Review article

Immunotherapeutic and pharmacological approaches for the treatment of Alzheimer’s disease

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Introduction

Alzheimer’s disease (AD) is one of the most common neurodegenerative diseases characterized pathologically by progressive neurodegeneration, subsequent to accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles of the microtubule-associated protein tau (Perl, 2010). This manifests clinically as progressive cognitive impairment, commonly amnestic in nature, with sporadic late-onset AD (LOAD) being the predominant cause of senile dementia (Vardarajan et al., 2014). Worldwide, it is estimated 1 in 10 people over the age of 65 have some form of dementia, with AD accounting for 50–70% of all cases (Qiu, Kivipelto, Von Strauss, 2009).

Understanding of AD pathology is incomplete. However, some of the most developed hypotheses reflect the dysregulation of the cholinergic system and glutamatergic system, in addition to protein aggregation (tau and amyloid) within the brain. Currently, prescribed treatments for AD are based upon the acetylcholine and glutamate dysregulation hypotheses as monotherapy with either acetylcholinesterase inhibitors (AChEIs) or memantine, respectively (National Institute for Health and Clinical Excellence, 2011). Therapies targeting tau and amyloid, most distinctively those exploring the effects of immunotherapy, are still under development but have yet to show clear clinical efficacy over that of current treatment regimens. According to a systematic review by Ehret and Chamberlin (2015), the optimal concentrations for the available acetylcholinesterase inhibitors...
Molino proposes a convincing model for AD that sees Aβ (Bredesen, Dong) found that BACE1 is upregulated in AD brains 2.7-fold compared with age-matched controls, including the 17 amino acid residues that overlap with the Aβ region. As such, Aβ also binds netrin-1, with the Aβ1–42 variant possessing a higher binding affinity than the Aβ1–17 fragment alone, although the authors comment that this is possibly only due to the inherently adherent properties of Aβ, and not necessarily biologically relevant.

Lourenço et al. (2009) demonstrated that production of Aβ is inhibited by APP–netrin-1 interaction, and that the netrin-1 binding-region of APP lies in its ectodomain (sAPPα region), including the 17 amino acid residues that overlap with the Aβ region. This upregulation of BACE1 is unregulated in AD brains 2.7-fold compared with age-matched controls, although this may not only reflect increased Aβ production, as BACE1 has multiple substrates (Chow et al., 2010). The next Aβ production mediator, γ-secretase, also has multiple substrates, including Notch receptors, and is composed of several components: presenilin 1 or 2 (PS1/PS2), nicastrin, Aph-1 and Pen2 (Chow et al., 2010). Genes coding for these presenilin components contain ~90% of mutations associated with early-onset familial AD (Dong et al., 2012). This upregulation of BACE1 and genetic risk associated with γ-secretase in AD, coupled with their pivotal role in Aβ production, make both enzymes prime candidates for inhibitory drugs.

Figure 1. APP structure and differential processing. In the plasma membrane, APP processing and subsequent cleavage occur via the non-amyloidogenic α-processing pathway, whereas amyloidogenic β-processing occurs in the endosomal membrane.

Based on such experimental evidence, however, Bredesen (2009) proposes a convincing model for AD that sees Aβ corrupting neural growth/death signalling by shifting the APP αβ processing ratio towards the β-pathway. Aβ (particularly Aβ42) acts as an anti-trophin, competing with APP for netrin-1

The amyloid hypothesis
At the centre of the amyloid hypothesis is the neuronal surface-membrane-bound protein, the amyloid precursor protein (APP) (Fig. 1). Physiologically, APP co-localizes to neuronal growth cones with the extracellular tropic molecule netrin-1 during neural development. Here, netrin-1 binds its main receptor, DCC (deleted in colorectal cancer), and co-receptor, APP, acting to navigate commissural axon outgrowth via chemoattraction (Rama et al., 2012). Netrin-1 is also expressed and active in the adult brain, including the hippocampus and dentate gyrus (Lourenço et al., 2009)—areas associated with memory formation, storage and major sites of AD pathology (Reilly et al., 2003).

APP undergoes either α or β processing to engender functionally polar intracellular signallings (Fig. 2) (Bredesen, 2009; Dong et al., 2012). Products from its α-pathway proteolysis regulate neurite outgrowth, synaptic plasticity, maintenance and spatial memory—all promoting memory retention and cell survival in cognitive neural pathways via netrin-1 interactions. Conversely, the β-pathway promotes neurite retraction, synaptic reorganization and neuronal apoptosis—promoting memory loss and reorganization (Bredesen, 2009). In particular, β-processing produces an anomalous Aβ variant with a 42-amino acid sequence, rather than normally produced Aβ 1–40, which possesses marked neurotoxicity and aggregative capacity, forming hallmark amyloid plaques in AD brains (Dong et al., 2012).
The acidic environment of the endosome activates β-secretase, which cleaves APP, shedding secretory APPα (sAPPα) and leaving a membrane bound β-C-terminal (β-CTF). Next, γ-secretase cleaves β-CTF, producing Aβ (38-43 amino acids long), and APP intracellular C-terminal domain (AICD— not shown). Aβ is then degraded in the lysosomal/late endosome by proteolytic enzymes; Aβ production— non-amyloidogenic pathway (green arrows). APP may also be restored to the plasma membrane via recycling endosomes. α-secretase cleaves APP through the Aβ region, shedding secretory APPx (sAPPx). Next, γ-secretase cleaves the remaining α-CTF into non-toxic peptide P3 and AICD (not shown); Aβ clearance— apolipoprotein E-mediated degradation (blue arrows). Activation of the peroxisome proliferator activated receptor γ (PPARγ) and the retinoid X receptor (RXR) cause the two to form a heterodimer, which then binds to the peroxisome proliferator response element (PPRE) DNA region, inducing transcription of APOE and abca1 genes. ABCA1 lipidates apoE, forming a high-density lipoprotein (HDL) particle. apoE in this particle binds secreted soluble Aβ, directing its LRP1-mediated endocytosis and autophagic degradation as in (3). Alternatively, Aβ is degraded by insulin degrading enzyme or neprilysin proteases, or removed from the brain parenchyma via de blood–brain barrier or interstitial fluid drainage pathway (not shown); Aβ-targeting drugs (yellow): Avagacestat binds and inhibits γ-secretase (3), thus preventing Aβ production. Rosiglitazone/piozilazone (RSG/PGZ) activate PPARγ (4), inducing apoE transcription and ultimately increasing Aβ clearance. Adapted from Bu (2009) and Mandrekar-Colucci et al. (2012).
via γ-secretase (Ghezzi, Scarpini and Galimberti, 2013). However, γ-secretase may still be a worthwhile target, with Li et al. (2007) suppressing γ-secretase activity by 30% via het erotzygous knockout of the γ-secretase subunit Aph-1 in AD-model mice, resulting in a 35 ± 16% reduction in Aβ burden. The authors concluded that such moderate suppression avoids adverse side effects associated with impairment of Notch-pathways. The γ-secretase inhibitor avagacestat builds on this success, inhibiting the Aβ cleavage function of γ-secretase with a 193-fold selectivity over Notch-processing (Gillman et al., 2010). Furthermore, Albright et al. (2013) demonstrated a single 200 mg dose of avagacestat caused a 47% decrease in cerebrospinal fluid (CSF) Aβ levels after 12 h in healthy human volunteers, in contrast with a steady increase in those participants in the placebo group. Moreover, the γ-secretase and inflammation modulator CHF5074 was also shown to decrease Aβ plaque burden and learning deficit in rodent models (Imbimbo et al., 2009; Porrini et al., 2015).

Although recent progress has been made by pharmaceutical companies in developing BACE1 inhibitors, some drugs have not made past phase I and II clinical trials. Trials involving small molecule BACE1 inhibitors such as RG7129 from Roche and LY2886721 from Lilly were either dropped or halted before reaching phase III, respectively. Whereas the development of RG7129 was terminated by Roche with no official explanation, LY2886721, an orally administered non-peptidic BACE1 inhibitor, showed promising results in animal models by reducing Aβ and successfully succeeded in phase I trials, however the continuous development was cancelled due to abnormal liver biochemistry being reported in a small number of phase II candidates (Vassar, 2014).

More recently, efforts have been placed upon the development of efficient Aβ immunotherapy. Aducanumab, a human monoclonal antibody selectively targeting aggregates and insoluble Aβ deposits, showed promising results in mice models and its latest clinical trial including AD patients, being the only drug in AD trials to suggest disease modification at a higher dose in Phase I trials (Sevigny et al., 2016). In this study, 165 patients with clinical AD diagnosis and positive Aβ PET scan were given monthly doses of 1, 3, 6 or 10 mg kg⁻¹ of aducanumab over the course of a year. The results showed the antibody readily transverses the blood–brain barrier and reduces Aβ aggregates in a time- and dosage-dependent manner. Moreover, in correlation with the decrease in Aβ burden, cognitive studies performed determined a steady improvement in cognition. Although the efficacy of aducanumab requires confirmation in larger studies, it provides further evidence that the development of immunotherapies as treatment for AD are a promising target route. In contrast, drugs such as solanezumab, an anti-amyloid monoclonal antibody similarly designed to target and bind to soluble Aβ peptide, showed no significant improvements to cognition or functional abilities in two Phase III trials (Doody et al., 2014). Bapineuzumab, another anti-amyloid monoclonal antibody candidate, was also unsuccessful during Phase III trials (Salloway et al., 2014). However, advantages and disadvantages are inevitable when exploring Aβ active and passive immunization, including variable antibody response and the necessity for repeated antibody infusions over time. Conversely, immunotherapy remains a promising target route and several clinical trials are still ongoing, having passed phase II of clinical development (Lannfelt, Relkin, Siemers, 2014).

**Microtubule-associated protein tau hypothesis**

Deposition of neurofibrillary tangles (NFTs) formed from the hyperphosphorylated microtubule-associated protein (MAP) tau correlates closely with AD progression. Tau is produced with alternative mRNA splicing of a single gene (Stoothoff and Johnson, 2005), approximately 80 serine/threonine and 5 tyrosine phosphorylation sites (Wang et al., 2013). This protein can be differentially posttranslationally modified (which includes, but is not limited to, aggregation, polyamination and nitration), with phosphorylation described as the most common under normal and pathological conditions. Although tau phosphorylation is present in the healthy brain, the concentrations are three to four times lower than those found in the AD brain, and the impact in biological function varies greatly depending on the phosphorylation site (Gong and Iqbal, 2008). Martin, Latypova, Terro (2011) reported that post-translational modifications are most likely responsible for excessive tau phosphorylation (hyperphosphorylation), which reduces its microtubule affinity and destabilizes neuronal cytoskeleton. However, tau hyperphosphorylation is a key mechanism for foetal neurodevelopment, where it is thought to contribute towards neuronal growth and plasticity by maintaining a less stable cytoskeleton (Lovestone and Reynolds, 1997). In the developed brain, aggregation of tau increases according to levels of hyperphosphorylated tau, also causing impairment in the functionality of other proteins (Götz, Ittner, Ittner, 2012). While tau hyperphosphorylation occurs prior to the development of NFTs, the mechanism behind its abnormal formation is not completely understood.

**Current pharmacological targets of tau**

Approaches including stimulation of tau disassembly and inhibition of tau aggregation are the most widely explored therapeutic treatments, and are based on the principle that an imbalance in phosphatases and kinases may lead to hyperphosphorylation of tau and to the development of NFTs (Mangialasche et al., 2010). Phosphoseryl/phosphothreonyl protein phosphatase (PP-2A), responsible for tau dephosphorylation and inhibition of phosphorylation-inducing kinases such as mitogen-associated protein kinase (MAPK), has been shown to directly impact AD pathogenesis and is a strong candidate for new pharmacological therapy (Mangialasche et al., 2010; Voronkov, Braithwaite, Stock, 2011; Götz, Ittner, Ittner, 2012). Downregulation of PP-2A and increased levels of PP-2A and endogenous inhibitors such as inhibitor 2 (I2) have been shown in post mortem evaluation of AD brains, which resulted in increased Aβ deposition and tau hyperphosphorylation in in vivo models (Voronkov,
Braithwaite, Stock, 2011). Over-activation of glycogen synthase kinase 3β (GSK-3β), involved in Aβ and tau processing, gene transcription and cell signalling, is hypothesized to be linked to tau hyperphosphorylation, Aβ deposition and memory impairment, all of which are observed in AD (Hooper et al., 2008). Furthermore, a study by Wen et al. (2008) has shown GSK-3β activity increases with aging, and its inhibition may reverse hyperphosphorylation both in vitro and in vivo (Engel, 2006).

Therapeutic treatments targeting PP-2A stimulation and GSK-3β inhibition are therefore highly desirable. Aβ-candidate drug memantine has been linked to decreased tau hyperphosphorylation by reversing PP-2A inhibition (Mangialasche et al., 2010), potentially reducing the necessity for combination therapy or simultaneous treatments. Lithium, one of the most studied pharmacological treatments for GSK-3β inhibition, has been correlated with decreased tauopathy and tau lesions in vivo (Nakashima et al., 2003; Noble et al., 2003; Pérez et al., 2003). However, both GSK-3β and PP-2A are essential for normal development and play key roles in cellular regulation, requiring further pharmacological investigation and development of pathological-specific drugs rather than broad targeting of these substrates.

Immunotherapy focusing on pathological tau removal is also a strong candidate for drug development. AADvac-1, active immunization therapy targeting tau peptides, has shown to decrease 95% of tau hyperphosphorylation in AD rodent models. The study also indicated levels of tau oligomers and NFTs were reduced, with clinical phenotype of models improving considerably (Kontsekova et al., 2014). Moreover, a double blind, phase I clinical trial conducted from June 2013 to March 2015 demonstrated positive results for AADvac-1, with 29 out of 30 patients developing the IgG immune response expected from the drug (Novak et al., 2016). In conjunction, these results offer promising routes for the development of novel tau immunotherapy.

**Conclusion**

Clinical trials in AD remain challenging due to the struggle to recruit participants, participant compliance and technological limitations related to sensitive markers/measures of cognitive or biochemical improvements as a result of treatment. Moreover, the development of therapeutics targeting multiple pathways responsible for the pathogenesis of AD is undoubtedly complex, and is yet to produce a disease-modifying agent. Acetylcholinesterase inhibitors such as rivastigmine, donepezil and galantamine, and the NMDA-receptor antagonist memantine are thus far the only therapeutics approved by the FDA and available for AD treatment. These treatments, however, offer no more than symptomatic relief over a short period of time, and have variable efficacy from patient to patient. Although any improvement must be considered a triumph, without efficacious treatments concentrating on the underlying pathology there is very little possibility of long-term recovery. Moreover, while passive and active immunization targeting either Aβ or hyperphosphorylated tau are still under development, most studies were halted after unsuccessful Phase III trials. There is, however, a growing expectation of those clinical trials persisting for longer periods and focusing on specific populations, therefore gathering more relevant data that could lead to the development of an effective treatment.

**Author’s biography**

[Timothy J Gaudet] Having attained a First in BSc (Hons) Biomedical Science, Timothy has directed the data handling skills he has learned towards finance. He is now a Director of a sportswear clothing company. They developed the study, provided essential materials, analysed data, wrote the article and had primary responsibility for final content. [Marcela Usmani Moraes] Having recently obtained a degree in BSc (Hons) Biomedical Science, Marcela currently works as a Research Assistant in a biochemistry laboratory. She has worked with Professor Myra Conway from her first academic year, completing two summer placements and a year placement in brain biochemistry, which have contributed towards the preparation of two manuscripts. Marcela has plans to pursue a PhD and continue her career in academia in the following years. They provided essential materials, analysed data, wrote the paper and had primary responsibility for final content.

**Conflict of Interest Statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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