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REVIEW

Neurotoxicity in Alzheimer's disease: is covalently crosslinked Aβ responsible?

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Abstract Alzheimer's disease is the most common form of dementia in the elderly, and is characterised by extracellular amyloid plaques composed of the β-amyloid peptide (Aβ). However, disease progression has been shown to correlate more closely with the level of soluble Aβ oligomers. Recent evidence suggests that these oligomers are covalently crosslinked, possibly due to the interaction of Aβ with redox-active metal ions. These findings offer new avenues for the treatment and prevention of disease, by modulating metal binding or preventing the formation of neurotoxic Aβ oligomers.

Keywords Alzheimer · Beta-amyloid · Aβ · Oligomer · Covalent crosslinks · Metal · Neurotoxicity

Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly, comprising more than 50% of reported cases (Small 2000). The major pathological hallmark of AD is protein deposition, particularly the extracellular accumulation of β-amyloid peptide (Aβ) in amyloid plaques. Recent research, however, has revealed the surprising finding that, although amyloid plaques are the most obvious pathological feature of AD and Aβ has been shown to be cytotoxic, plaque burden does not correlate with disease progression. Instead, the severity of the disease correlates with the level of soluble Aβ oligomers [see Fig. 1, (McLean et al. 1999)]. The stability of these oligomers, coupled with the demonstrated ability of Aβ to undergo copper-induced redox chemistry, has led to the proposal that they are chemically crosslinked. That is, a covalent bond has formed between monomeric Aβ units.

At present, AD has a global incidence of 22 cases per 1,000 people in adults over the age of 75. The disease is characterised by neuronal death, oxidative damage, the hyperphosphorylation and intracellular deposition of the microtubule-associated protein tau, and, as noted above, the extracellular deposition of Aβ (Small 2000).

Aβ is cleaved from the amyloid precursor protein (APP) by proteases known as secretases (Small 2000). Controversy exists as to the pathogenic process of AD and the role of Aβ therein, but even those researchers who suggest that Aβ over-production or reduced clearance is not the primary cause of late-onset AD allow the peptide some role in the pathogenic process (e.g. Maurer and Hoyer 2006; Lee et al. 2007; Webber et al. 2007). Establishing the aetiology of AD has been complicated by the fact that γ-secretase can cleave APP at a number of residues, creating a pool of Aβ species of 38–46 residues long. The major species, $A\beta_{1-40,}$ is 40 residues long, but a 42 residue species, $A\beta_{1-42,}$ is more hydrophobic, has a greater tendency to aggregate, shows increased neurotoxicity, is more redox active and will seed aggregation of other Aβ species in the brain (Small 2000). Aβ also displays N-terminal truncations, including the modification of Glu-3 into pyroglutamate, which has been

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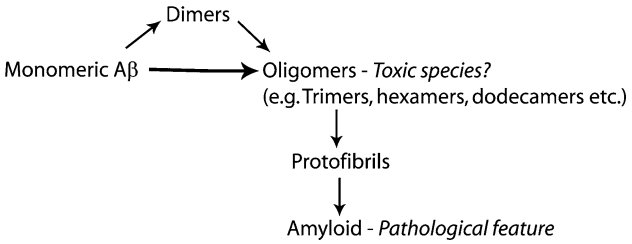

Fig. 1 Diagram of the proposed cascade leading from the formation of monomeric Aβ to amyloid deposition in AD. Although amyloid is the main pathological feature in AD, soluble oligomers are believed to be the main toxic species. Aggregation is believed to be transition metal dependant

shown to be a major component of amyloid plaques (Harigaya 2000).

Aβ oligomers

Soluble Aβ oligomers are defined as those species that remain in the supernatant following centrifugation at 100,000 × g. These oligomers are resistant to degradation into monomers by compounds such as hexafluoroisopropanol (HFIP), urea, sodium dodecyl sulphate (SDS) and formic acid (Podlisny et al. 1995; Walsh et al. 2002; Lesne et al. 2006). Aβ oligomerisation is essential to the pathology of AD: Aβ is a normal cellular product, and is only considered harmful when aggregated (Walsh et al. 2005; Cohen et al. 2006). It can assume a number of different aggregation states, and much controversy exists over which of these states is "the" toxic agent in AD. Trimers, hexamers, dodecamers and a host of other soluble assembly states up to the protofibril level have all been implicated as the potential toxic agent (Lambert et al. 1998; Lesne et al. 2006; Townsend et al. 2006; Wu et al. 2006). However, the concept of a single toxic state has been challenged by the observation that different aggregation states induce neurotoxicity by distinct mechanisms or have different cellular effects (Deshpande et al. 2006). Additionally, monomeric Aβ is capable of adopting a wide variety of conformations depending on its environment, which can result in structural polymorphisms in higher aggregation states (Petkova 2005). This finding suggests that a specific isoform of, for example, trimer, not trimers per se, may be the most toxic Aβ species. Townsend et al. (2006) demonstrate this by showing that oral administration of *scyllo*-inositol neutralises the synaptotoxic effects of Aβ trimers without destabilising them or otherwise influencing their profile on Western blots.

The aggregation of the Aβ peptide appears to be driven by interaction between Aβ and metal ions, particularly copper and zinc, for which the peptide has a high binding affinity (Atwood et al. 2000). This observation accounts for the tendency of amyloid plaques to be focused on synapses, where bioavailable metals are present at elevated concentrations ([Cu^{2+}] ~15 μM; [Zn^{2+}] ~ 300 μM). One form of metal coordination that can drive peptide aggregation is the formation of histidine bridges, where the imidazole side-chain of a His residue in a Cu-bound Aβ can also coordinate to the copper of a second Aβ peptide [see Fig. 2, (Smith et al. 2006)]. This forms a metal coordination site similar to the active site of SOD1, an enzyme involved in the neutralisation of superoxide radicals. Metal–induced aggregation is generally non-covalent and can be reversed by the addition of chelators (Cherny 1999). However, coordination of metals, particularly copper, by Aβ can also lead to the formation of covalent bonds (Atwood 2004; Barnham et al. 2004). Copper coordination induces redox chemistry, which leads to the oxidative modification of Aβ. One such modification is the formation of covalent dityrosine crosslinks. Another is the oxidation of the methionine at residue 35 of Aβ, which has been shown to profoundly affect the oligomerisation and toxicity of the peptide (Bitan 2003).

Proposed mechanisms of toxicity: dityrosine, membrane binding and radical formation

A detailed mechanism for the formation of dityrosine Aβ adducts is proposed in Barnham et al. (2004) and summarised in Fig. 3. In short, when Aβ binds copper (coordinated to histidine residues at positions 6, 13 and 14 and the tyrosine at position 10), it is capable of activating oxygen in the presence of a reducing substrate such as ascorbate, dopamine or thiols such as glutathione, and catalyses the reduction of oxygen to H_2O_2. The final step of this reaction involves the transfer of a hydrogen atom of the side-chain hydroxyl group of Tyr-10 to the nascent H_2O_2, forming a tyrosyl radical. While the Aβ/Cu catalyst can be regenerated by further reaction with the original reducing substrate, two tyrosyl radicals can also react to form dityrosine. Thus, the two peptides become covalently linked. Further support for the toxicity of these dityrosine-based oligomers has been shown by investigating a mutant peptide of Aβ where

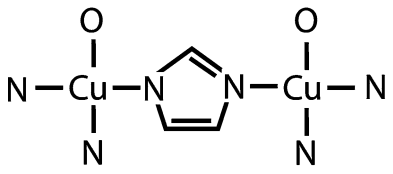

Fig. 2 Illustration of Cu^{2+} coordination by Aβ, where the metal is believed to be coordinated by His-6, His-13 and His-14 and Tyr-10. The imidazole side chain of a His residue bridges between two Cu^{2+} atoms to form dimeric Aβ

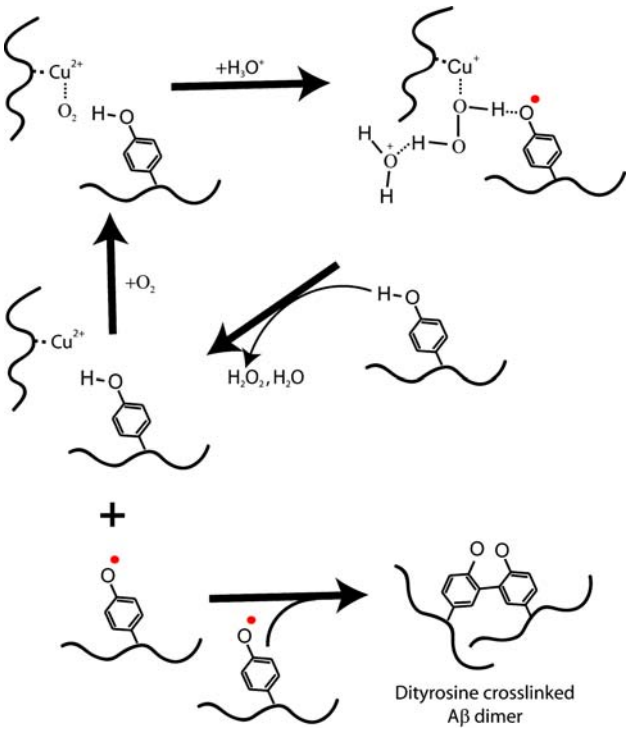

Fig. 3 Simplified diagram of the Aβ/Cu-catalysed production of H_2O_2 from O_2 and ascorbate. This reaction can generate tyrosyl radicals which can crosslink to form dityrosine crosslinked Aβ dimers

alanine is substituted for the tyrosine at position 10 (Y10A). The mutant Aβ peptide is incapable of generating dityrosine crosslinks, and although it produced significant amounts of H_2O_2 and was shown to aggregate with a similar propensity as the wildtype Aβ, Y10A did not induce any neurotoxicity (Barnham et al. 2004).

It has also been suggested Aβ toxicity is due to the catalytic formation of reactive oxygen species (ROS) from membrane-associated peptides. Experiments with an M35V mutant Aβ peptide (methionine to valine at position 35) have shown increased toxicity despite levels of H_2O_2 production similar to wild type Aβ. This is believed to be due to increased membrane binding (Ciccotosto et al. 2004). In vivo, AD brains display a high level of oxidative stress and ROS-mediated injury. ROS-mediated damage is detected in the brain primarily as an increase in lipid peroxidation, and increased levels of malondialdehyde, 4-hydroxynonenal, 8-hydroxydeoxyguanosine, protein carbonyls and, most importantly, increased dityrosine levels (Hensley et al. 1998).

The intricate relationship between Aβ, copper, radical and ROS formation and neurotoxicity has led to the development and use of compounds to modulate metal homeostasis and so prevent neurodegeneration. The metal ligand clioquinol (5-chloro-7-iodo-quinolin-8-ol) has been successfully used in vitro, in animal models and in small clinical trials. Clioquinol and similar compounds have been shown to inhibit the in vitro generation of H_2O_2 by Aβ, and reverse the aggregation of the peptide both in vitro and from human brain post-mortem specimens. In animal models, oral administration of clioquinol markedly reduced the accumulation of Aβ (Cherny et al. 2001). Further metal-protein attenuating compounds are in development.

Future directions

Despite this progress, the chemical nature of the toxic oligomeric Aβ species is still unknown, the exact nature of inter-molecular crosslinking and its method of formation has not been established. Whether this crosslinking is due to the cellular processing of APP and Aβ or to the intrinsic chemical nature of the peptide remains to be determined. As noted above, Aβ can be induced in vitro to form dityrosine crosslinks via a mechanism involving metal-catalysed redox chemistry. It is interesting to note that, of the mammalian proteins with intra-molecular crosslinks described in the literature (for example, cytochrome c oxidase, catalase and lysyl oxidase), many involve copper-based enzymes or enzymes associated with reactive oxygen species [reviewed in (Okeley and van der Donk 2000)]. Aβ, of course, is also associated with both ROS production and copper. However, although data supporting dityrosine formation in vivo have been published (Hensley et al. 1998), it has not yet been shown that dityrosine Aβ adducts can be isolated from in vivo sources. It has also been suggested that the oligomers may be linked by an N^{ε}-(γ-glutamyl)-lysine bond mediated by tissue transglutaminase. High levels of this enzyme have also been described in Huntington's disease and Parkinson's disease brains, and tissue transglutaminase is also activated during apoptosis (Ho et al. 1994; Nemes et al. 2001; Citron et al. 2002). If this is the case, clioquinol and other MPACs may be effective treatments due to secondary effects on Aβ production, such as metal-based modulation of secretase activity. Alternately, Aβ monomers may become crosslinked by other reactive cellular products, such as carbohydrates or aldehydes, including those produced by the effects of ROS created by Aβ/Cu complexes. Understanding how Aβ oligomerisation occurs is an essential step in understanding and preventing AD, so further research into the peptide's chemistry is essential.

References

Atwood CS, Perry G, Zeng H, Kato Y, Jones WD, Ling K, Huang X, Moir RD, Wong D, Sayre LM, Smith MA, Chen SG, Bush AI (2004) Copper mediates dityrosine cross-linking of Alzheimer's amyloid-ß. Biochemistry 43:560–568

Atwood CS, Scarpa RC, Huang X, Moir RD, Jones WD, Fairlie DP, Tanzi RE, Bush AI (2000) Characterization of copper interactions

with alzheimer amyloid beta peptides: identification of an attomolar-affinity copper binding site on amyloid beta1–42. J Neurochem 75:1219–1233

Barnham KJ, Haeffner F, Ciccotosto GD, Curtain CC, Tew D, Mavros C, Beyreuther K, Carrington D, Masters CL, Cherny RA, Cappai R, Bush AI (2004) Tyrosine gated electron transfer is key to the toxic mechanism of Alzheimer's disease beta-amyloid. FASEB J 18:1427–1429

Bitan G, Tarus B, Vollers SS, Lashuel HA, Condron MM, Straub JE, Teplow DB (2003) A molecular switch in amyloid assembly: Met35 and amyloid beta-protein oligomerisation. J Am Chem Soc 125:15359–15365

Cherny R, Legg JT, McLean CA, Fairlie DP, Huang X, Atwood CS, Beyreuther K, Tanzi RE, Masters CL, Bush AI (1999) Aqueous dissolution of Alzheimer's disease Abeta amyloid depositis by biometal depletion. J Biol Chem 274:232223–232228

Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30:665–676

Ciccotosto GD, Tew D, Curtain CC, Smith D, Carrington D, Masters CL, Bush AI, Cherny RA, Cappai R, Barnham KJ (2004) Enhanced toxicity and cellular binding of a modified amyloid b peptide with a methionine to valine substitution. J Biol Chem 279:42528–42534

Citron BA, Suo Z, SantaCruz K, Davies PJA, Qin F, Festoff BW (2002) Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. Neurochem Int 40:69–78

Cohen E, Bieschke J, Perciavalle RM, Kelly JW, Dillin A (2006) Opposing activities protect against age-onset proteotoxicity. Science 313:1604–1610

Deshpande A, Mina E, Glabe CG, Busciglio J (2006) Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. J Neurosci 26:6011–6018

Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG (2000) Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. Biochem Biophys Res Comm 276:422–427

Hensley K, Maidt ML, Yu Z, Sang H, Markesbery WR, Floyd RA (1998) Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. J Neurosci 18:8126–8132

Ho GJ, Gregory EJ, Smirnova IV, Zoubine MN, Festoff BW (1994) Cross-linking of beta-amyloid precursor catalyzed by tissue transglutaminase. FEBS Lett 349:151–154

Lambert MP, Barlow AK, Chromy BA, Edwards C, Fred R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Ab1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95:6448–6453

Lee H, Zhu X, Castellani RJ, Nunomura A, Perry G, Smith MA (2007) Amyloid-beta in Alzheimer disease: the null versus the alternate hypothesis. J Pharmacol Exp Ther 321:823–9

Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH 2006) A specific amylid-beta protein assembly in the brain impairs memory. Nature 440:352–357

Maurer K, Hoyer S (2006) Alois Alzheimer revisited: differences in origin of the disease carrying his name. J Neural Transm 113:1645–1658

McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL (1999) Soluble Pool of Aß Amyloid as a Determinant of Severity of Neurodegeneration in Alzheimer's Disease. Ann Neurol 46:860–866

Nemes Z, Fesus L, Egerhazi A, Keszthelyi A, Degrell IM (2001) N(epsilon)(gamma-glutamyl)lysine in cerebrospinal fluid marks Alzheimer type and vascular dementia. Neurobiol Aging 22:403–406

Okeley NM, van der Donk WA (2000) Novel cofactors via post-translational modifications of enzyme active sites. Chem Biol 7:R159–171

Petkova AT, Leapman RD, Guo Z, Yau W, Mattson MP, Tycko R (2005) Self-propagating, molecular-level polymorphism in Alzheimer's ß-amyloid fibrils. Science 307:262–265

Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ (1995) Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J Biol Chem 270:9564–9570

Small DH (2000) Recent findings on the biology of Alzheimer's disease. Res Pract Alzheimers Dis 3:27–33

Smith DP, Smith DG, Curtain CC, Boas JF, Pilbrow JR, Ciccotosto GD, Lau TL, Tew DJ, Perez K, Wade JD, Bush AI, Drew SC, Separovic F, Masters CL, Cappai R, Barnham KJ (2006) Copper-mediated amyloid-beta toxicity is associated with an intermolecular histidine bridge. J Biol Chem 281:15145–15154

Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers. J Physiol 572:477–492

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturaly secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416:535–539

Walsh DM, Townsend M, Podlisny MB, Shankar GM, Fadeeva JV, El Agnaf O, Hartley DM, Selkoe DJ (2005) Certain inhibitors of synthetic amyloid beta-peptide (Ab) fibrillogenesis block oligomerization of natural Ab and thereby rescue long-term potentiation. J Neurosci 25:2455–2462

Webber KM, Casadesus G, Atwood CS, Bowen RL, Perry G, Smith MA (2007) Gonadotropins: a cohesive gender-based etiology of Alzheimer disease. Mol Cell Endo 260–262:271–275

Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, Link CD, Luo Y (2006) Amyloid beta-induced pathological behaviors are suppressed by *Gingko biloba* extract EGb 761 and ginkolides in transgenic *Caenorhabditis elegans*. J Neurosci 26:13102–13113