The Unexpected Role of Aβ1-42 Monomers in the Pathogenesis of Alzheimer’s Disease

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Abstract. Amyloid-β (Aβ) has been proposed as a biomarker and a drug target for the therapy of Alzheimer’s disease (AD). The neurotoxic entity and relevance of each conformational form of Aβ to AD pathology is still under debate; Aβ oligomers are considered the major killer form of the peptide whereas monomers have been proposed to be involved in physiological process. Here we reviewed some different effects mediated by monomers and oligomers on mechanisms involved in AD pathogenesis such as autophagy and tau aggregation. Data reported in this review demonstrate that Aβ monomers could have a major role in sustaining the pathogenesis of AD and that AD therapy should be focused not only in the removal of oligomers but also of monomers.

Keywords: Alzheimer’s disease, Aβ monomers, Aβ oligomers, autophagy, tau protein

INTRODUCTION

Alzheimer’s disease (AD) is the most common age-related disease [1], and it has become a very serious social and health problem with the increase of life expectancy. Indeed, the risk of AD increases dramatically in individuals above the age of 70, and it is predicted that the incidence of the disease could further increase by 3-fold over the next 50 years [2].

Extracellular amyloid plaques formed by aggregated amyloid-β peptides (Aβ) and intracellular neurofibrillary tangles composed by polymers of altered tau protein are the two main pathological hallmarks of the disease.

According to the amyloid cascade hypothesis, a series of clues indicate that the accumulation of Aβ in the brain is the primary and early event that induces neuronal degeneration, characterized by accumulation of conformational altered and aggregated tau protein.

Aβ, a 39–43 residue polypeptide, is cleaved from the amyloid-β protein precursor (AβPP) by β- and γ-secretases and consists of a largely hydrophilic N-terminal domain (1–16) and a C-terminal hydrophobic domain [3]. The predominant Aβ species end at 40 and 42 residues; the latter shows a greater propensity for aggregation and is considerably more neurotoxic because of two additional hydrophobic amino acids [3].

Although many details in the pathogenesis of AD remain elusive, Aβ has been proposed as a biomarker and a drug target for the therapy, being expected to
ameliortate the accuracy of early diagnosis, and to investigate the influence of drugs on Aβ removal and aggregation. The neurotoxic entity and relevance of each conformational form of Aβ to AD pathology is still under debate; Aβ oligomers are considered the major killer form of the peptide [4] but a role for fibrillar form of Aβ to neurotoxicity cannot be ignored [5]. Monomers instead have been proposed to be involved in physiological process. Their role in the pathogenesis of AD is unknown. Here we reviewed some different effects mediated by monomers and oligomers on mechanisms involved in AD pathogenesis.

**Aβ MONOMERS AND OLIGOMERS**

Aβ monomers are predominantly α-helical and random coil in structure. Aβ42 monomers are highly prone to aggregation and they form a wide range of soluble oligomers which vary in morphology and size from dimers to trimers and then up to large prefibrillar structures [6].

Monomers are the prevalent species of the lag phase and fibrils dominate at the final plateau, while during the growth phase their concentrations are similar. The concentrations of any intermediates, small aggregates or oligomers, appear low at all time [7].

Monomers have been proposed to be involved in physiological processes. There is concern in the field about technical limitations of working with such small peptides and their derivatives. The problem in studying Aβ42 monomers derives by their tendency to aggregate as well as by the heterogeneity of the peptide solution that can assume different conformational states [8]. Despite these technical limitations, some researchers developed methods to obtain a homogeneous population of Aβ42 monomers [9–11]. These pure monomeric preparations have been found able to protect neurons by trophic deprivation and excitotoxicity [9] through the activation of the phosphatidyl-inositol-3-kinase pathway. More recently, it was also demonstrated that Aβ42 monomers mediated the glucose uptake in neurons by selectively activating the member of the insulin receptor superfamily, IGF-IRs, and promoting the translocation of glucose transported Glut3 to the plasma membrane from the cytosol [12]. These results suggest a positive role of Aβ42 monomers that would be important for neuronal survival; therefore, therapeutic approaches should take into account this neuroprotective function.

The oligomeric forms of Aβ are the major toxic agents in AD [13–15]. Interestingly, concentration of Aβ42 oligomers are higher in plasma of AD patients than control subject [16]. Several pathogenetic mechanisms of Aβ42 oligomers have been proposed. Oligomers are able to bind neurons and directly induced cell death mediating phagocytosis and oxidative stress [17, 18]. They can also impair the electrochemical signals by forming small channels [19–21] or by interfering with the cell signaling pathways [22] and cause neuronal death. Finally, it has been demonstrated that oligomers can accumulate in mitochondria, damaging the respiratory chain [23]. During the Aβ aggregation process, small prefibrillar aggregates are first formed and then these assemble into protofibrils and protofilaments. The level of Aβ polymers with fibrillar conformation also correlates with AD onset and severity, thus although oligomers are believed to be more toxic to cells, strong evidence indicates that the fibril formation is related to the rates of disease progression of AD patients [5].

We found that Aβ42 monomers at physiological concentrations upregulated BACE1 activity [24] suggesting that the limit between the physiological and pathological functions of Aβ is very subtle. Our recent studies showed different effects of Aβ42 monomers and oligomers in autophagy, apoptosis, and tau aggregation.

**Aβ42 MONOMERS VERSUS OLIGOMERS IN AUTOPHAGY AND APOPTOSIS**

Autophagy and apoptosis are two mechanisms closely involved in the pathogenesis of AD.

The misfolded proteins, in particular those that trend to form aggregates, are directed to autophagy, a degradation system in which substrates are segregated into autophagosomes which are then fused with lysosomes for degradation into amino acids [25]. In turn, an increased level of apoptosis, the programmed cell death that leads to the destruction of cells and organelles through the activation of catabolic pathways, has been found increased in neurodegenerative disease such as AD [26].

The role of autophagy and its connection with apoptosis in AD pathogenesis is far from being clear. This relationship has many facets since autophagy in some cases represents a mechanism for adaptation to stress conditions that suppresses apoptosis, while in other cases, it is an alternative death mechanism.
Some studies reported that the induction of apoptosis by rapamycin, an inducer of autophagy, significantly reduces the permeability of mitochondrial outer membrane, which represents a crucial event to mediate apoptotic cell death [27]. On the other hand, stressors are able to induce damage in apoptotic machinery, for example inhibiting caspase activities, mediating autophagic cell death [28, 29].

Given the central role of Aβ in the pathogenesis of AD, it is plausible that it can play a role in linking the two mechanisms. Thus, it is well known that impairment of autophagy leads to Aβ accumulation in vacuoles and cell death. AβPP and Aβ peptides colocalized in autophagosomes in AD cellular and murine models [30, 31]. Moreover, it has been reported that the accumulation of Aβ42 and p62, a marker of the autophagic flux, precedes the derangement of autophagic clearance and mediates the lysosomal impairment [32]. On the other hand, strong evidence indicates that Aβ is also produced during autophagy [30]. Probably, physiologically, autophagy does not influence the production of Aβ because of the efficient clearance of lysosomal degradation [33]. In pathological conditions, autophagy becomes a site for AβPP processing and Aβ generation, thus many autophagic vacuoles are found in AD brains particularly in dystrophic neurites [34] and in perikarya of neurons containing tangles [35]. Moreover, in dystrophic neurites, an accumulation of phagophores has been shown, suggesting that their maturation to lysosomes may be impaired in AD [30].

We recently obtained data shedding light on the interaction between autophagy and apoptosis in response to oligomeric as well as monomeric forms of Aβ42 [36]. We demonstrated that oligomers induce apoptosis allowing the formation of a complex between the anti-apoptotic protein Bcl-2 and Beclin1, a protein implicated in the autophagosomes formation [37]. Other authors demonstrated that the regulation of this complex represents a crucial mechanism by which cells turn off autophagy [38], leading to apoptosis. The mechanism through which the inhibition of autophagy predisposes cells to apoptosis is not completely clear and could be due to a bioenergetics deficiency [39] or to oxidative stress induction [40]. The latter mechanism is in agreement with our previous reports demonstrating that oligomers, but not monomers, increase oxidative stress in different cellular models [41]. On the other hand, monomers lead to autophagy and hamper the formation of the complex formed by Bcl-2 and Beclin 1, through activation of the JNK pathway and inducing Bcl-2 phosphorylation [42]. Monomers also cause a significant accumulation of autophagosomes and also a reduction of lysosomal activity and an accumulation of substrates that are not digested such as BACE1 [36]. These findings confirm our previous data demonstrating that Aβ42 monomers upregulate BACE1 expression interfering with its lysosomal degradation, inducing a cycle of Aβ production [43]. We also found that monomers of Aβ42, but not oligomers, inhibit the activity of Uch-L1, an abundant neuronal enzyme that mediates the proteosomal degradation; our data suggest that Uch-L1 inhibition interferes with the lysosomes as demonstrated by the decrease of cathepsin D, a marker of lysosomal activity [43].

**Aβ42 MONOMERS VS OLIGOMERS ON TAU AGGREGATION AND PHOSPHORYLATION**

The causal relationship between ‘plaques and tangles’, i.e., whether and how Aβ induces the formation of intracellular altered and aggregated protein tau, is a crucial and a much-debated issue.

We investigated whether Aβ42 could modify the conformation and/or the phosphorylation of tau protein to render it more prone to aggregate [44]. Previous data reported three major mechanisms through which Aβ peptides may induce tau aggregation: 1) Aβ phosphorylates tau through the activation of specific kinases and this event alters the ability of tau to bind tubulin [45, 46]; 2) Aβ interferes with proteasomal degradation of tau, thus increasing the free-state of the protein [47]; 3) Aβ aggregates exert a nuclear effect on tau [3, 4]. The latter hypothesis is supported by the notion that tau pathology often coexists with cerebral amyloidosis [48, 49].

We demonstrated that Aβ42 monomers, but not oligomers, intraventricularly injected in mice expressing wild type human tau, produce a pathological conformational change of tau protein [44]. In the same experimental model, we also found that monomers induce phosphorylation of pathological tau epitopes activating GSK3β, JNK, and ERK kinases and that the inhibition of these kinases rescues the tau conformational change [44]. Finally, we investigated whether the observed modification of tau mediated by Aβ monomers could be ascribed to an increase of tau protein levels. It is well known that the increase of total tau is a condition that favors phosphorylation and conformational change of tau,
as demonstrated with mutant tau [50]. We found that Aβ monomers inhibit its proteasomal degradation. Thus, Aβ monomers alter tau conformation through two different mechanisms: hyperphosphorylation and increase of protein levels [44].

CONCLUSIONS

Results of our previous works suggest that oligomeric neurotoxicity is higher than in monomers, possibly through the production of reactive oxygen species or others mediators, and kill neurons inducing apoptosis. Monomers, on the other hand, are able to modulate AβPP processing, increase BACE1 activity sustaining its continuous production and favoring tau aggregation. Thus, both Aβ species may be considered relevant in the pathogenesis of AD. Our results suggest that AD therapy may be focused not only in the removal of oligomers but also of monomers and help to develop new therapeutic approaches to treat the disease.

DISCLOSURE STATEMENT

Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/17-0581r1).

REFERENCES

[1] Selkoe DJ (2001) Alzheimer’s disease: Genes, proteins, and therapy. Physiol Rev 81, 741–766.
[2] Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer’s disease. Alzheimers Dement 3, 186–191.
[3] Guo JP, Arai T, Miklozy J, McGeer PL (2006) Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer’s disease. Proc Natl Acad Sci USA 103, 1953–1958.
[4] Bolmont T, Clavaguera F, Meyer-Luehmann M, Herzig MC, Radde R, Staufenbiel M, Lewis J, Hutton M, Tolnay M, Jucker M (2007) Induction of tau pathology by intracellular infusion of amyloid-beta-containing brain extract and by amyloid-beta deposition in APP x Tau transgenic mice. Am J Pathol 171, 2012–2020.
[5] Kepp KP (2012) Bioinorganic chemistry of Alzheimer’s disease. Chem Rev 112, 5193–5239.
[6] Ha C, Ryu J, Park CB (2007) Metal ions differentially influence the aggregation and deposition of Alzheimer’s beta-amyloid on a solid template. Biochemistry 46, 6118–6125.
[7] Arosio P, Knowles TP, Linse S (2015) On the lag phase in amyloid fibril formation. Phys Chem Chem Phys 17, 7606–7618.
[8] Teplow DB, Lazo ND, Bitan G, Bernstein S, Wytenbach T, Bowers MT, Baumketner A, Shea JE, Urbanc B, Cruz L, Borreguero J, Stanley HE (2006) Elucidating amyloid-beta-protein folding and assembly: A multidisciplinary approach. Acc Chem Res 39, 635–645.
[9] Giuffrida ML, Caraci F, Pignataro B, Cataldo S, De Bona P, Bruno V, Molinaro G, Pappalardo G, Messina A, Palmigiano A, Garozzo D, Nicoletti F, Rizzarelli E, Copani A (2009) Beta-amyloid monomers are neuroprotective. J Neurosci 29, 10582–10587.
[10] Beeg M, Stravalaci M, Bastone A, Salamina M, Gobbi M (2011) A modified protocol to prepare seed-free starting solutions of amyloid-β (Aβ1-40) and Aβ1-42 from the corresponding depsipeptides. Anal Biochem 411, 297–299.
[11] Stravalaci M, Beeg M, Salamina M, Gobbi M (2011) Use of surface plasmon resonance to study the elongation kinetics and the binding properties of the highly amyloidogenic Aβ(1–42) peptide, synthesized by depsipeptide technique. Biosens Bioelectron 26, 2772–2775.
[12] Giuffrida ML, Tommasello MF, Pandini G, Caraci F, Battaglia G, Busceti C, Di Pietro P, Pappalardo G, Attanasio F, Chiechio S, Bagnoli S, Nacimiaz B, Sorbi S, Vigneri R, Rizzarelli E, Nicoletti F, Copani A (2015) Monomeric β-amyloid interacts with type-1 insulin-like growth factor receptors to provide energy supply to neurons. Front Cell Neurosci 9, 297.
[13] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416, 535–539.
[14] Calamai M, Pavone FS (2011) Single molecule tracking analysis reveals that the surface mobility of amyloid oligomers is driven by their conformational structure. J Am Chem Soc 133, 12001–12008.
[15] Liu C, Sawaya MR, Cheng PN, Zheng J, Nowick JS, Eisenberg D (2011) Characteristics of amyloid-related oligomers revealed by crystal structures of macrocyclic β-sheet mimics. J Am Chem Soc 133, 6736–6744.
[16] Zhou L, Chan KH, Chu LW, Kwan JS, Song YQ, Chen LH, Ho PW, Cheng OY, Ho JW, Lam KS (2012) Plasma amyloid-β oligomers level is a biomarker for Alzheimer’s disease diagnosis. Biochim Biophys Acta 1822, 1741–1751.
[17] Zheng L, Terman A, Hallbeck M, Delhvari N, Cowburn RF, Benedikz E, Kågedal K, Cedazo-Minguez A, Marcusson J (2011) Macrautothagy-generated increase of lysosomal amyloid β-protein mediates oxidant-induced apoptosis of cultured neuroblastoma cells. Autophagy 7, 1528–1545.
[18] Ohnishi T, Yanazawa M, Sasahara T, Kitamura Y, Hiroaki H, Fukazawa Y, Kii I, Nishiyama T, Kakita A, Takeda H, Takeuchi A, Arii Y, To A, Komura H, Hiroa H, Satomura K, Inoue M, Muramatsu S, Matsui K, Tada M, Sato M, Saijo E, Shigemitsu Y, Sakai S, Umetu Y, Goda N, Takino N, Takahashi H, Hagiwara M, Sasawaki T, Isagaki S, Nakamura Y, Nabeshima Y, Teplow DB, Hoshi M (2015) Na-K-ATPase α3 is a death target of Alzheimer patient amyloid-β assembly. Proc Natl Acad Sci USA 112, E4465–4474.
[19] Wang Y, Song M, Hou L, Yu Z, Chen H (2012) The newly identified K+ channel blocker talatisamine attenuates beta-amyloid oligomers induced neurotoxicity in cultured cortical neurons. Neurosci Lett 518, 122–127.
[20] Cotella D, Hernandez-Enriquez B, Wu X, Li R, Pan Z, Leveille J, Link CD, Oddo S, Sesti F (2012) Toxic role of
K+ channel oxidation in mammalian brain. *J Neurosci* **32**, 4133-4144.

[22] Rush T, Buisson A (2014) Reciprocal disruption of neuronal signaling and Aβ production mediated by extrasympathetic NMDA receptors: A downward spiral. *Cell Tissue Res* **356**, 279-286.

[23] Atamna H (2009) Amino acids variations in amyloid-beta peptides, mitochondrial dysfunction, and new therapies for Alzheimer’s disease. *J Bioenerg Biomembr* **41**, 457-464.

[24] Piccini A, Borghi R, Guglielmotto M, Tamagno E, Cirmena G, Garuti A, Pollero V, Cammarata S, Fornaro M, Messa M, Colombo L, Salmona M, Perry G, Tabaton M (2012) β-amyloid 1-42 induces physiological transcriptional regulation of BACE1. *J Neurochem* **122**, 1023-1031.

[25] Mizushima N, Levine B, Cuervo AM, Kionsky DJ (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069-1075.

[26] Tower J (2015) Programmed cell death in aging. *Ageing Res Rev* **23**(Pt A), 90-100.

[27] Ravikumar B, Berger Z, Vacher C, O’Kane CJ, Rubinsztein DC (2006) Rapamycin pre-treatment protects against apoptosis. *Hum Mol Genet* **15**, 1209-1216.

[28] Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y (2004) Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat Cell Biol* **6**, 1221-1228.

[29] Madden DT, Egger L, Bredesen DE (2007) A calpain-like protease inhibits autophagic cell death. *Autophagy* **3**, 519-522.

[30] Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Mohan PS, Mercken M, Farmery MR, Tjernberg LO, Jiang Y, Duff K, Uchiyama Y, Näslund J, Mathews PM, Cataldo AM, Nixon RA (2005) Macroautophagy—a novel Beta-amyloid peptide-generating pathway activated in Alzheimer’s disease. *J Cell Biol* **171**, 87-98.

[31] Lünenmann JD, Schmidt J, Schmid D, Barthel K, Wrede A, Dalakas MC, Minz C (2007) Beta-amyloid is a substrate of autophagy in sporadic inclusion body myositis. *Ann Neurol* **61**, 476-483.

[32] Steele JW, Ju S, Lachenmayer ML, Liken J, Stock A, Kim SH, Delgado LM, Alfaro IE, Bernales S, Verdile G, Bharadwaj P, Gupta V, Barr R, Friss D, Diolos G, Wang R, Ringe D, Procter AA, Martins RN, Ehrlich ME, Yue Z, Petsko GA, Gandy S (2006) Uch-L1 is dependent on NF-κB activation and impaired BACE1 lysosomal degradation. *Aging Cell* **5**, 914-923.

[33] Oddo S, Caccamo A, Cheng D, LaFerla FM (2009) Genetically altering Abeta distribution from the brain to the vasculature ameliorates tau pathology. *Brain Pathol* **19**, 421-430.

[34] Holton JL, Ghiso J, Lashley T, Rostagno A, Guerin CJ, Gibb G, Houlden H, Aylng H, Martinian L, Anderton BH, Wood NW, Vidal R, Plant G, Frangione B, Revesz T (2001) Regional distribution of amyloid-Bri deposition and its association with neurofibrillary degeneration in familial British dementia. *Am J Pathol* **158**, 515-526.

[35] Friedman LG, Qureshi YH, Yu WH (2015) Promoting autophagic clearance: Viable therapeutic targets in Alzheimer’s disease. *Neurotherapeutics* **12**, 94-108.