The role of amyloid β in the pathogenesis of Alzheimer's disease

Barnabas James Gilbert

Correspondence to
Barnabas James Gilbert,
Medical Sciences Division,
University of Oxford,
Green Templeton College,
43 Woodstock Road,
Summertown,
Oxford OX2 6HG, UK;
barnabas.gilbert@gtc.ox.ac.uk

Received 2 February 2013
Revised 3 March 2013
Accepted 4 March 2013
Published Online First
23 March 2013

ABSTRACT

The amyloid-β peptide (Aβ) is widely considered to be the major toxic agent in the pathogenesis of Alzheimer's disease, a condition which afflicts approximately 36 million people worldwide. Despite a plethora of studies stretching back over two decades, identifying the toxic Aβ species has proved difficult. Debate has centred on the Aβ fibril and oligomer. Despite support from numerous experimental models, important questions linger regarding the role of the Aβ oligomer in particular. It is likely a huge array of oligomers, rather than a single species, which cause toxicity. Reappraisal of the role of the Aβ fibril points towards a dynamic relationship with the Aβ oligomer within an integrated system, as supported by evidence from microglia. However, some continue to doubt the pathological role of amyloid β, instead proposing a protective role. If the field is to progress, all Aβ oligomers should be characterised, the nomenclature revised and a consistent experimental protocol defined. For this to occur, collaboration will be required between major research groups and innovative analytical tools developed. Such action must surely be taken if amyloid-based therapeutic endeavour is to progress.

INTRODUCTION

Alzheimer's disease (AD) accounts for up to 70% of all diagnosed cases of dementia, an age-related condition which affects approximately 36 million people worldwide and will cost the UK economy over £23 billion in 2013.[1–3] AD is characterised by progressive cognitive decline which classically affects several cognitive domains including memory, visuospatial skill and executive function.[4 5] Later stage disease is associated with complex behavioural and psychological needs which often require specialist care.[6] There is marked, selective neuronal degeneration and synaptic loss, particularly in the hippocampus, amygdala and temporal neocortex.[7] These changes are accompanied by the formation of extracellular senile plaques and intraneuronal neurofibrillary tangles which comprise the cytoskeletal τ protein.[8]

Among the many factors involved in AD pathogenesis, the major toxic agent is thought to be the amyloid-β peptide (Aβ).[9] Aβ exists within a growing family of amyloid polypeptides which, following their misfolding, can interact inappropriately with specific cell types to invoke degenerative disease.[10] This is the case in Huntington's, Parkinson's and Prion diseases.[10] In AD, the 4 kDa Aβ monomer is cleaved from the amyloid precursor protein (APP) in the neuronal membrane by the secretase complex.[11] The monomer transitions from a random coil or α helix conformation to a β hairpin. This facilitates a dynamic nucleation-dependent polymerisation reaction which forms short, soluble, metastable intermediates called oligomers. These assemble to form an oligomeric nucleus which can be rapidly extended by monomer addition to form curvilinear protofibrils. Finally, protofibrils are bundled together to form the large, insoluble, cross β-sheet fibrils which accumulate in plaques.[12–17]

Despite many studies in recent years, identifying a true toxic Aβ species has proved contentious. Debate has centred on the potential toxicities of the Aβ fibril and oligomer, raising the inevitable question whether a single or multiple species are toxic. Hardy and Higgins[18] put forward the amyloid cascade hypothesis of AD more than 20 years ago, positioning the insoluble Aβ fibril as the primary toxic species. More recently, widespread support has been generated for the toxic Aβ oligomer hypothesis which proposes that oligomeric intermediates are toxic from an early stage in AD.[19] By monitoring neurotoxicity in terms of both cell death and synaptic plasticity, a heterogeneous array of brain-derived and synthetic oligomers have been identified as neurotoxic:[10] compact dimers and trimers;[20 21] small, globular Aβ-derived ligands;[22 23] doughnut-like, annular Aβ oligomers;[24] and large amylospheroids,[25] to name but a few. Despite support from numerous experimental paradigms, important questions linger regarding the role of the Aβ oligomer. Reappraisal of the role of the Aβ fibril has shed new light on how fibrillar and oligomeric species might integrate to induce toxicity. However, some continue to doubt the pathological role of amyloid β, instead proposing a protective role. This review seeks to critique the current status of the amyloid hypotheses and to make logical suggestions which may accelerate the quest for a successful amyloid-based therapy for AD.

EARLY EVIDENCE OF FIBRILLAR TOXICITY

As early as 1959, the long (>200 nm) and narrow (6–10 nm) structure of the amyloid fibril was revealed by electron microscopy.[26] x-Ray diffraction studies later showed a highly ordered, cross β-sheet arrangement which was stabilised by hydrogen bonds between polypeptide backbones.[27 28] Fibril-containing plaques were identified by histopathological studies in the affected tissues of various amyloid diseases, including AD.[29]

Following the original formulation of Hardy and Higgins[18] of the amyloid cascade hypothesis in the early 1990s, a wide body of in vitro evidence began to suggest that the Aβ fibril might be toxic. In a landmark study in 1993, Carl Cotman and colleagues found that synthetic Aβ preparations which '*exhibited significant aggregation*' were toxic to rat

To cite: Gilbert BJ. *J Clin Pathol* 2013;**66**:362–366.

hippocampal neurones in culture.[30] This was quantified using a cell viability assay which monitored the morphological appearance of photographed cells.[30] When the aggregation state of Aβ was partially reversed, toxicity fell. These findings were strengthened by the observations of Bruce Yankner and coworkers[31] who noted dystrophic neurite formation occurring prior to neuronal death when Aβ was applied to these neurones. Lorenzo and Yankner[32] showed that synthetic Aβ fibrils were toxic to hippocampal neurones, while amorphous, non-amyloidogenic Aβ aggregates were not. Moreover, toxicity could be attenuated by Congo red, a dye known to inhibit fibril formation.[32] Markedly, this was the first study to overtly state that these effects were mediated by the Aβ *fibril*.

Crucially, much of this early in vitro evidence failed to exclude the possibility of oligomeric toxicity. Lorenzo and Yankner[32] had noted that solutions containing only the Aβ monomer were innocuous, but always considered the aggregation process in terms of its end product, the Aβ fibril, without considering that an intermediate species might be toxic. These early investigators failed to account for the artificial air–water interface present in vitro, which is now known to induce conformational changes in Aβ which promote fibrillogenesis.[33] Cell culture experiments also lack the macromolecular crowding present in vivo and poorly replicate the cell–cell and synaptic interactions present in the AD brain.[7 34]

For those who maintained that the culprit of AD toxicity had been found, there remained several unavoidable conundrums. First, plaque load and distribution correlated poorly with symptom severity in the AD patient.[35] Second, in various APP transgenic mouse models, neuronal loss and associated behavioural abnormalities were shown to occur prior to plaque formation.[36–39] Third, Aβ fibrils derived from the AD brain lacked consistent toxic effects in rat and human neurones in vitro.[40] As pressure continued to mount, new evidence of a toxic Aβ oligomer began to emerge.

THE TOXIC OLIGOMER HYPOTHESIS

Interest in the Aβ oligomer originated from a study conducted by Dennis Selkoe's group in 1995.[41] Selkoe showed that Chinese hamster ovary cells expressing a mutant form of the human *APP* gene could secrete small Aβ oligomers in culture. These were shown by immunoprecipitation assay to weigh 6, 8 and 12 kDa, alongside the established 4 kDa monomer. Rigorous controls were performed to prove that these cell-derived Aβ species had not arisen during immunoprecipitation. However, the oligomeric bands were detected at lower levels than the monomer, causing the investigators to use high APP-expressing cell lines in subsequent experiments.

Researchers soon began to conduct studies in models which more closely mimicked the environment of the AD brain. One such model is the human APP (hAPP) transgenic mouse,[36–39] engineered to overexpress various mutant forms of APP associated with familial AD. hAPP mice develop plaques with much of the associated neuropathology of human AD. Lesné *et al*[38] found that a dodecameric 56 kDa Aβ species (Aβ*56) generated from a Swedish-mutated hAPP could strongly disturb memory in *Tg2576* mice expressing the peptide. This effect was maintained when Aβ*56 was purified from the brains of *Tg2576* mice and injected into the brains of young rats. Similar results have since been attained in the *J20* and *3xTg-AD* transgenic mouse models.[39 42]

However, the study of Lesné *et al*[38] highlights various issues facing oligomeric research. Not only is the nomenclature highly nuanced, often varying according to laboratory preference, but investigating a single species could prove ineffective if multiple species are in fact toxic. The hAPP transgenic mouse model is also limited; foremost, neuronal loss and atrophy tend to be slight compared with the overwhelming cell loss seen in the AD brain.[43] Performance in rodent behavioural tests such as the Morris water maze can be affected by many factors, including age, handling and sleep cycle,[44] any of which could affect the potential memory or cognitive effects of the Aβ species under investigation.

In order to reduce the use of live animals, investigators began to probe oligomeric toxicity using semi-in vivo organotypic brain slice cultures.[45] These slices are developed from the neonatal brain tissue of mice or rats and maintain the anatomical relations and synaptic qualities of the rodent brain.[45] The regionally selective toxicity of Aβ oligomers in mouse cerebral slices was found to mirror the degenerative patterns common in AD.[46] Live-dead toxicity assays also revealed marked atrophy in the entorhinal cortex and hippocampal CA1 region, with sparing of cerebellar neurones.[46]

In order to better understand the memory loss associated with AD, the Selkoe group used brain slice cultures to monitor changes in synaptic plasticity.[47] By adding Aβ oligomers extracted from the cortex of AD patients to rat hippocampal slices, they showed that the memory-related processes of long-term potentiation (LTP) and long-term depression can be attenuated and enhanced, respectively.[47] The role of the Aβ oligomer in LTP is now known to be intricately related to the NMDA-type glutamate receptor (NMDA-R). Using an amplicon vector to knock-down NMDA-Rs in cultured hippocampal neurones, Decker *et al*[48] found that they could avert the oxidative stress that otherwise arose in the presence of Aβ oligomers. This was likely due to the prevention of upstream changes in calcium homeostasis.[48] Randomised controlled trials have since shown the NMDA-R antagonist memantine to be reasonably effective in treating late stage disease.[49 50]

However, even in organotypic slice cultures, individual cells can be difficult to track, afferent nervous pathways and blood vessels may be severed, and resistance to ischaemic damage is low.[51] Findings from the postmortem *human* brain may therefore be more reliable and there have been a number of poignant findings. By using dot blot assays to compare extracts of homogenised frontal cortex from five AD patients versus healthy, age-matched controls, Gong *et al*[52] found that the levels of soluble Aβ oligomers could rise as much as 70-fold in the AD brain. However, postmortem brain tissue is limited in availability and its extraction and preservation can cause protein degradation.[53]

One area of research which continues to strengthen the toxic oligomer hypothesis and unites many of these experimental paradigms is the interaction of Aβ oligomers with the intracellular τ protein. Although τ plays a key role in microtubule stabilisation under physiological conditions,[54] the intraneuronal accumulation of Aβ oligomers can activate signalling pathways which cause τ hyperphosphorylation and subsequent cytoskeletal changes resulting in neuronal dysfunction.[42 54–56] Notably, τ knockdown in cultured hippocampal neurones can limit the cytoskeletal disruption and neuritic dystrophy induced by brain-derived Aβ oligomers.[54] Further, hippocampal slices from τ knockout mice are resistant to the LTP inhibition induced by synthetic Aβ oligomers,[56] although such species are known to correlate poorly with brain-derived oligomers in toxicity assays.[57] Both Aβ and τ pathologies can also be eliminated via intrahippocampal injection of a specific anti-Aβ oligomer *antibody* in *3xTg-AD* mice.[42] It thus appears that Aβ oligomers, acting in concert with τ, can increase the severity of disease.

REAPPRAISING THE ROLE OF THE Aβ FIBRIL: AN INTEGRATIVE MODEL

In light of this assembling evidence for a toxic Aβ oligomer, the role of the Aβ fibril in AD pathogenesis is being reappraised. Some maintain that the Aβ fibril is an inert species, representing only an inactive reservoir of smaller, readily diffusible oligomers; others suggest that the Aβ fibril, while not inherently toxic, may induce neurotoxicity indirectly. As the notion of an amyloid cascade suggests, fibrillogenesis itself may provide a source of toxic factors, as supported by evidence that fibrillar growth enhances toxicity.[58]

One intriguing line of study in microglia formulates an integrative model in which the Aβ fibril binds to a surface receptor complex and is endocytosed and targeted to the cell lysosomes, where it is partially digested by lysosomal enzymes to release fragmented oligomers.[59–62] These oligomers act to perturb the lysosomal membrane and are released into the cytosol where, among a host of potential interactions, they bind to proteasomes to cause dysfunction and cell death.[63] Sequestered lysosomal calcium and hydrolase enzymes are released with downstream apoptotic effects.[64 65]

These observations are supported by the inflammation hypothesis of AD which posits active, phagocytic microglia as the primary cause of toxicity.[66] This hypothesis proposes that Aβ oligomers trigger conversion to a pro-inflammatory phenotype in early AD, while Aβ fibrils induce a later, chronic inflammatory state.[66] According to this model, Aβ fibrils invoke a vicious cycle of cell recruitment and cell death, as dying microglia release many Aβ species which attack surrounding neurones and newly recruited inflammatory cells.[66]

THE ALTERNATE HYPOTHESIS

Perry and colleagues propose a controversial alternate hypothesis in which Aβ functions not as an initiator of disease, but rather as a protective response to neuronal insult.[67] According to this theory, Aβ attenuates oxidative stress in vivo by acting as an antioxidant to prevent neuronal apoptosis.[68] There is some supportive evidence: first, the levels of Aβ and reactive oxygen species in the AD brain may be negatively correlated;[69] second, Aβ has been shown to prevent neuronal death following injection of iron or saline;[70] and, third, in the plasma and cerebrospinal fluid (CSF), Aβ has been shown to protect lipoproteins from oxidation.[71] However, the alternate hypothesis remains controversial. If true, vast swathes of AD research would be discredited and the focus for research should shift to the dominant upstream processes of oxidative stress and inflammation.

SUGGESTIONS FOR INVESTIGATORS

If the field is to finalise the nature of the toxic species, logical steps must be taken to ensure that future experiments are conducted in conditions resembling those in vivo. Investigators should describe the source and aggregation state of all Aβ species used and should characterise the Aβ species present *after* each experiment, since metastable oligomers can associate with other Aβ species to form new structures which alter the toxicity of an Aβ solution over time.[72] Of the numerous methods presently used to characterise Aβ species, only techniques accessible to all laboratories should be employed. Further, the use of natural, brain-derived oligomers should be encouraged and synthetic oligomers, which may lack toxicity,[57] should be avoided.

Highly prioritised on the research agenda should be the structural characterisation and longitudinal profiling of all Aβ oligomers present in the AD brain, as this may ultimately facilitate presymptomatic diagnosis of AD. Soluble oligomers could be isolated from the CSF of at-risk patients via lumbar puncture.[73] Specific CSF oligomers could then be quantified using an analytical tool such as the NanoMonitor assay designed by Sierks *et al*.[74] This combines the imaging resolution of atomic force microscopy with highly selective 'nanobodies'.[74] Another powerful approach would be to develop widespread, cost-effective biomarkers which could be used to monitor the Aβ load in the live AD brain, using techniques such as positron emission tomography. Pittsburgh Compound-B is an example of one such biomarker, although its use is currently limited by its complex synthesis and short half-life.[75 76]

Due to ambiguity in the current nomenclature, a simplified, structural basis for naming oligomers might accelerate progress. A new scheme could adapt the model of Kayed *et al*,[77] classifying Aβ oligomers as either spherical 'prefibrillar' or rod-like 'fibrillar' structures, as recognised by conformation-dependent antibodies. If all oligomers were classified according to these criteria, specific on-pathway species could be subclassified according to their number of subunits or molecular weight. Although presently hindered by a lack of structural differences between species, the development of a wide panel of oligomer-specific antibodies would aid this classification process.

Finally, while in vivo experiments should be encouraged, further in vitro studies should seek to remove the air–water interface as this accelerates fibrillogenesis. Hydrophilic occlusion plugs are sufficiently hydrophilic to prevent amyloid peptides transitioning from the monolayer to the multilayer adsorption state which otherwise favours aggregation.[33]

WHY ARE AMYLOID-BASED CLINICAL TRIALS FAILING?

Clinical trials have examined numerous facets of amyloid biology: production, aggregation and clearance by immunotherapy. However, high-profile, late-phase clinical trials continue to fail. After the AN-1792 vaccine was discontinued in 2002 due to the occurrence of meningoencephalitis in 6% of subjects,[78] the monoclonal antibody bapineuzumab was designed to resemble the antibody stimulated by the vaccine.[78] Although bapineuzumab held significant therapeutic promise in early-phase trials, it failed to show cognitive or behavioural benefits in a major phase III trial in 2012.[79 80] Solanezumab has also failed to slow memory decline in patients with mild-to-moderate AD.[80]

For the field to progress and the pharmaceutical industry to keep investing in clinical trials, innovative trial designs are required which gauge therapeutic efficacy quickly and accurately. Biomarkers should replace traditional measures of cognitive function. Since plaque development occurs over many years, the timing of clinical intervention is important. Several preventative studies are set to begin in 2013 in patients identified at an increased risk of AD. For example, the Alzheimer's Prevention Initiative will evaluate the effects of crenezumab in subjects with presenilin 1 gene mutations which are known to cause AD in middle age.[81]

CONCLUSIONS

Although toxic roles have been postulated for numerous polypeptides in Alzheimer's pathogenesis, Aβ appears to be the dominant neurotoxic peptide. A multitude of studies over the past two decades have affirmed that the neurotoxic effects of the Aβ fibril and oligomer are diverse and complex. Some have even supported an alternate hypothesis in which amyloid species mediate a protective response. However, the weight of evidence presently favours the Aβ *oligomer* as the more toxic species. Its toxicity may relate to a greater capacity for diffusion and larger

collective surface area for interacting with neurones and glial cells. The role of the Aβ fibril in Alzheimer's pathogenesis might best be considered in terms of its dynamic relationship with the Aβ oligomer, rather than as an independent entity. This hypothesis is strengthened by evidence of their integrative function in microglia.

It is likely a huge array of oligomers, an 'Aβ soup',[19] which causes toxicity, rather than a single species. Further progress may require revision of the oligomeric nomenclature and clarification of a consistent experimental protocol. This could be achieved through online conferences between the heads of major research groups, as typified by the Alzforum 'webinar' model. Perhaps the most crucial area for further progress should be the development of accurate and accessible analytical tools to study and to monitor Aβ species in the AD brain. Such action must surely be taken if amyloid-based diagnostic and therapeutic endeavour is to progress.

Key messages

- The amyloid-β peptide (Aβ) is thought to be the principal pathogenic agent in Alzheimer's disease.
- Evidence supports a toxic role for a wide array of Aβ oligomers.
- For the field of amyloid-based therapy to progress, all Aβ oligomers should be characterised, the nomenclature revised and a consistent experimental protocol defined.
- This will necessitate collaborative research effort and the development of innovative analytical tools.

Acknowledgements The author thanks Dr Letitia Jean for constructive discussions during the planning stage.

Contributors The author was responsible for the planning, conduct and reporting of the work described in the article. The author is responsible for the overall content as the guarantor.

Competing interests None.

Provenance and peer review Not commissioned; internally peer reviewed.

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Barnabas James Gilbert

J Clin Pathol 2013 66: 362-366 originally published online March 23, 2013
doi: 10.1136/jclinpath-2013-201515