ELSEVIER

Neurobiology of Aging 33 (2012) 499–509

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Amyloid β1–42 oligomer inhibits myelin sheet formation in vitro

Makoto Horiuchi[a,b], Izumi Maezawa[c], Aki Itoh[a,b], Kouji Wakayama[a,b], Lee-Way Jin[c], Takayuki Itoh[a,b,1,*], Charles DeCarli[a,1]

[a] Department of Neurology, University of California Davis, School of Medicine, Sacramento, CA, United States
[b] Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children Northern California, Sacramento, CA, United States
[c] M.I.N.D. Institute and Department of Pathology, Department of Internal Medicine, University of California Davis Cancer Center, University of California Davis, Sacramento, CA, United States

Received 4 February 2010; received in revised form 30 April 2010; accepted 9 May 2010

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 (Aβ) protein-mediated damage to oligodendrocytes could be another mechanism. To test this hypothesis, we studied effects of soluble Aβ in oligomeric form on survival and differentiation of cells of the oligodendroglial lineage using highly purified oligodendroglial cultures from rats at different developmental stages. Aβ oligomer at 10 μM or higher reduced survival of mature oligodendrocytes, whereas oligodendroglial progenitor cells (OPCs) were relatively resistant to the Aβ oligomer-mediated cytotoxicity. Further study revealed that Aβ oligomer even at 1 μM 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 Aβ 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 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 (Aβ) protein aggregation is posited to be the pathologic first step of the AD process (Haass and Selkoe, 2007), Aβ protein can also be found in relatively high concentration in cerebral white matter (Roher et al., 2002). However, it remains to be

* Corresponding author at: 610A Shriners Hospitals for Children Northern California, 2425 Stockton Boulevard, Sacramento, CA 95817, United States. Tel.: +1 916 453 2266; fax: +1 916 453 2288.
E-mail address: takito@ucdavis.edu (T. Itoh).
[1] Both authors contributed equally to this work.

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2010.05.007

clarified whether Aβ 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 Aβ 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 Aβ 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 Aβ oligomer, which could occur at physiologically relevant concentrations of Aβ 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) antibody was from Chemicon (Temecula, CA).

2.2. Preparation of unaggregated and oligomeric Aβ solusions and Aβ fibrils

Solutions of seedless, unaggregated Aβ1–42 and Aβ1–42 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 Aβ1–42 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 Aβ1–42 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 Aβ1–42 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 Aβ1–42 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 Aβ1–42 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 μg/mL insulin, 50 μg/mL apo-transferrin, 30 nM sodium selenite, 20 nM progesterone, and 100 μM 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