Alzheimer's disease: many failed trials, so where do we go from here?

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative brain disorder associated with relentlessly progressive cognitive impairment and memory loss. AD pathology proceeds for decades before cognitive deficits become clinically apparent, opening a window for preventative therapy. Imbalance of clearance and buildup of amyloid β and phosphorylated tau proteins in the central nervous system is believed to contribute to AD pathogenesis. However, multiple clinical trials of treatments aimed at averting accumulation of these proteins have yielded little success, and there is still no disease-modifying intervention. Here, we discuss current knowledge of AD pathology and treatment with an emphasis on emerging biomarkers and treatment strategies.

INTRODUCTION

Alzheimer's disease (AD) is a progressive, irreversible disabling neurodegenerative disorder characterized by memory loss, cognitive dysfunction and behavioral changes.[1–3] It is the leading cause of dementia, affecting approximately 46.8 million people globally.[4–6] At the molecular level, pathological changes in the brain that are hallmarks of AD include intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein and insoluble extracellular β-amyloid (Aβ) plaques.[7 8] Pathological changes in the brain precede clinical symptoms and, therefore, diagnosis, by decades.[9–11] The debilitating effects of AD impose an enormous social, emotional and economic burden on patients and their families.[6 12] The etiology of AD remains unclear and, despite research programs costing billions of dollars and numerous successful approaches in mouse models, at this time, there are no effective treatments for AD.[13–16] Mild symptomatic benefits are all that can be offered. This symposium will explore the underlying reasons for failure in developing effective drugs and will suggest possible new directions to improve prognosis for this devastating disease.

AMYLOID PRECURSOR PROTEIN (APP) PROCESSING AND Aβ FORMATION

The amyloid cascade hypothesis is a widely accepted model of AD pathogenesis that postulates an imbalance between production and clearance of the Aβ peptide leading to brain deposition of Aβ as the cause of AD.[17 18] Accumulation of Aβ occurs in the AD brain, and in familial forms of the disease, this is thought to be due to overproduction, whereas in most late-onset forms of the disease, it is thought to be due to insufficient clearance.[19] Based on the amyloid cascade hypothesis and the presence of Aβ in the AD brain, numerous treatments have been devised to reduce Aβ load as a way to halt or slow AD progression. Despite multiple failures, the hypothesis continues to drive the development of potential AD treatments.[20 21]

The proteolytic processing of APP determines whether Aβ will be generated (figure 1).[22] The three enzymes that control this process are α-secretase, β-secretase (β-site APP-cleaving enzyme (BACE) 1) and γ-secretase. APP can enter either an amyloidogenic or a non-amyloidogenic pathway, depending on which of these secretases act on it. The amyloidogenic pathway begins with BACE1 releasing a soluble APP fragment and leaving in the membrane a 99 amino acid C-terminal fragment (CTF-β).[23] This fragment is then cleaved by γ-secretase at slightly different positions, producing $Aβ_{1-40}$ and $Aβ_{1-42}$, as well as a cytoplasmic peptide, the amyloid precursor protein intracellular domain (AICD). The non-amyloidogenic route starts with APP cleavage by α-secretase in the middle of the Aβ domain, freeing a soluble ectodomain (sAPP-α) and a membrane-bound C-terminal APP fragment of 83 amino acids (CTF-α). Further cleavage within the transmembrane domain by the γ-secretase complex yields the p3 peptide fragment and the AICD.

RODENT MODELS

Transgenic mice overexpressing APP have been used in myriad studies and have proven to be a valuable in vivo model, contributing tremendously to our understanding of AD pathophysiology.[24–29] However, there is a disconnect between efficacy of treatment in rodent models and failure when attempts are made to translate to humans. In theory, Aβ removal or interference with Aβ aggregation will improve the signs and symptoms of AD. Unfortunately, this holds true in mice, but for reasons that we have

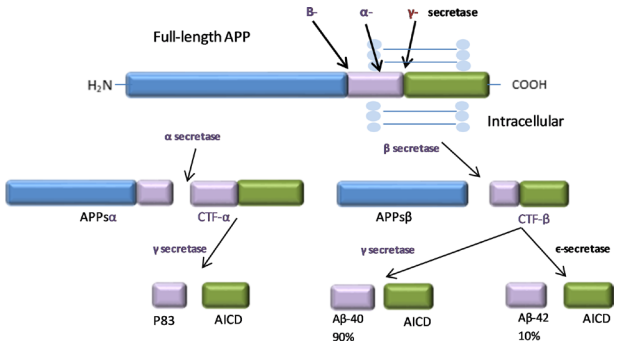

Figure 1 APP processing via non-amyloidogenic and amyloidogenic pathways. The left side of the figure shows non-amyloid-forming reactions in which APP is cleaved by α-secretase to form sAPP-α, then by γ-secretase to yield P83 and AICD (amyloid intracellular domain). The right side of the figure shows amyloid-forming reactions in which APP is cleaved by BACE1 to form sAPP-β, then by γ-secretase to yield Aβ and AICD. AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; BACE1, β-site APP-cleaving enzyme; CTF-β, C-terminal fragment; sAPP-β, soluble APP fragment.

to work out, it does not yield the same improvement in people[28 30–32] (box 1).

ALZHEIMER'S PATHOLOGY AND IMAGING

The AD brain is notable for the presence of senile plaques of insoluble $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ and NFTs of tau, a microtubule-associated protein that is normally involved in axonal transport.[33 34] The plaques are composed primarily of fibrillar Aβ deposited extracellularly. Neuritic plaques, a subset of senile plaques, are most closely linked to synaptic loss, have a dense core and are found associated with dystrophic neurons and activated microglia. Tangles are intracellular and consist of paired-helical filaments of hyperphosphorylated tau protein.[35 36] These pathological changes occur decades before initial clinical symptoms manifest.[37 38]

Aβ pathology can be imaged in human brain tissue in vivo by positron emission tomography (PET) using amyloid-β radiotracers.[39 40] The use of fluorodeoxyglucose-PET has allowed documentation of decreased glucose metabolism in the AD brain, generally occurring earliest in the posteromedial cortex and also in the temporal regions and correlating with neuronal or synaptic loss.[41 42]

PET also allows for in vivo evaluation of tau, and this is important because it has been shown that NFTs correlate with cognitive decline in AD better than amyloid.[43–45] The PET tracer fluorine 18–labeled [18F] flortaucipir (AV1451) binds to the paired helical filaments of tau in NFTs and correlates well with cerebrospinal fluid (CSF) tau content.[46–48] A recent report found that older persons carrying the ApoE4 allele have greater taupathy in the medial temporal area of the brain, indicating that ApoE4 status may affect not only brain Aβ but also tau. However, the link among ApoE4, tau and AD is not established here.[49] At this time, imaging for neither Aβ nor tau is currently used in routine clinical practice, and CSF measures are also not routine.[50]

The American College of Radiology and the American College of Neurology each recommend structural imaging, such as non-contrast MRI in the evaluation of patients suspected of having AD.[51 52] Atrophy of the brain is a frequent finding on MRI in AD and correlates poorly to the degree of cognitive impairment.[53] Hippocampal atrophy is a validated AD biomarker.[54] In general, the pattern of progression begins with early atrophy in the superior temporal region and the hippocampus, then subsequently in the amygdala and the remaining temporal regions and even later in the frontal association cortices.[55 56] Ventricular enlargement accompanies atrophy of the gyri and expanded sulci, indicating loss of both grey and white matter. As expected, a decrease in brain weight has been observed.

Although imaging can show signs of incipient dementia before it becomes clinically overt, by the time imaging is abnormal, substantial neuronal loss has occurred.[57] Further, progression from mild cognitive impairment to AD may be predicted with reasonable accuracy, but this does not lead to better outcome.[58]

Box 1 Problems with current strategies to develop Alzheimer's disease (AD) treatment

- Treatment success in AD animal models has not translated to humans.
- Multiple attempts to use anti-amyloid antibodies by drug companies have been unsuccessful, and some have given up, rather than trying to go in another direction.
- No good biomarker to predict who will develop AD. Accurate prediction allows intervention before too much neuronal loss.
- No way to screen potential treatments for human efficacy other than 5 or 10 years of administering to humans or trying it on animals.
- Lack of direct access to the neurons in the brain of the affected individual.
- No personalized approach or precision medicine as we see for many cancers.

CURRENT AND FUTURE TREATMENT: THE OBSTACLES TO SUCCESS

There is no disease-modifying therapy for AD at this time.[15 59] Only modest symptomatic relief for between 6 and 18 months can be achieved, generally by using cholinesterase inhibitors to prevent degradation of acetylcholine and by using the glutamate receptor antagonist memantine to attenuate excitotoxicity.[60] Acetylcholine is a neurotransmitter and neuromodulator that plays a critical role in forming and retrieving memories and maintaining attention. Cholinesterase inhibitors are the first-line medications in the treatment of AD. They slow the degradation of acetylcholine in the synaptic cleft, thus treating the symptoms of AD by increasing cholinergic function in the brain.[61] The currently available cholinesterase inhibitors most frequently prescribed are donepezil, rivastigmine, and galantamine.[62 63] Although cholinesterase inhibitors can slow symptoms of AD dementia, they do not alter the inevitable neurodegeneration and cognitive decline.[64] The N-methyl-D-aspartate antagonist memantine may delay cognitive decline, although not as effectively as cholinesterase inhibitors,

Table 1 AD treatments targeting Aβ and results in human trials

Drug	Action	Route of administration	Key findings
Aducanumab[108]	High-affinity, fully humanized monoclonal antibody that binds aggregated forms of Aβ	Intravenous	Did not slow cognitive decline, but a larger dataset showed high dose associated with reduced clinical decline in early AD
Crenezumab[109]	Fully humanized monoclonal antibody against human Aβ1–40 and Aβ1–42	Intravenous or subcutaneous	Interim analysis showed unlikely to reach primary endpoint of change from baseline on a clinical dementia assessment
Bapineuzumab[110]	Humanized monoclonal antibody binds the 5 N-terminal residues of Aβ and clears both fibrillar and soluble forms	Intravenous or subcutaneous	Did not meet primary endpoints, no treatment effect on cognitive or functional outcomes.
Solanezumab[111]	Humanized monoclonal antibody binds the mid-domain of Aβ and clears monomers	Intravenous	Did not meet primary endpoint of change from baseline on a cognitive assessment scale
Elenbecestat[112]	Small molecule BACE1 inhibitor	Oral	Unfavorable risk-to-benefit ratio
Verubecestat[80]	Small molecule inhibitor of BACE1 and BACE2	Oral	Modest worsening in mean cognition scores versus placebo
Atabecestat[113]	Small molecule inhibitor of BACE1	Oral	Halted due to liver toxicity
Lanabacestat[114]	Small molecule inhibitor of BACE1	Oral	Faster cognitive decline with drug than placebo
Semagacestat[115]	Inhibitor of γ-secretase	Oral	Interim analysis showed worsening of cognitive function and excess skin cancers.

AD, Alzheimer's disease; BACE, β-site APP-cleaving enzyme.

and can be helpful in controlling agitation.[65–67] Memantine, either as part of a multidrug regimen or by itself, is most clinically beneficial in people with moderate-to-severe AD.[68] Memantine and anticholinesterase therapies are often combined, but it is not clear whether these are superior to the cholinesterase inhibitor alone.[69]

Based on observational studies, lifestyle and health behaviors may delay or slow cognitive deterioration in AD.[70] Modifiable factors that may benefit persons at risk of AD include physical activity, maintaining good nutrition, controlling blood pressure and diabetes, and pursuing social activities.[71–73] Avoiding obesity and tobacco and alcohol use are all beneficial to general health and possibly as measures to postpone clinical manifestations of AD.[74–76]

According to the amyloid hypothesis, Aβ removal or interference with Aβ aggregation will improve the signs and symptoms of AD.[77 78] Unfortunately, what is effective in mice, for reasons that are numerous and yet to be fully explained, does not translate to humans. Further, some anti-amyloid treatments have caused unacceptable side effects, such as meningoencephalitis, brain edema, and brain microhemorrhage. The many failures in clinical trials of active and passive immunotherapies to remove Aβ have led some pharmaceutical companies to close their neurology divisions and cease working on AD[79 80] (table 1).

Another impediment to designing treatments is the lack of early predictors of who will develop AD (box 1). Identification of patients with AD before they have undergone significant neurodegeneration becomes crucial if we are to preserve cognitive function.[79] Current methods have limited accuracy in predicting progression to AD, and the search for better biomarkers and imaging techniques is ongoing.[81–84] Currently, CSF biomarkers for AD that are measured in practice for some patients are Aβ42, total tau, and phosphorylated tau.[85 86] Immunoassays can detect species of tau based on site of phosphorylation, and while CSF p-tau181 is used commonly, p-tau217 is being studied for its potential to be a superior biomarker for AD.[50 87 88]

Our lab and others are looking for early biomarkers. One possibility is micro(mi)RNAs, small endogenous non-protein-coding RNAs that influence the post-transcriptional regulation of gene expression and are involved in many neuronal processes.[89 90] A number of miRNAs show differential levels in the circulation and CSF in AD. These include miR-133b and miR-193a-3p, which are downregulated in AD serum and miR-206, which is elevated in AD plasma.[91–93] The predictive accuracy of any of these miRNAs has yet to be proven or brought into clinical use.

Not only can miRNAs serve as biomarkers but also they may be targets for treatment because they can affect signaling pathways crucial to neuronal function.[94] Circulating miRNAs are often carried in exosomes, small extracellular vesicles shed from all cells that contain cellular proteins, mRNA transcripts, miRNAs and lipids from their cell of origin.[95] They are a fundamental mechanism of communication in the nervous system, allowing bidirectional cell signaling.[96] The miRNAs within exosomes can transfer between neurons and microglia and can influence their phenotype. They can affect genes involved in Aβ generation.[97] Extraction of exosomes that are shed from the neurons of the brain and central nervous system of humans with and without AD, may allow us to distinguish miRNAs from brain neurons that are altered by AD. This knowledge could then be used to identify signaling pathways relevant to nerve health and synaptic function that are modulated in AD and potentially to manipulate these in a beneficial direction to mitigate negative effects.

Our group is exploring a human cell culture model of AD that may circumvent or complement the need for rodents. It is not practical to do human brain biopsies and study brain cells in culture. If we could, that would be comparable to our approach to cancer.[98] We can, however, approximate neurons from the brain using human induced pluripotent stem cells from patients clinically diagnosed with AD and differentiated to neural stem cells and then further differentiated to human cerebral cortical neurons.[28 99]

Neurons do not exist in isolation. To put a brain model together, multiple brain cell types, including microglia, are required because inflammation is undoubtedly part of the process of AD. With injury, the microglia proliferate and transform into active 'brain macrophages', also called reactive microglia. We know that amyloid and tau activate microglia.[100 101] In our model, we are looking at neurons and microglia together. The level of the major synaptic protein synaptophysin is significantly lower in these AD-derived neurons, and our unpublished results show that they respond differently than neurons from non-AD subjects to both direct exposure to high glucose and to conditioned medium from microglia exposed to high glucose.[102]

Briefly, we cultured HMC3 human microglial cells in normal and high-glucose conditions, then transferred conditioned medium from HMC3 to human-induced pleuripotent stem cell-derived neurons from AD versus non-AD donors (Axol Biosciences) and found an increase in APP and low density lipoprotein receptor-related protein 1 (a receptor that facilitates Aβ clearance) only in AD neurons in the presence of high glucose ($p<0.01$ vs normal glucose), while healthy donors showed no change or reduced expression under high-glucose conditions.[103] This preliminary work shows that immune-modulating glial-type cells influence gene expression in neuronal cells and that the AD neuronal cell response differs from the non-AD response. The AD-derived neurons may be more prone to dysfunction from compromised Aβ handling in high glucose, linking diabetes and AD. Disruptions in neuronal response to insulin, insulin signaling and glucose homeostasis may occur in some patients with AD, and this could be a cause of damage to neurons.[104 105] This is an active area of research.[106] Our study is in progress, and as we and others continue to look deeper into mechanisms of neurotoxicity in AD, breakthroughs may be on the horizon.

CONCLUSIONS

AD is a common neurodegenerative disorder that leads inexorably to deterioration of cognitive functions, memory loss and ultimately death.[107] The need to develop new treatments is urgent, and innovative thinking must prevail over repeated attempts to use the same approaches that have failed previously. In the interim, there are fundamental steps to take now: encourage patients with AD and healthy persons to eat a high-quality diet, engage in regular physical activity, increase social connections and intellectual activities, avoid head trauma and minimize heart disease risk factors: hypertension, obesity, high cholesterol and diabetes (keep A1C in normal range). These seem like very basic lifestyle behaviors that apply almost universally, but so many of us do not put in the effort to adhere to them.

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