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REVIEW

Brain amyloid β protein and memory disruption in Alzheimer's disease

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Abstract: The development of amyloid-containing neuritic plaques is an invariable characteristic of Alzheimer's diseases (AD). The conversion from monomeric amyloid β protein (Aβ) to oligomeric Aβ and finally neuritic plaques is highly dynamic. The specific Aβ species that is correlated with disease severity remains to be discovered. Oligomeric Aβ has been detected in cultured cells, rodent and human brains, as well as human cerebrospinal fluid. Synthetic, cell, and brain derived Aβ oligomers have been found to inhibit hippocampal long-term potentiation (LTP) and this effect can be suppressed by the blockage of Aβ oligomer formation. A large body of evidence suggests that Aβ oligomers inhibit N-methyl-D-aspartate receptor dependent LTP; additional receptors have also been found to elicit downstream pathways upon binding to Aβ oligomers. Amyloid antibodies and small molecular compounds that reduce brain Aβ levels and block Aβ oligomer formation are capable of reversing synaptic dysfunction and these approaches hold a promising therapeutic potential to rescue memory disruption.

Keywords: Alzheimer, amyloid, oligomer, long-term potentiation, NMDA

Amyloid β protein and Alzheimer's disease

Alzheimer's disease (AD) is pathologically characterized by the presence of intracellular neurofibrillary tangles and extracellular neuritic plaques.[1] Neurofibrillary tangles are mainly composed of hyperphosphorylated tau protein while neuritic plaques are formed by a gradual accumulation of amyloid β protein (Aβ). Aβ is produced by the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase.[1–3] The APP gene encodes three different splicing isoforms, APP770, APP751 and APP695. The ectodomain of APP contains Kunitz protease inhibitor region (KPI) that accounts for the major difference in precursor sizes. Earlier studies have shown that KPI containing APP are present in dystrophic neuritis and may be associated with Aβ production and senile plaque formation.[4] The final cleavage by the γ-secretase determines the length of Aβ peptides. Among various Aβ isoforms, the most common ones are 40-residue Aβ (Aβ40) and 42-residue Aβ (Aβ42). The γ-secretase complex contains four components, presenilin 1 (PS1), nicastrin, anterior pharynx defective-1 (APH-1) and presenilin enhancer-2 (PEN-2).[5] Two aspartate residues located at the transmembrane domain 6 and 7 of PS1 constitute the active site of the γ-secretase.[6] Nicastrin was found to associate with the C-terminal fragments of APP and required for γ-secretase activity.[7,8] Genetic screens in *Caenorhabditis elegans* revealed two additional components of the γ-secretase components, APH-1,[9] and PEN-2.[10]

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The temporal sequence of deposition for different Aβ species is critical for understanding the pathogenesis of neuritic plaques in brains.[11] While the number of neuritic plaques may not be correlated to the severity of dementia in a linear fashion, the levels of Aβ42 are closely associated with the disease.[12] Among a subset of nondemented subjects who carry classic AD pathology, as determined by Aβ immunoreactive plaques and thioflavin histofluorescent plaques, the concentration of insoluble Aβ is similar to those from AD patients. The soluble pool of Aβ, which could be both extracellular and intracellular, differentiates AD from nondemented subjects and shows a strong inverse corelationship with synapse loss.[13] Measuring the soluble and insoluble Aβ pools from another subset of AD and control subjects indicated that levels of total and insoluble Aβ differentiate AD from control subjects although not the disease severity. Likewise, the soluble pool of Aβ is increased threefold in AD subjects and correlates with disease severity.[14] Pathological comparison of nondemented subjects, or those at the very early stage of dementia, with demented subjects suggests that an increase in Aβ40 and Aβ42 correlates with the progression of dementia and precedes apparent tau pathology in the frontal cortex of the brain.[15] Plasma Aβ42 is also elevated in patients carrying familial Alzheimer disease-linked mutations in PS1, PS2 and APP genes.[16] All autosomal dominant mutations have been found in PS and APP genes, and missense mutations in PS and APP genes account for the majority of early onset familial AD cases. Most of these familial AD (FAD) patients have a very early onset of disease, reflecting an increase of both peripheral and cerebral deposition of Aβ42. Among most AD patients, evidence suggests that a small pool of soluble Aβ may contain the toxic form of Aβ that causes neurodegeneration. This pool of Aβ may contribute to the large static pool of insoluble Aβ and form neuritic plaques over time.

Oligomeric Aβ and Aβ42

Biochemical analysis of the soluble pool of Aβ has revealed a fraction of Aβ specimens to migrate as 6–8 kDa on electrophoresis gel. Extracted from aged human cortical samples, these sodium dodecyl sulfate (SDS)-stable Aβ species corresponding to the size of dimers are detected by Western blot analysis using antibodies against Aβ. Similar SDS-stable Aβ dimers are also detected in brain lysates from PDGF-driven hAPP PDAPP transgenic mice that over express human APP gene.[17]

The Aβ dimer and higher molecular weight Aβ trimer have been similarly discovered in the media of Chinese hamster ovary (CHO) cells expressing human APP.[18] These oligomeric Aβ have been immunoprecipitated with a number of Aβ antibodies, and the authenticity of Aβ peptide forming the dimer and trimer has been confirmed by amino acid sequencing.[18] When CHO cells coexpress APP and a FAD-linked mutant PS1 or PS2 gene, oligomeric Aβ has been detected in the culture media that contains high levels of Aβ42 monomers. These results clearly indicate that increase in Aβ42 monomers facilitate the aggregation and formation of Aβ oligomers.[12,19]

The significance of Aβ42 has been elucidated in an earlier report on the biochemical and pathological comparison of AD brains.[20] A third of brains with no congophilic angiopathy have been found to be carrying a majority of Aβ species ending at residue 42.[20] The same amount of Aβ42 has also been found in brains with substantial congophilic angiopathy, although these brains contain far more Aβ40. Immunohistochemical staining has revealed that Aβ42 is primarily located in the senile plaques, while Aβ40 is mainly in blood vessel walls.[20] The association of Aβ42 in neuritic plaques and disease onset appears to be the outcome of oligomer formation at the early stages. Biophysical analysis of synthetic Aβ peptides ending at 40 and 42 pinpoints the critical amino acids at residue 41 and 42, isoleucine-41 (Ile-42) and alanine-42 (Ala-42). Aβ40 monomers and oligomers can quickly reach equilibrium; however, Aβ42 preferentially forms pentamer/hexamer followed by subsequent conversion to early protofibrils. The addition of residue Ile-41 to Aβ40 facilitates the formation of pentamer/hexamer and the residue Ala-42 is needed for further formation of protofibrils.[21] The major subcellular compartment that contains dimeric Aβ is the lipid raft, which carries a quarter of the brain's Aβ40 and Aβ42. Dimeric Aβ is detected in lipid rafts from transgenic (Tg) Tg2576 mouse brains at 6 months when no amyloid plaque are present although memory impairment starts to emerge. The levels of dimeric Aβ continue to increase, and then apolipoprotein E (ApoE) and phosphorylated tau start to accumulate in the lipid rafts. When animals are two years or older, a 500 fold increase of dimeric Aβ has been found in the lipid rafts. Importantly, similar increases of dimeric Aβ, ApoE and phosphorylated tau are also found in brains of AD patients.[22]

Aβ oligomers and memory impairment

A disconnection of memory impairment and plaque formation has been explored in transgenic mice overexpressing wild type (wt) or mutant APP. Loss of synaptophysin-immunoreactive

(SYN-IR) presynaptic terminals in specific brain regions has been correlated to cognitive decline in AD and used as an index for comparing wt and mutant APP transgenic mice. In transgenic mice expressing high levels of wt APP, there is no plaque formation even when large amounts of Aβ42 peptides are generated. This causes a significant decrease of SYN-IR presynaptic terminals, which is inversely correlated to Aβ42 levels.[23] In transgenic mice overexpressing FAD linked (APP V717F) mutant APP, SYN-IR presynaptic terminals decrease before the appearance of amyloid plaques.[24] Significant deficits in synaptic transmission have been detected by electrophysiological recordings from the hippocampus of these mutant transgenic mice in the absence of plaques. When the Swedish mutation is introduced to APP, those transgenic mice generate more Aβ peptide with relatively lower expression levels of Swedish mutant APP. The young transgenic mice do not develop plaques although they do show increased synaptic transmission deficits.[24] Apparently, Aβ peptides exhibit neurotoxicity independent of plaque formation.

Although the neurotoxic effect of Aβ42 is observed in very young mice, there is a significant delay in any behavioral effect upon injection of aggregated Aβ42 into the hippocampi of rats. After rats were trained in two-lever operant chambers under an alternating lever cyclic-ratio schedule, aggregated Aβ42 was injected into the CA3 area of the hippocampus. Severe deficits were shown in behavioral tests at 30 days post injection, with much greater symptoms at 50 days post injection.[25] Upon intracerebroventricular injection of Aβ42 into rat brains, low-frequency stimulation induces long-term depression (LTD), while the low-frequency stimulation alone is not sufficient. Therefore, Aβ42 promotes a long-lasting reduction in synaptic strength and causes disruption of the processing that relies on hippocampal synaptic plasticity.[26]

The synthetic Aβ peptides have been used to generate low molecular weight oligomers like Aβ-derived diffusible ligands (ADDLs). At nanomolar concentrations, ADDLs are neurotoxic *in vitro*. ADDLs are found to inhibit hippocampal long-term potentiation (LTP) and affect neural signal transduction.[27] Synthetic ADDLs have been correlated to synapse loss and memory failure and their properties are highly similar to those Aβ oligomers detected in brains using antibodies raised against synthetic Aβ oligomers. When compared to control brains, more than a 70 fold increase of oligomer Aβ has been found in the frontal cortex of AD patients. Both synthetic ADDLs and brain derived oligomeric Aβ are found attached to cultured hippocampal neurons and bound to dendrite surfaces.[28] When rat hippocampal slices were preincubated with ADDLs, Tetanus-induced LTP and reversal of LTD were strongly inhibited, while LTD was not affected.[29]

Aβ oligomers are formed inside cells and subsequently secreted into the media. Microinjection of Aβ oligomer-containing cell media clearly inhibits hippocampal LTP in rats. The inhibitory effect could be blocked by removing all Aβs with Aβ antibodies. However, inhibition could not be blocked in media pretreated with insulin-degrading enzyme that specifically targets Aβ monomers, though not oligomers. On the other hand, γ-secretase inhibitors used to treat cells at dosages which slightly reduce Aβ monomer production lead to a reduction in oligomeric Aβ formation. Media from γ-secretase inhibitor-treated cells contain Aβ monomers, although not oligomers, and fail to disrupt LTP.[30] The low number of oligomers, including the dimmers, trimers or tetramers, but not monomers, are found to: block the hippocampal LTP; do not affect presynaptic vesicle release; and fail to affect LTP in juvenile mice and brain-derived neurotrophic-factor-induced LTP in the adult hippocampus.[31] Importantly, Aβ dimers isolated from brains of AD subjects have shown the same effect.[32]

In APP transgenic mice Tg2576, little or no neuronal cell loss appears concurrently with the accumulation of Aβ and memory impairment. Tg2576 mice younger than 6 months old have normal memory, middle-aged mice (6–14 months old) show memory deficits in the absence of neuronal loss and striking neuropathology, and mice at 14 months old develop abundant neuritic plaques. While small Aβ dimers and trimers derived from cultured cells are known to specifically disrupt cognitive function,[33] a unique form of Aβ oligomer, termed Aβ*56, has been purified from the brains of middle-aged (6–14 month old) Tg2576 mice that impairs memory function once it is administered to young rats. Because Tg2576 mice start to develop plaques after 14 months, the accumulation of soluble Aβ*56 in the brains of middle-aged Tg2576 mice may be responsible for cognitive deficit, which is independent of plaque formation.[34] However, it is not clear whether Aβ*56 is unique to Tg2576 or a universal Aβ oligomer species that can be identified across most middle aged transgenic mouse lines that develop neuritic plaques at a later time.

From Aβ to synaptic deficits: possible mechanisms

A number of mechanisms of action have been proposed for the effect of Aβ on synaptic signaling. In cultured cortical neurons, Aβ has been found to promote the endocytosis of N-methyl-D-aspartate (NMDA) receptors and the

reduction of Aβ by γ-secretase inhibitor which suppresses the internalization of NMDA receptors. On the other hand, lower levels of cell surface NMDA receptors are found in APP transgenic mice that carry high levels of Aβ. α-7 nicotinic receptors (nAChRs), protein phosphatase 2B and the striatal-enriched protein (STEP) tyrosine phosphatase that are required for endocytosis of NMDA receptors; which is correlated to the dephosphorylation of the NMDA receptor-2B at Tyr1472.[35] Aβ has been shown to inhibit NMDA receptor dependent LTP although not the NMDA receptor independent LTP or LTD. This inhibition requires the activation of microglia and involves inducible nitric oxide synthase (iNOS) and superoxide. These inflammatory related reactions seem to be necessary for the Aβ mediated inhibition of LTP; which could be prevented in the presence of minocycline (inhibition of microglia activation), nicotinamide adenine dinucleotide phosphate-H oxidase inhibitor, or in iNOS knock out mice.[36]

In differentiated cultures of hippocampal neurons, synthetic ADDLs bind to excitatory pyramidal neurons although not γ-aminobutyric acid (GABA)-inergic neurons and are associated with postsynaptic density complexes containing NMDA receptors. It decreases expression of memory-related receptors (NMDA and ephrin-B2) and causes abnormal spine morphology and a significant decrease in spine density.[37] The NMDA-evoked cell firing rate has been studied in CA1 neurons in the rat and Aβ42 significantly increases NMDA responses, an effect which is irreversible.[38] The peptide Aβ25–35 shows a similar effect. On the other hand dendritic spine loss in the presence of Aβ oligomers is reversible once Aβ is eliminated by antibodies. When exposed to a physiological concentration of Aβ oligomers (a picomolar concentration), the density of dendritic spines decreases, and the active synapses are reduced. Furthermore, the activity of NMDA receptors is required for Aβ oligomer to affect dendritic spine morphological changes, and NMDA receptor-mediated calcium influx into dendritic spines decrease in the presence of Aβ oligomers.[39]

Application of Aβ to astrocytes *in vitro* leads to an inhibition of glutamate uptake along with rapid depolarization of astroglial membranes. Infusion of Aβ via microdialysis in the rat magnocellular nucleus basalis (MBN) leads to an acute increase of the extracellular concentration of excitatory amino acid neurotransmitters and enhances the intracellular accumulation of Ca^{2+} in the injection area. The effect of Aβ in the MBN can be suppressed by the NMDA receptor channel blocker dizocilpine maleate MK-801, suggesting multiple occurring events upon Aβ insult, eg, astroglial depolarization, extracellular glutamate accumulation, intracellular Ca^{2+} increase, and NMDA receptor activation.[40] On the other hand, an earlier study shows that MK-801 at the dose that produces a similar inhibition of NMDA potentials shows no effect on LTP, while Aβ produces a significant inhibition of NMDA receptor-mediated synaptic potentials.[41] Therefore, it is possible that Aβ affects multiple pathways leading to the inhibition of LTP, and it is important to understand whether these downstream pathways are among the main contributors to the effect of Aβ on LTP.

Besides NMDA receptors, Aβ also promotes endocytosis of synaptic α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPAR), which causes the loss of dendritic spines.[42] Consistent with this finding, double knock-in mice expressing mutant APP and presenilin genes show age-dependent downscaling of AMPAR-mediated evoked currents. Accordingly, age-related deficits in LTP/LTD are also found in the double knock-in mice.[43]

A receptor for advanced glycation end products (RAGE) dependent pathway has been proposed for Aβ mediated synaptic dysfunction. Inhibition of LTP by Aβ is abolished in slices from anti-RAGE antibody-treated wild type mice. Furthermore, no inhibition has been observed in slices from RAGE knock-out mice or transgenic mice expressing a dominant negative mutant of RAGE. Since activation of p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation is decreased by antibodies against RAGE, suppression of LTP inhibition has been achieved by blocking p38 MAPK. These studies illustrate a RAGE-dependent pathway that involves Aβ induced activation of p38 MAPK to impair LTP.[44] On the other hand, Aβ has been shown to activate the complement cascade and generate complement component C5-derived anaphilatoxin C5a, which activates MAPK and mediates neuroprotection.[45,46] The complex pathways downstream of Aβ reflect direct and indirect activation of MAPK pathways[47] and may have different effect on synaptic function.

Recently, cellular prion protein (PrP(C)) has been found to bind to Aβ oligomers, and the infectious prion protein PrP(Sc) conformation is not required for the interaction.[48,49] The binding of Aβ oligomers to PrP(C) can be blocked by anti-PrP antibodies, and the inhibitory effect on LTP is suppressed.[48] Whether PrP(C) plays a critical role in recognizing Aβ oligomers and mediating downstream synaptic response is not clear, as conflicting results have been reported on LTP in Aβ oligomer-treated hippocampal slices from PrP null mice.[48,49]

Pharmacological inhibition of ion translocation by the Na^+, K^+-adenosine triphosphatease has been observed in the presence of micromolar concentrations of Aβ1-42.[50] Inhibition of calcineurin activity by FK506 or cyclosporine A reveals the calcineurin-dependent inhibition of LTP by Aβ42.[51] The disruptions in Ca^{2+} signaling are caused not only by Aβ oligomers but also by amyloid-type oligomers like prions and polyglutamine. Amyloid oligomers increase intracellular Ca^{2+}, and Ca^{2+} signals induced by Aβ42 oligomers are derived from extracellular and intracellular Ca^{2+} sources. Treating a fluorescent dye loaded neuroblastoma cell line with Aβ oligomers leads to an increase in membrane permeability and the leakage of anionic dye. When membrane permeability is increased in the presence of Aβ or other oligomers, cells with a high transmembrane concentration gradient of Ca^{2+} ion are extremely vulnerable.[52]

Additional pathways that are implicated in Aβ mediated synaptic deficits include altered serine/threonine protein kinase (Akt), glycogen synthase kinase3β (GSK3β) together with the phosphatase and tensin homolog (PTEN). Exposing organotypic slice culture to synthetic Aβ25–35 peptide leads to an initial increase then decrease in phosphorylation of Akt and GSK3β, followed by an increase of PTEN protein after one day of exposure.[53]

In animals, analysis of APP transgenic mice reveals an upregulation of nAChRs along with a decrease of the MAPK in hippocampus. The phosphorylation of cyclic adenosine monophosphate regulatory element binding (CREB) protein is decreased by high levels of Aβ42. Therefore, Aβ42 may depend on nAChRs to downregulate MAPK and the phosphorylation of CREB protein.[54] Accordingly, activation of extracellular signal-regulated kinase/MAPK, Ca^{2+}/calmodulin-dependent kinase II, and the phosphatidylinositol 3-kinase-activated protein Akt/protein kinase B is disrupted in the presence of soluble Aβ. The insulin receptor family of tyrosine kinase has been found to mediate Aβ induced synaptic deficits and soluble Aβ binds to insulin receptors and affects its autophosphorylation in the presence of insulin. The Aβ mediated kinase inhibition is similar to that achieved by an antagonist of the insulin receptor family of tyrosine kinases.[55]

Rescuing synaptic deficits: amyloid based therapeutic intervention

Multiple approaches have been applied to decrease Aβ oligomer-induced synaptic dysfunction. Cyclohexanehexol stereoisomers have been identified to prevent the formation of Aβ oligomers and reduce AD-like pathologies in transgenic mouse brains. Impaired cognition and synaptic function are reduced in the presence of cyclohexanehexol inhibitors.[56] Among these stereoisomers, small molecular weight scyllo-inositol is found to suppress the inhibitory effect of Aβ oligomers on LTP in mouse hippocampus. Cerebroventricular injection of Aβ oligomers derived from APP overexpressing CHO cells into rat impairs learned performance on a complex lever-pressing task. This impairment can be rescued in animals drinking scyllo-inositol containing water.[57] Apparently, small molecular compounds that block the oligomer formation suppress the inhibitory effect of Aβ on LTP.[58,59]

The rescue of LTP inhibition by Aβ oligomers can also be achieved by a monoclonal antibody against Aβ. Earlier studies have shown that a monoclonal antibody against the mid region of Aβ (m266) reverses memory deficits in an object recognition task and a holeboard learning and memory task.[60] Injection of monoclonal Aβ antibody after intracerebroventricular injection of Aβ secreted from APP overexpressing cells prevents the inhibition of LTP by Aβ oligomers. Interestingly, active immunization shows partial effects that correlate positively with levels of Aβ antibodies.[61] In another study, the memory loss in Tg2576 mice can be fully reversed upon intraperitoneally dosing with BAM-10, an antibody recognizing N-terminus of Aβ. BAM-10 neutralizes Aβ assemblies in the brain and prevents them from disrupting cognitive function.[62] Furthermore, soluble Aβ oligomer induced spine loss could be rescued by Aβ specific antibodies; this reversible event provides a promising approach for therapeutic intervention of AD.[39]

The most advanced clinical trial with antibody based Aβ reduction therapy is at Phase III with bapineuzumab, a humanised anti-amyloid-β monoclonal antibody. This was based on earlier findings that vaccination with a synthetic Aβ reduces plaque load in transgenic mice.[63] However, active vaccination with AN1792 (β-amyloid [Aß]1-42) failed clinical trial, and recent studies with passive vaccine bapineuzumab showed some effect on Aβ load. Three ascending dose groups at 0.5, 1 and 2 mg/kg of bapineuzumab were administrated up to 6 times in mild to moderate AD patients at 13 weeks apart. Positron emission tomography (PET) scanning using Carbon-11-labelled Pittsburgh compound B (^{11}C-PiB) was carried out to measure cortical fibrillar amyloid-β load in AD patients. Administration of bapineuzumab for 78 weeks led to a reduction of ^{11}C-PiB retention. Currently there is no imaging based method to measure soluble Aβ oligomers in human brains,

and it is not clear why bapineuzumab only improves cognitive function in apolipoprotein-E4 non-carriers.

Non-vaccine based therapeutic approaches include γ-secretase inhibitors[64] and modulators. R-flurbiprofen is a non-steroidal anti-inflammatory drug and a γ-secretase modulator, and treatment of transgenic mice with R-flurbiprofen efficiently reduces Aβ levels.[65] However, R-flurbiprofen failed Phase III clinical trials for the treatment of AD. Semagacestat[66–69] is a γ-secretase inhibitor and was shown to be effective in reducing plasma Aβ during a recent Phase II clinical trial. Begacestat, a γ-secretase modulator, reduced both Aβ40 and Aβ42 in transgenic mouse brains and reversed cognitive deficits.[70]

Conclusion

The amyloid hypothesis illustrates a multi-step cascade originating from excessive Aβ generation to final neuropathological hallmarks found in brains of Alzheimer's patients.[71] While potential targets have been explored to block the cascade and prevent the onset of the disease, identification and validation of Aβ species that are most toxic to neurons continues to be a challenge. Aβ oligomers physically or functionally interact with multi-components that play intrinsic roles in pathways leading to LTP inhibition, and potential targets related to these pathways show tremendous promise for therapeutic intervention. Current efforts focus on removing monomeric and oligomeric Aβ to rescue synaptic dysfunction, and these approaches are highly promising as they have reversed Aβ-induced synaptic deficits. In conclusion, reducing oligomeric Aβ formation as well as minimizing its toxicity to synapses will provide enhanced protection against neuronal loss and eventually delay memory impairment.

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Disclosure

The author reports no conflicts of interest relevant to this research.

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