009; Yoshita et al., 2006). Our current findings, however, are limited to in vitro studies reported herein. It will, therefore, be important to extend these findings to the intact animal model as well as further study of human tissues.

Recognizing the important role of axons to connect distributed neural circuits within the human brain, preservation of A β protein-mediated white matter degeneration may prove to be a new pathway for novel therapeutic discovery.

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Fig. 1.

(A) Results of the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay after 48 hours of incubation with amyloid β ($A\beta$)1–42 oligomer at the concentrations indicated. MTT reduction was significantly reduced in mature oligodendrocytes (MO) at 0.1 μ M or higher, but not in oligodendroglial progenitor cells (OPC, closed circle). (B) Results of the standard lactate dehydrogenase (LDH) assay after 48 hours of incubation with $A\beta$ 1–42 oligomer at the concentrations indicated. Actual cell death was detected only in mature oligodendrocytes treated at 10 μ M. Data are mean $\pm$ SD. **, $p < 0.01$ in comparison with the control (0 μ M).

Fig. 2.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formazan exocytosis in mature oligodendrocytes (A and B) and oligodendroglial progenitor cells (C and D) in the presence (B and D) or absence (A and C, control) of amyloid β ($A\beta$) $_{1-42}$ oligomer at $1 \mu\text{M}$. Time-lapse images were taken at indicated time points after addition of MTT to the culture medium. Arrowheads (B) indicate typical needle-like crystals of exocytosed MTT formazan formed adjacent to the cell surface. Scale bar; $50 \mu\text{m}$.

Fig. 3. (A–D) Rat oligodendroglial progenitor cells (OPCs) were in vitro differentiated for 4 days in the differentiation medium alone (A and C, control), or the medium containing amyloid β ($A\beta$)1–42 oligomer (B and D, 1 μ M), and then immunocytochemically labeled for myelin basic protein (MBP). A representative picture from randomly taken image fields is shown for each experimental condition at low (A and B) and high magnifications (C and D). Typical myelin sheets are indicated by the two flanking arrows in (C). (E) O4-positive or MBP-positive areas in mature oligodendrocyte (MO) cultures differentiated in the differentiation medium alone or the medium containing the reverse peptide $A\beta$ 42–1, unaggregated $A\beta$ 1–42 ($A\beta$ 1–42 monomer), or $A\beta$ 1–42 oligomer ($A\beta$ 1–42 oligo) at 1 μ M were measured, and the results are presented as percentages of the whole image areas. (F) Percentages of MBP-immunopositive cells at 4 days after in vitro differentiation in the same experimental groups as in (E). Scale bars; 50 μ m in A and B, 80 μ m in (C) and (D). Data are mean $\pm$ SD. * $p < 0.05$ and ** $p < 0.01$ in comparison with the data from control.

Fig. 4.

Rat oligodendroglial progenitor cells (OPCs) were in vitro differentiated for 4 days in the differentiation medium alone (A–C, control), or the medium containing amyloid β ($A\beta$)1–42 monomer (D–F, 1 μ M). Cells were then immunocytochemically labeled for O4 (red) and proteolipid protein (PLP) (green). Scale bar; 50 μ m.

Fig. 5.

$A\beta$ 1–42 oligomer did not inhibit upregulation of myelin basic protein (MBP) and proteolipid protein (PLP) after induction of in vitro differentiation. (A) Oligodendroglial progenitor cells (OPCs) were transferred to the differentiation medium and maintained in medium alone (open circle), amyloid β ($A\beta$)1–42 oligomer (1 μ M, closed circle), or the reverse peptide $A\beta$ 42–1 (1 μ M, open triangle). The reverse transcribed complementary DNA (cDNA) levels were quantified by quantitative polymerase chain reaction (PCR) at the indicated time points. At time 0, data from controls are only shown. The data are plotted as ratios to copy numbers of antiglyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA on a logarithmic scale. Calculated results from 3 independent experiments at 72 hours confirmed no statistical significance among 3 experimental groups (right panels). (B) MBP and PLP protein levels were comparable when oligodendrocytes were maintained in the differentiation medium alone (C), the medium containing $A\beta$ 1–42 oligomer (1 μ M, $A\beta$), or the reverse peptide $A\beta$ 42–1 (1 μ M, Rv) for 4 days. Protein samples (20 μ g) were immunoblotted for MBP and PLP. The subsequent immunoblots for GAPDH is shown for equal protein loading.

Fig. 6.

(A–F) Representative images of double-staining for galactocerebroside (A and D, green in C and F) and unesterified cholesterol (B and E, blue in C and F) with H8H9 hybridoma supernatant and filipin, respectively, at day 4 in the differentiation medium alone (control, A–C) and with A β 1–42 oligomer (1 μ M) (D–F). Cholesterol was concentrated in the plasma membrane, particularly in the bleb-like structures of the processes (arrows in D–F) in the presence of A β 1–42 oligomer, whereas cholesterol was evenly distributed in the galactocerebroside-positive myelin sheets in control cultures. (G) and (H) Immunocytochemistry for myelin basic protein (MBP) and O4 of oligodendrocytes at 4 days in the differentiation medium alone (control, G) and with exogenous cholesterol (5 μ M, H). Scale bars; 50 μ m.

Fig. 7.

(A–D) Effects of amyloid β ($A\beta$)1–42 oligomer on process formation and F-actin distribution in differentiating immature oligodendrocytes. Oligodendroglial progenitor cells (OPCs) were transferred to the differentiation medium, maintained for 2 days in medium alone (A), $A\beta$ 1–42 oligomer (B, 1 μ M), the reverse peptide $A\beta$ 42–1 (C, 1 μ M), or unaggregated $A\beta$ 1–42 (D, 1 μ M), and then labeled with O4 antibody (green) and Texas red-X phalloidin (red). White lines in the insets in (A) and (B) indicate the F-actin-abundant territories of oligodendroglial processes. Scale bar; 50 μ m. The accumulated quantitative results of the F-actin-abundant territories are summarized in (E). Immature oligodendrocytes differentiating in the presence of $A\beta$ 1–42 oligomer at 1 μ M ($A\beta$ 1–42oligo) had significantly smaller F-actin-abundant territories than those in control cultures. Data are mean $\pm$ SD. ** $p < 0.01$ in comparison with the data from control.