Pharmacotherapeutic targets in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by an increasing impairment in normal memory and cognitive processes that significantly diminishes a person's daily functioning. Despite decades of research and advances in our understanding of disease aetiology and pathogenesis, there are still no effective disease-modifying drugs available for the treatment of AD. However, numerous compounds are currently undergoing pre-clinical and clinical evaluations. These candidate pharmacotherapeutics are aimed at various aspects of the disease, such as the microtubule-associated τ-protein, the amyloid-β (Aβ) peptide and metal ion dyshomeostasis – all of which are involved in the development and progression of AD. We will review the way these pharmacological strategies target the biochemical and clinical features of the disease and the investigational drugs for each category.

Keywords: Alzheimer's disease • τ • amyloid-β • metals • therapeutics

Current pharmacotherapies for the treatment of AD

Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly population, affecting approximately 35–40 million patients worldwide [1], and is the third leading cause of death in developed countries [2]. As such, AD represents a major socio-economic problem, which requires better diagnostic tools, management and effective therapies in order to ease the burden of this disease. While there are advances being made in all these areas, particularly with the identification of new biomarkers and the development of novel brain imaging compounds for the early detection of disease, it is clear that an effective treatment for AD is as elusive as ever. To date, the only Food and Drugs Administration (FDA)-approved drugs for the treatment of AD patients are the acetylcholinesterase inhibitors (AChEIs) tacrine, donepezil, galantamine and rivastigmine, and the non-competitive

N-methyl-D-aspartate (NMDA)-receptor antagonist memantine. The AChEIs exert their affect by preventing the enzymatic degradation of the neurotransmitter acetylcholine (AChE), resulting in increased AChE concentrations in the synaptic cleft and enhanced cholinergic transmission [3]. Memantine, however, protects neurons against NMDA receptor activation-mediated glutamate excitotoxicity [4–6] and also inhibits τ-hyperphosphorylation and aggregation [7]. A new approach, using combination therapy of donepezil and memantine, has been reported to have significant beneficial effects on cognitive function, activities of daily living and behaviour [8]. Meanwhile, potent and more selective AChEIs (Huperzine A, Neuro-Hitech Inc., New York, NY, USA) and NMDA-receptor antagonists (Dimebon, Medivation Inc., San Francisco, CA, USA) are being assessed.

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However, irrespective of the form of therapy utilized, the current approaches for the treatment of AD provide only temporary symptomatic relief and do not inhibit and/or reverse the underlying disease mechanisms. This stresses the urgent need for disease-modifying drugs for AD – small, easily administrated, well-tolerated, bioavailable compounds that cross the blood-brain barrier (BBB) and have little or no adverse effects and/or contraindications. There are currently more than 50 compounds in various stages of clinical investigation for the treatment of AD (www.alzforum.org) including: statins [9–12], peroxisome proliferator-activated receptor-γ agonists [13–16], non-steroidal anti-inflammatory drugs [17–19], neurotrophic molecules and even metabolic or nutritional drinks (Ketasyn™, Accera, Broomfield, CO, USA; Souvenaid™, Danone Research-Centre for Specialized Nutrition, respectively, Palaiseau, France). In addition, there are many more candidate molecules that are at the pre-clinical stage of development and are likely to proceed into clinical trials. Most of these pharmacological agents have been designed and/or developed based upon a notion that has been dominating the AD field for the past two decades – the 'amyloid cascade hypothesis'. This theory claims that the metabolism of the amyloid-β (Aβ) peptide (both generation and clearance) is the main initiator of AD, which together with the downstream formation of the τ-protein aggregates, leads to neuronal and synaptic dysfunction and loss, microglial activation and neuronal death [20, 21]. Thus, most of the pharma-cological agents being developed target one or both of the principal cerebral proteins implicated in the pathogenesis of AD: τ and Aβ. In this review, we will provide a broad overview of the therapeutic approaches currently being developed for the treatment of AD.

AD pharmacotherapies targeting τ

Neurofibrillary tangles (NFTs), which are found in AD and other forms of dementia, consist of insoluble, intra-neuronal inclusions [22, 23] comprised paired helical filaments that are formed from hyperphosphorylated τ [24, 25]. Hyperphosphorylation of the microtubule-associated τ-protein is likely to result from an imbalance in kinase and phosphatases activities, and leads to destabilization of microtubules [26], loss of neuronal cytoskeletal architecture and/or plasticity [27], impaired neuronal transport, dystrophy and ultimately neuronal cell death [28, 29]. Based on these findings, small molecules that interfere with the formation of τ-aggregates, selectively inhibit τ-kinases and/or activate τ-phosphatases are being pursued as therapeutic targets (see Fig. 1).

Modulators of τ kinases or phosphatases

The biological function of the microtubule-associated τ-protein [30] is regulated by several kinases and phosphatases [31–33]. An imbalance in activity between kinases and phosphatases results in the abnormal phosphorylation of 38 or more serine and/or threonine amino acids on τ in the AD brain [34–37]. Phosphorylation of a tyrosine residue at position 18 (Tyr[18]) on τ by the tyrosine kinase fyn has also been reported [38]. Decreased mRNA levels [39] and activity of the main τ-protein phosphatases (PP)1 and PP2A, as well as other τ-phosphatases such as PP2B and PP5, have been observed in AD [40–42]. This can lead to a direct reduction in τ-dephosphorylation or indirect hyperphosphorylation by the inability of these phosphatases to inhibit τ-hyperphosphorylation by different kinases [43], therefore τ-phosphatases have been proposed as therapeutic targets [44]. Major kinases, whose protein levels and activities are reported to be up-regulated in AD and other tauopathies [45–48], involved in the phosphorylation of τ include glycogen synthase kinase (GSK)-3, cyclin-dependent protein kinase-5, casein kinase-1, protein kinase A (cyclic adenosine monophosphate (cAMP)-dependent protein kinase), protein kinase C, calcium and calmodulin-dependent protein kinase-II, microtubule-affinity regulation kinase and mitogen-activated protein kinase family members [49–53]. These proteins have also been suggested as therapeutic targets for AD. Recent reports have highlighted the importance of GSK-3β in the developments of both τ and Aβ pathologies in AD and concluded that this kinase is a vital drug target for the treatment of AD and other neurodegenerative diseases [54–57]. Several animal studies, for example, have demonstrated that the inhibition of GSK-3β activity by lithium [58] results in decreased levels of both Aβ (in PDAPP mice) and τ-phospho-rylation, τ-aggregation and NFT formation (in JNPL3 mutant τ-mice) [59–61]. Other GSK-3β inhibitors are being developed, such as AR-A014418 [61], as well as other kinase inhibitors [62–67]; however, this approach is hindered due to the ubiquitous expression of these kinases, their pleiotropic activities in countless cellular functions and the low selectivity of inhibitors for specific kinases, isoforms of a particular kinase, cellular compartment and/or pathological, rather than physiological, activity of the kinase [68–70].

τ aggregation inhibitors (TAIs)

Screening for TAIs started in the early 1990s with reports on the ability of phenothiazines [71], anthraquinones [72] and low molecular weight N-phenylamine derivatives [73] to prevent τ-aggregation and associated toxicity in cell lines [74]. The most clinically advanced TAI is AL-108 or NAP (Allon Therapeutics Inc., Vancouver, BC, Canada), which is an intra-nasal formulation of an 8 amino-acid peptide (NAPVSIPQ) derived from the biological activity-dependent neuroprotective protein secreted by the brain in response to various insults [75]. Studies in transgenic mice suggest that AL-108 interacts with microtubules, reduces τ-hyperphosphorylation and increases soluble τ levels leading to an improvement in cognition [76, 77]. Data from a recently completed phase IIa trial evaluating AL-108 in 144 patients with amnestic mild cognitive impairment demonstrated that it is safe and well tolerated, and the high dose (15 mg twice a day) resulted in a significant and lasting improvement in short term and working

Fig. 1 Pharmacotherapeutic strategies for the treatment of Alzheimer's disease targeting τ. Schematic representation of the anti-τ targets for potential pharmacotherapies: (*i*) Modulators of τ-kinases or phosphatases, (ii) τ-aggregation inhibitors (TAIs). Abbreviations: MT (microtubule); NFTs (neurofibrillary tangles); PHFs (paired helical filaments).
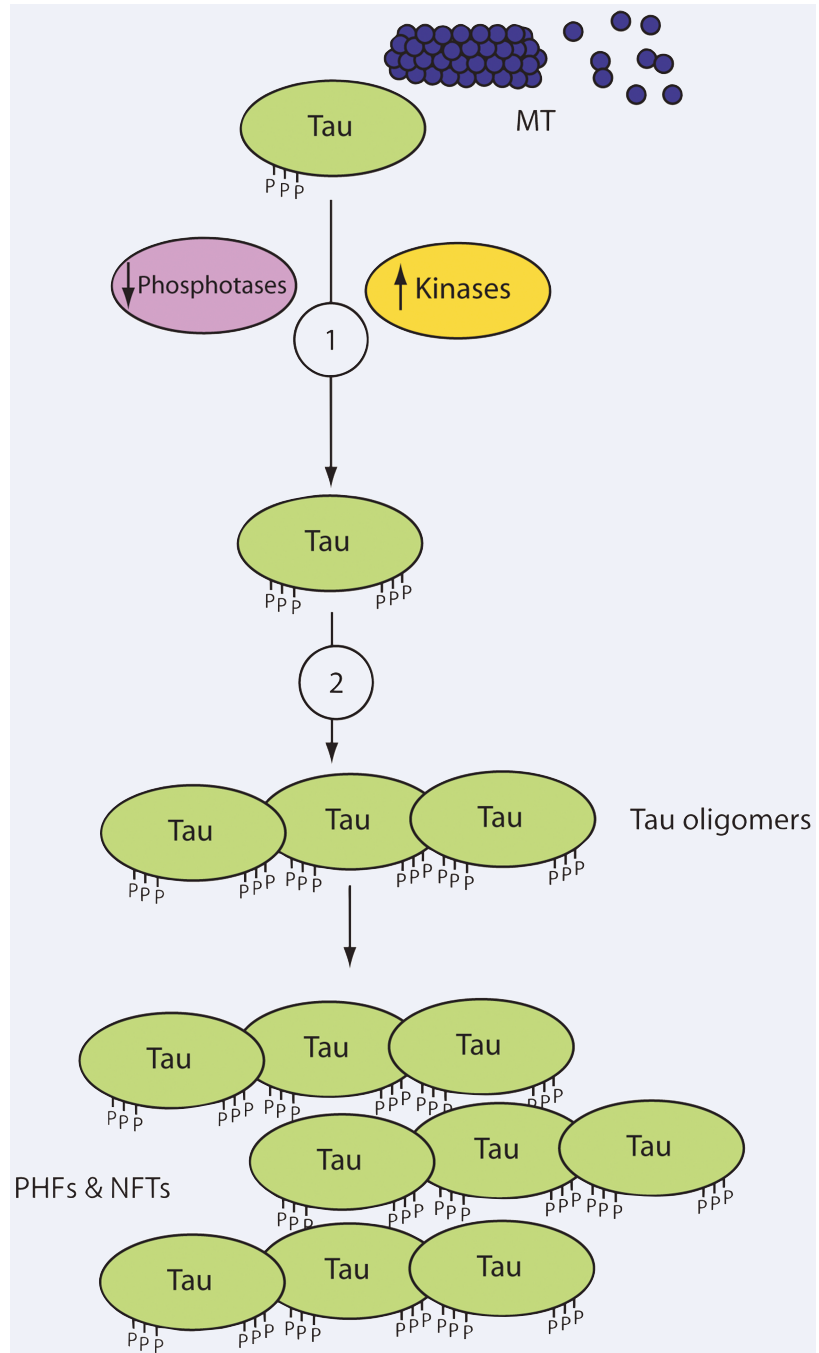
memory (but not in tests that involved executive functions). AL-108 is now being tested as a treatment for other neurodegenerative diseases, mental disorders and ocular disease. An intravenous (IV) formulation of NAP, known as AL-208, is also under clinical investigation for mild cognitive impairment associated with coronary artery bypass graft surgery as well as other indications [78].

A recently announced TAI is Rember™ (TauRx Therapeutics Ltd., Singapore), which has methylthioninium chloride (MTC; also known as the histochemical dye methylene blue) as its active constituent. It is proposed that this compound is not only able to prevent the oligomerization and self-aggregation of τ, but also dissolve pre-formed τ-oligomers and paired helical filaments into truncated

τ-fragments, which can then be naturally degraded and eliminated (http://www.taurx.com/). At the 11th International Conference on Alzheimer's Disease (ICAD, Chicago, 2008), pre-clinical data (O1–06-04, P2–383, P2–428) and results of a recently completed 24-week, multi-centred, randomized, double-blind, dose-ranging (30, 60 or 100 mg, three times per day), placebo-controlled phase IIb trial followed by a 60-week, blinded, active treatment extension study were presented (O3–04-07, P4–347, P4–384). Patients with moderate AD who received MTC at 60 mg three times/day showed a significant improvement in the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) scores, compared to placebo control, at the end of the 24-week-long trial. This result was further verified after 50 weeks of treatment and again at the conclusion of the trial (84 weeks in total). Another measure of the drug's efficacy that was utilized was single photon emission computed tomography (SPECT) analysis at week 24 compared to baseline, which revealed that the regional cerebral blood flow decline seen in the hippocampus and entorhinal cortex of individuals treated with placebo, was not observed in individuals treated with MTC (60 mg three times/day). Despite these seemingly encouraging results, great reservations have been expressed, mainly due to unusual trial design and an unconventional method of analysis. However, TauRx Therapeutics Ltd. has announced that it intends to take Rember™ into a phase III clinical trial, and that it is already testing a second generation TAI molecule, LMT-X, in τ-transgenic animal models.

AD pharmacotherapies targeting Aβ

Although the exact mechanism is still unclear, it is widely believed that dysfunctional Aβ metabolism is the underlying cause for the neurodegeneration and dementia observed in AD. Therefore, a leading strategy for the development of AD pharmacotherapies is modulation of Aβ production, aggregation and/or clearance. It is assumed that altering these processes will stop and/or reverse the pathological neuronal loss and the clinical cognitive decline. We will briefly summarize key findings of the major AD pharmacological strategies being development to target various aspects of Aβ metabolism (see Fig. 2).

Inhibitors and/or modulators of the secretases

The amyloid precursor protein (APP) is an evolutionary conserved type I transmembrane glycoprotein [79] that belongs to a family of proteins, including amyloid protein precursor-like protein1 (APLP1) and APLP2 [80, 81]. Both the amino and carboxyl terminals of APP can be divided into several regions, each with its own characteristics and functions [82]. The overall function of APP is unclear; however, it is believed to be important during the development of the CNS and in response to stress or injury [83]. APP has been suggested to act as a cell-surface receptor and may also

be involved in cell adhesion and/or neurite outgrowth [84, 85]. APP is synthesized in the endoplasmic reticulum, undergoes N- and O-glycosylation in the Golgi, and is translocated from the trans-Golgi network to the cell surface *via* the secretory pathway [86]. During and/or after trafficking, APP undergoes degradation *via* the ubiquitin–proteasome system [87] and/or various forms of autophagy [88, 89]. Neuronal macroautophagy induction and impaired clearance of several autophagy intermediates is evident in the AD brain, leading to an overproduction and accumulation of intracellular Aβ in autophagic vacuoles [90, 91].

APP also undergoes proteolytic processing through either the non-amyloidogenic or the amyloidogenic pathways [92]. During the non-amyloidogenic pathway, the membrane-bound enzyme α-secretase cleaves APP within its Aβ domain, resulting in the extracellular secretion of soluble APP-α (sAPP-α) and the production of a short membrane-bound COOH-terminal fragment (CTF), α-CTF or C83 [93]. Subsequent γ-secretase cleavage of C83 results in the secretion of a 3-kD peptide termed p3 out of the cell [94], and release of the APP intracellular domain (AICD) into the cytoplasm [95]. Enzymes that have been suggested to have α-secretase activity include members of a disintegrin and metalloprotease family of proteins, ADAM 10 and ADAM 17 or TACE (tumour necrosis factor-α converting enzyme) [96–98]. The amyloidogenic pathway is initiated when β-secretase, identified as the aspartyl protease β-site APP cleaving enzyme (BACE1, Asp-2 or memapsin-2) [99, 100], cleaves APP at the N-terminal part of the Aβ domain. This cleavage leads to the extracellular release of sAPPβ, while the β-CTF or C99 fragment remains membrane bound. Sequential γ-secretase cleavage of C99, at the C-terminal of Aβ, allows the shedding of the AICD and the secretion of Aβ species of variable length, into the lumen or extracellular space [101]. γ-Secretase is thought to be an intramembranous-cleaving polytopic aspartyl protease [102], comprised a complex of presenilin1 (PS1), presenilin2 (PS2), nicastrin, aph-1 and pen-2 [103–105]. The presenilins (PSs) are transmembrane homologue proteins [106], which have been shown to be essential for the γ-secretase cleavage of APP [107, 108] as well as other type I proteins [109]. Mutations in PSs have been shown to alter APP processing and Aβ levels in mice [110] and are associated with the inheritance of early onset familial AD in human beings [111].

Following their discovery and characterization, the APP secretases became attractive targets in the quest for an AD treatment. The logic behind modulating the APP secretases is two fold: stimulating α-secretase cleavage in order to direct APP processing towards the non-amyloidogenic pathway or suppressing β- and/or γ-secretase cleavage in order to reduce the amount of Aβ produced. It has been shown that muscarinic AChE-receptor agonists can foster α-secretase processing of APP to subsequently result in a reduction in Aβ levels [112, 113]. This has been further demonstrated in animal models of AD, where the treatment of triple transgenic mice [114] with the M1 AChR agonist NGX267 (TorreyPines Therapeutics, La Jolla, CA, USA) resulted in reduced $Aβ_{1–42}$, reduced amyloid load and decreased τ-phosphorylation as well as improved behaviour [115]. Numerous β- and γ-secretase inhibitors and/or modulators have also been designed; however,
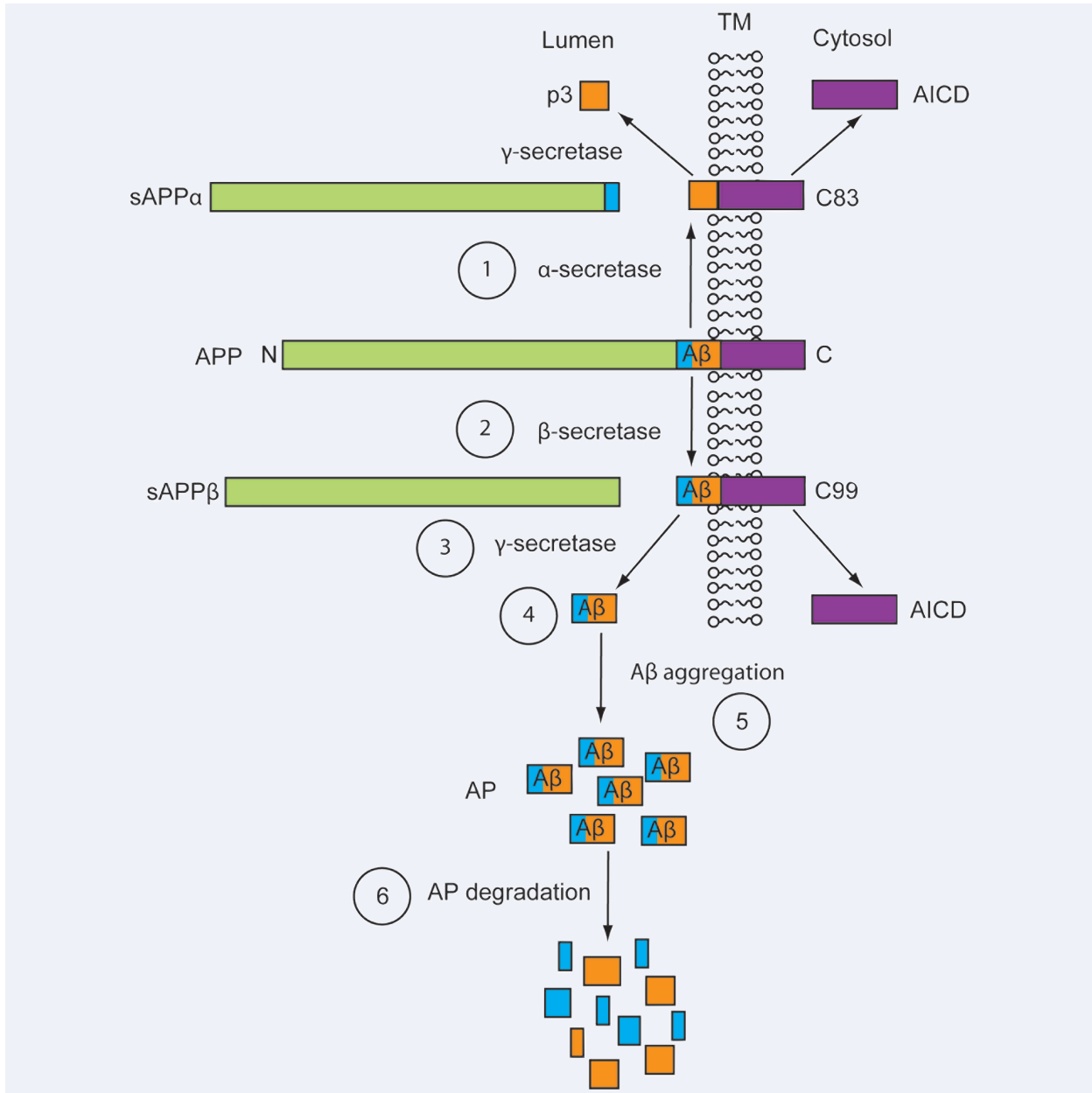
Fig. 2 Pharmacotherapeutic strategies for the treatment of Alzheimer's disease targeting Aβ. Schematic representation of the anti-amyloidogenic targets for potential pharmacotherapies: (*i*) α-secretase activators, (*ii*) β-secretase modulators/inhibitors, (*iii*) γ-secretase modulators/inhibitors, (*iv*) Aβ immunotherapy, (*v*) Aβ aggregation inhibitors, (*vi*) Amyloid-plaque degredation enhancers. Abbreviations: Aβ (amyloid-β), AICD (APP intracellular domain); AP (amyloid plaque); APP (amyloid precursor protein); sAPPα (soluble APP-α); sAPPβ (soluble APP-β); TM (trans-membrane).

the majority of these agents are not specific for the secretase cleavage of APP and thus may prevent the cleavage and processing of additional substrates, which could result in various adverse effects [116, 117]. At the moment, the β-secretase inhibitor TAK-070 (Takeda Pharmaceutical Co. Ltd., Osaka, Japan) is undergoing a phase I clinical trial. A number of γ-secretase-targeting compounds are in early clinical development, including a selective

γ-secretase inhibitor (BMS-708163; Bristol-Myers Squibb, New York, NY, USA) and a γ-secretase modulator (E2012; Eisai Inc., Woodcliff Lake, NJ, USA). The most advanced compound, however, is the γ-secretase inhibitor hydroxyl-valeryl monobenzocaprolactam/ LY450139 dihydrate (Eli Lilly, Indianapolis, IN, USA). A 40-week, multi-centre, randomized, double-blinded, dose escalation, placebo-controlled, parallel assignment phase II study (safety, tolerability

and biomarker assessment) with LY450139 dihydrate in individuals with mild-to-moderate AD showed that individuals who received either the low (100 mg/day) or high (140 mg/day) dose of the drug had a significant (~60%) decrease in plasma $A\beta_{1-40}$ compared to placebo; however, $A\beta_{1-40}$ changes in cerebrospinal fluid (CSF) were not statistically significant [118]. Recruitment of approximately 1,500 individuals for a phase III trial to study the effects of LY450139 dihydrate (100 or 140 mg per day) on the rate of cognitive and functional decline *versus* placebo over a 2-year period has begun, with the clinical trial estimated to be complete in the first quarter of 2012.

A focal point at ICAD 2008 was the announcement by Myriad Genetics (Salt Lake City, UT, USA) that the most extensive (1,649 patients treated over 18 months in a phase III) AD clinical trial ever to be completed (tarenflurbil/Flurizan™ 800 mg/twice daily or placebo) had failed to demonstrate significant differences in any of its outcome measures, including ADAS-Cog and Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS-ADL) scores. Thus, the γ-secretase modulator Flurizan™ was ineffective in slowing disease progression. The failure of this trial has raised many issues within the AD research community with the main question being whether or not β- and/or γ-secretase modulators should still be considered as a therapeutic target. Many scientists believe that a wiser strategy to targeting Aβ production is to target Aβ after it has been synthesized.

Aβ aggregation inhibitors

As described above, Aβ is constitutively synthesized at the membrane surface by proteolytic cleavage and is then secreted [119]. Aβ typically ranges between 38 and 43 amino acid residues in length with $A\beta_{1-40}$ and $A\beta_{1-42}$ being the most prominent types in AD [120]. Following its secretion, extracellular Aβ can later be internalized back into the cell by poorly understood molecular mechanisms. Recently, it was reported that in the absence of apolipoprotein E (ApoE), $A\beta_{1-42}$ is internalized in axons of primary neurons *via* a clathrin-independent endocytic pathway involving lipid rafts [121]. The rapid turnover of Aβ in the brain [122, 123] suggests efficient clearance and/or degradation mechanism(s) of the peptide are in place. Detection of Aβ in plasma and CSF [124], implies that Aβ can be transported from the CNS across the BBB into the periphery. In this regard, a few receptors (involved in cholesterol and/or lipid metabolism) have been suggested to mediate Aβ efflux from the brain, including MDR1-P-glycoprotein (P-gp/ABCB1) [125], receptor for advanced glycation end products (RAGE) [126] and the extensively studied low-density lipoprotein receptor-related protein (LRP). Aβ has been shown to bind directly to LRP-1 and LRP-2/megalin or indirectly, by binding to their ligands: apolipoprotein J and E (ApoJ and ApoE, respectively) and α2-macroglobulin (α2 M) [127–129]. Aβ-LRP1/2 complexes can be internalized and delivered to the endosomal/lysosomal compartments, where they either undergo autophagy in a similar manner to APP, or they may undergo transcytosis into the CSF or plasma [130, 131]. Aβ is finally eliminated

through the kidney and liver *via* LRP [132, 133] or by liver X receptor [134–136]. Alternatively, Aβ can be catabolized *via* enzymatic degradation [137]. To this end, several classes of enzymes have been identified, including the serine proteases plasmin and tissue plasminogen activator [138–140], and the metalloproteases neprilysin [141–144], insulin degrading enzyme [145–148], as well as the zinc-dependent endothelin-converting enzyme 1 [149, 150] and matrix metalloproteinases 2 and 9 (MMP2 and MMP9, respectively) [151–153].

The fact that Aβ is normally produced in the body throughout life, is present in various organs and bodily fluids, and that the body has evolved sophisticated mechanisms for its metabolism (as detailed above) suggest that Aβ has a physiological role [154]. Although the function of Aβ is yet to be elucidated, Aβ has been proposed as an acute-phase apolipoprotein with metal-binding and antioxidant activities [155–160]. The idea that Aβ has a functional role leads us to the conclusion that with old age, and more specifically with the late onset of AD, Aβ either losses its physiological function or gains a pathological function [155, 156]. There are several theories as to factor(s) that may turn Aβ from being a physiological to a pathological agent; however, none of these hypotheses are definite and all of them still have many caveats. However, it has been consistently demonstrated that Aβ exerts neurotoxic and synaptotoxic affects both *in vitro* [161] and *in vivo* [162]. Researchers have turned to the study of Aβ structure in search of clues as to its toxic effects. It was found that soluble Aβ monomers assume a random coil or α-helix conformation; however, in AD they undergo a structural change into a pleated β-sheet [163]. This induces the peptide to form low molecular weight oligomers, higher molecular weight complexes (protofibrils and amyloid-β derived diffusible ligands or ADDLs), mature fibrils and amyloid plaques (APs) in the neuropil and the vasculature [164–166]. *In vitro* studies have shown that amyloidogenesis and fibrillogenesis can be affected not only by the type of Aβ produced and its conformation, but also by factors such as time, concentration, temperature, pH and metal ion concentration [167]. For many years it was believed that the toxic effects of Aβ were a result of the mature Aβ fibrils; however, recent studies suggest that low molecular weight, soluble, oligomeric forms of $A\beta_{1-42}$ rather than $A\beta_{1-40}$ [168] are more neurotoxic than the mature Aβ fibrils [169–173]. Indeed, the severity of AD correlates more closely with cerebral concentrations of soluble Aβ rather than insoluble Aβ load (reviewed by Lesne and Kotilinek [174]). As our understanding of Aβ structure improves and with the advent of more advanced techniques, the development of inhibitors of Aβ oligomers will improve [175]. Candidate drugs in this category are synthetic peptides based on the $A\beta_{17-21}$ sequence, with the five-amino-acid β-sheet breaker peptide Ac-LPFFD-NH2 (iAβ5p) as its lead compound [176, 177], the discontinued tramiprosate/Alzhemed™ (Neurochem Inc.) and ELND-005/AZD-103 (developed as a joint venture by Elan Pharma International Ltd., Dublin, Ireland and Transition Therapeutics, Toronto, ON, Canada). Tramiprosate/Alzhemed™ is in fact a variant of the amino acid taurine (3-amino-1-propanylsulfonic acid [3-APS]) [178], which prevents sulphated glycosaminoglycans from promoting the

oligomerization of soluble Aβ [179], but at the same time also enhances non-toxic τ-aggregation *in vitro* [180, 181]. Unfortunately, pre-clinical studies of tramiprosate/ Alzhemed™ in TgCRND8 mice did not include an investigation of τ-pathology or any behavioural testing. Phase II trial results showed the only significant effect of tramiprosate/Alzhemed™ treatment was a dose-dependent reduction in CSF Aβ$_{1-42}$, but had no significant impact on CSF Aβ$_{1-40}$ and τ, or on psychometric scores [182, 183]. Despite these disappointing results, the investigational drug progressed into a phase III trial in Northern America, which was recently declared by the FDA to have failed. As a result, the European Phase III study of tramiprosate/Alzhemed™ has been abandoned and the compound is being marketed as a nutraceutical, although a phase II trial for its use as a preventative of hemorrhagic stroke in patients with cerebral amyloid angiopathy (CAA) is ongoing. Another investigational drug, ELND-005/AZD-103 (Transition Therapeutics, Toronto, ON, Canada and Elan, Dublin, Ireland), is an orally administered compound that crosses the BBB and is believed to break-down Aβ aggregates and prevent further Aβ oligomerization from taking place. In transgenic mouse models of AD, ELND-005/AZD-103 treatment improved their spatial memory performance in the Morris Water Maze. In several phase I studies, single and multiple ascending doses of ELND-005/AZD-103 were shown to have good safety, tolerability and pharmacokinetic profiles. At present, ELND-005/AZD-103 is undergoing an 18-month phase II trial in 340 patients with mild-to-moderate AD in order to confirm its safety and to evaluate its efficacy on cognition and functionality.

Another approach has been to try and characterize the mechanism(s) involved in the neurotoxicity of Aβ as a basis for developing pharmacotherapeutics that modulate these processes. Aβ-associated neurotoxicity may be attributed to various factors [184], including: Aβ interactions with intracellular target(s) and/or extracellular Aβ interaction with membrane surface receptor(s), cholesterol, lipids and lipoproteins [185, 186]. Activation of microglia and inflammatory factors [187] and induction of apoptosis by Aβ-mediated activation of cysteine aspartyl proteases termed caspases [114, 188, 189] have also been proposed to have neurotoxic effects. Berman and colleagues recently demonstrated that Aβ oligomer-induced neurotoxicity is due to the destabilization of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P$_2$) metabolism [190]. Another proposed mechanism of Aβ toxicity is the promotion of ion-channel formation and calcium ion (Ca^{2+}) influx [191]. This theory gained support from pre-clinical and early clinical trials with different neuronal L-type calcium channel blockers, such as S-312-d, nimodipine and MEM 1003 (Memory Pharmaceuticals, Montvale, NJ, USA) [192–195]. However, meta-analysis of clinical studies revealed that nimodipine only slows down the disease progression and may be effective only in certain types of dementia [196]. As for MEM 1003, late last year Memory Pharmaceuticals announced that the drug failed to show changes in ADAS-Cog scores between treated and control mild-to-moderate AD patients in a phase IIa trial, yet the company is still testing the efficacy of MEM 1003 in individuals with bipolar disorder (www.memorypharma.com).

Passive or active immunization

A novel and controversial approach to treating AD is based on vaccine therapy. Transgenic mouse models of AD actively immunized with Aβ [197–200] or passively immunized with humanized anti-Aβ antibodies [201–208] showed reduced Aβ and τ-pathology, neutralized soluble Aβ oligomers, attenuated synaptic degeneration and improved synaptic plasticity, all of which were accompanied by improved learning. Immunization against Aβ thus appeared to be the much-anticipated breakthrough in the development of AD therapeutics, in addition to being the primary test of the amyloid cascade hypothesis. An active immunization strategy was rapidly advanced into clinical trials by Elan, and following successful completion of the phase I trial, a phase IIa trial with AN-1792/Betabloc was initiated by Elan/Wyeth. This study was terminated after four patients presented with symptoms consistent with autoimmune meningoencephalitis [209, 210] and by the end of 2002 there were 18 known cases [211]. A subsequent autopsy analysis of a phase I study patient, who died 20 months after the first inoculation, indicated evidence of encephalitis [212]. This, together with three later autopsy cases of AN-1792-immunized AD patients, highlighted the persistence of CAA despite the removal of Aβ from plaques [213], consistent with observations from studies in PDAPP mice [214, 215] and monkeys [216]. A follow-up study of a further 36 patients demonstrated that many developed anti-Aβ antibodies, which was consistent with a slowing in the rate of cognitive decline 12 months after completion of the trial [217]. Patients with the highest titres also displayed the greatest slowing in cognitive decline [218]. While encouraging, MRI scans of the antibody responders revealed a reduction in total brain volume and the rates of cognitive decline in the non-responders appeared more rapid than typical [219]. However, a composite neuropsychological performance study has shown that the patients developing Aβ antibodies showed improvements in memory, attention and concentration, along with decreases in the level of τ-protein in CSF [220]. The most recent data to emerge from the original immunization trial, however, appear to confound some of these earlier reports. The long-term clinical follow-up of 80 patients demonstrated that, despite a varied degree of Aβ plaque removal, there was no prevention of progressive neurodegeneration and no evidence for improved survival [221]. Of note, seven of the eight immunized patients that underwent autopsy, including two patients with near complete removal of plaques, had severe end-stage dementia prior to death [221]. Despite its tragic outcome, valuable lessons learnt from this failed trial have lead researchers to develop more selective, advanced immunotherapies [222–225], including another active Aβ vaccine developed by Elan and Wyeth (Madison, NJ, USA) (ACC-001). Affiris GmbH (Vienna, Austria) is also developing an active immunization program with AFFITOPE AD01 (phase I study due to be completed in November 2008) and AFFITOPE AD02 (recruitment stage for a phase I trial due to be completed in early 2009).

The development of intravenous recombinant humanized anti-Aβ monoclonal immunoglobulins (IVIg), which avoid the induction

of an immune response, continues in parallel. Two small, independent phase I investigations of AD patients with IVIg over six months proved to be safe, stopped the cognitive deterioration and in most cases even resulted in a slight improvement of ADAS-Cog scores [226]. Examples of passive vaccines against Aβ in various stages of research and development are: phase I (V950, Merck, Whitehouse Station, NJ, USA; PF-04360365, Pfizer, New York, NY, USA), completed phase II (LY2062430, Eli Lilly, Indianapolis, IN, USA), and ongoing parallel phase II and III (AAB-001/Bapineuzumab, Elan with Wyeth, Madison, NJ, USA). Data from a phase II study with LY2062430 indicate that the monoclonal antibodies lead to elevated levels of $A\beta_{1-40}$ and $A\beta_{1-42}$, both in serum and CSF; however, SPECT analysis did not reveal any reduction in APs and no improvement in cognition was detected. Despite this, the company has announced its intention to commence a phase III study with LY2062430 in the coming year. With regards to AAB-001/bapineuzumab, modified intent-to-treat (MITT) interim analysis of phase II studies showed no significant changes in ADAS-Cog and Disability Assessment Scale for Dementia in the total study population and no statistically significant changes in any of the cognitive or functional efficacy endpoints in the ApoE4 carrier sub-group. In fact, a significant elevation in ventricular volume was observed in ApoE4 carriers treated with the drug. However, *post hoc* MITT analysis of the results did show statistically significant differences from baseline in ADAS-Cog, the Neuropsychological Test Battery and the Clinical Dementia Rating Sum of Boxes, as well as the Brain Boundary Shift Integral in the non-ApoE4 carrier sub-group treated with AAB-001/bapineuzumab compared to placebo. It should be noted that individuals treated with the drug experienced significantly more cases of cataracts, deep vein thrombosis, syncope, seizures and pulmonary embolism, as compared to placebo control patients. Importantly, vasogenic edema was observed only in drug-treated patients and mostly in ApoE4 carriers treated with the highest dose of the drug (2.0 mg/kg). The significance of the results, however, will only be made clear once a final analysis is done after the completion of all phase II and III trials.

The metal hypothesis of AD

It is evident that both Aβ and τ are involved in the development and progression of AD; however, pharmacological strategies directed at these targets have not yet proven to be disease modifying in human studies. In particular, several investigational drugs that target Aβ have failed to show any correlation between a reduction in amyloid burden and improvement in cognitive functions in large-scale clinical trials (as mentioned above). While such data might indicate that the 'amyloid hypothesis' of AD is not necessarily the correct one, there remains considerable debate as to whether it has yet to be truly tested in the clinic. Numerous factors have been proposed to account for the poor performance of several frontline drugs, including: patient confounds (*e.g.* ApoE genotype, overall rates of cognitive decline in placebo groups),

trial design (*e.g.* is a 'treatment' protocol, as opposed to a 'prevention' protocol, the best way to test the hypothesis) and drug penetration (*e.g.* it is suggested that Flurizan may have failed because of a poor pharmacodynamic profile). While the debate over the validity of the amyloid cascade hypothesis will no doubt continue, it remains likely that there are other critical factors playing a role in AD pathogenesis.

Metal ions are one such possibility, as cerebral concentrations of zinc (Zn), copper (Cu) and iron (Fe) ions are significantly elevated in AD, compared to age-matched controls [227–230], and metals have been implicated in several other neurodegenerative diseases [231–234]. Here, we will review the various events in AD pathogenesis in which metal ions are involved, and then discuss the pharmacotherapeutics being developed to modulate metal ions in AD.

There is an increasing amount of evidence suggesting that τ and NFTs may in some way be involved in, or regulated by, metal metabolism. Zinc ions (Zn^{2+}) [235] and the iron regulatory protein-2 [236], for example, have been found to co-localize with NFT-containing neurons. Addition of Zn^{2+} to mouse and human neuroblastoma cells (N2a and SH-SY5Y, respectively) induces τ-hyperphosphorylation [237], whereas the opposite result is seen in hippocampal neurons with the addition of pyrolidium dithiocarbamate (PDTC) [238] or iron citrate ($FeC_6H_5O_7$) [239]. Ferric ions (Fe^{3+}) and cupric ions (Cu^{2+}) can bind to various 'repeat' motifs on τ, thus altering the protein's conformation, promoting its phosphorylation [238] and inducing its aggregation [240–242]. In the case of iron, this effect can be reversed by reducing Fe^{3+} to Fe^{2+} (ferrous ions) [243]. As for APs, they have been shown to be enriched with Cu (400 μM), Zn (1 mM) and Fe (1 mM) [114, 176, 192–194], suggesting that there may be an interaction between metals, APP and Aβ that may influence Aβ aggregation and Aβ-associated toxicity.

It has been demonstrated that APP contains putative zinc and copper-binding domains (CuBD) both in its ectodomain and in its Aβ sequence (see Fig. 3). Little is known about the APP Zn-binding domain; however, it has been established that its CuBD consists of a tyrosine (Tyr^{168}), a methionine (Met^{170}) and two histidine ($His^{147, 151}$) residues that are able to coordinate Cu^{2+} and reduce it to Cu^+ [244]. The similarities between the CuBD on APP and Cu chaperone proteins suggest that APP may play a role in metal homeostasis [245]. This notion has recently gained support from findings that the translation of APP mRNA is governed by the binding of an iron-regulatory element to its 5′-untranslated region such that in an Fe-enriched environment APP translation is up-regulated, whereas it is down-regulated in response to an Fe-deficient milieu [246, 247]. Moreover, increasing Cu levels *in vitro* can shift APP processing towards the non-amyloidogenic pathway and result in decreased Aβ production [222–225]. This may result from an increase in GSK-3β phosphorylation, which activates phosphatidylinositol-3-kinase (PI3K) to result in the secretion of MMPs that can degrade Aβ [225]. In addition, genetically modified animal models of AD provide vital clues as to the affects of APP and Aβ on metal-ions and *vice versa*. Tg2576 mice that over-express the Swedish double mutant APP_{695} (K-670-N and M-671-L)

Fig. 3 Copper binding domains on APP. APP contains two high-affinity copper binding domains: one on its N-terminus and the other on the Aβ sequence. Highlighted in red are the copper binding ligands in the CuBD and in the $Aβ_{1-42}$ sequence. Abbreviations: Aβ (amyloid-β); APP (amyloid precursor protein); CuBD (copper binding domains); TM (trans-membrane).
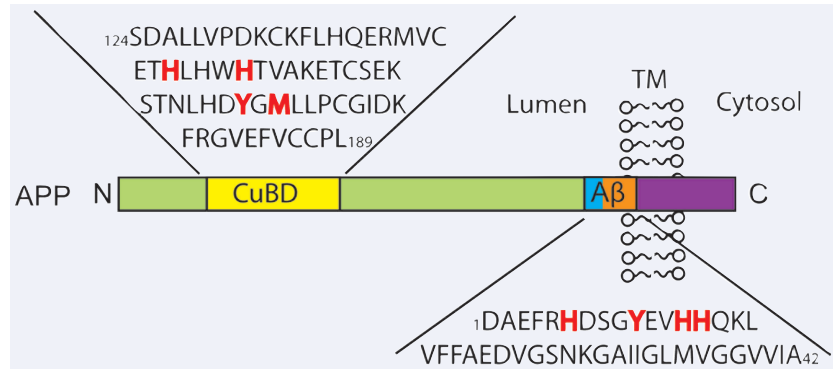
exhibit AD-related behavioural and cognitive changes (memory and spatial learning impairments) [248] and AD-related pathology (substantially elevated levels of full-length APP, CTFs and cerebral extracellular Aβ) [249]. However, their cerebral Cu (but not Fe) levels are significantly reduced [224, 250]. C100 mice over-express Aβ and the C-terminal of APP, yet have significantly lower levels of both Cu and Fe in the brain [250]. Conversely, APP (and APLP2) knockout mice have raised brain and liver Cu levels [251] and develop reactive cerebral gliosis and locomotor-behavioural changes with age [252]. These studies all suggest a role for APP in metal homeostasis. As a further demonstration that metal homeostasis is important in the pathogenesis of AD, when $APP_{Swe}/PS1_{P-264-L}$-expressing mice, which also have ~15% lower brain Cu levels compared to non-transgenic controls, are crossed with TxJ 'toxic milk' mice (that have a mutated ATPase7b transporter and a consequent elevation in Cu levels), the resulting progeny have markedly reduced AP load and Aβ levels [224]. Similarly, increasing dietary copper intake in APP23 mice (carrying the Swedish mutation of human APP_{751}, regulated by the murine Thy-1.2 promoter [253]) resulted in reduced Aβ levels and a prolonged lifespan [222]. Conversely, increasing dietary Cu intake in normal rabbits resulted in elevated Aβ levels and impaired learning [134, 254]. Thus, metal homeostasis appears to be intimately involved in Aβ metabolism.

These *in vivo* studies are supported by a wealth of *in vitro* data demonstrating that low concentrations of Zn^{2+} promote the rapid aggregation of Aβ at physiological pH [255–259]. At mildly acidic pH, Cu^{2+} (and Fe^{3+}) have also been shown to induce Aβ precipitation [227, 230, 260–262]. These data suggest that the synaptic cleft is an ideal location for Aβ metallation and aggregation, as neurotransmission results in peak concentrations of ~300 μM Zn^{2+} [263, 264] and up to 100 μM Cu^{2+} [265–267]. This is supported by observations of a significant reduction in plaque formation in a transgenic mouse model of AD (Tg2576) lacking the zinc transporter 3 (ZnT3) protein ($Tg2576/ZnT3^{-/-}$) [268, 269], which is responsible for zinc enrichment and transport into pre-synaptic vesicles [270, 271]. The complicated process of Aβ aggregation makes it is difficult to characterize the binding of metals to Aβ, and while there have been numerous reports on the affinity and stoichiometry of Aβ-metal binding, results have varied depending on:

the Aβ source (mouse, rat or human), Aβ sequence or length ($Aβ_{x-16/28/40/42}$), Aβ species (monomers, oligomers, etc.), as well as the reaction conditions (sample preparation, type and concentration of buffer, pH, incubation time and/or technique used). Most researchers are in agreement that Aβ binds Cu^{2+} and Zn^{2+} in a 1:1 ratio [272–276]; however, there have also been reports of Zn^{2+} binding to Aβ in a 2:1 [277] and 3:1 stoichiometry [278], and of Cu^{2+} binding to Aβ in a 2:1 ratio when copper is in excess [279, 280]. Mounting evidence indicates that the Aβ:metal ions ratio modulates not only Aβ conformation (random coil, α-helix or β-sheet) and aggregation [281–283], but also the morphology of the Aβ aggregates (amorphous, non-fibrillar or fibrillar) [280, 284, 285]. There is also an ongoing debate as to the binding affinity and kinetics of Aβ to Cu^{2+} and Zn^{2+}, with dissociation constants (K_D) ranging from nM to μM for Cu^{2+}-Aβ [272, 286, 287] and for Zn^{2+}-Aβ [255, 276, 287–291]. A novel study has even suggested an initial, weak Zn^{2+}-$Aβ_{40}$ complex, which quickly turns into a high-affinity complex, possibly due to a conformational change of the peptide [287]. In order to resolve the issues above, it is imperative that the metal-binding site(s) of Aβ and APP are defined and that the relationship between the structural features of the protein and its function in health and disease can be elucidated. Recent studies [287, 292] utilizing the electrospray-ionization mass spectrometry, Raman spectroscopy, electron paramagnetic resonance, circular dichroism, nuclear magnetic resonance, X-ray diffraction and extended X-ray absorption fine structure spectroscopies have determined the coordination of Cu and Zn by His^6, His^{13}, His^{14} [163, 255, 262, 272, 284, 286, 293–301] and a fourth ligand. The fourth donor could be Tyr^{10} [293, 301] and/or Glu^{11} [288, 302] for Zn^{2+}, or Tyr^{10} [293, 296] and/or Asp^1 [272, 298, 299] for Cu^{2+}. Interestingly, mouse and rat Aβ contains three amino acid substitutions (R-5-G, Y-10-F and H-13-R), which prevent the formation of intermolecular histidine bridges [293, 303, 304] and therefore do not allow metal-induced Aβ aggregation *in vitro* [256, 260] and cerebral Aβ deposits *in vivo* [305].

In summary, the above findings demonstrate APP and/or Aβ play a major physiological role in regulating metal-ion levels. This cumulative data has lead Bush, Tanzi and colleagues to propose 'the metal theory of AD' [306], which stipulates that age-related endogenous metal dyshomeostasis in the brain allows binding of

redox-active metal ions (Cu^{2+} and Fe^{3+}) to Aβ. This can lead to neurotoxicity as Cu^{2+} stabilizes the neurotoxic, oligomeric Aβ species [307–309], induces the covalent di-tyrosine crosslink of Aβ [274, 286, 287, 310–317] and promotes the generation of SDS-resistant copper-derived diffusible ligands [278, 286, 316]. Metallated-Aβ also has an increased affinity for the phospholipid heads of the membrane bilayer [318, 319], which acts as a reductant in the production of reactive oxygen species (ROS) *via* Fenton and Haber–Weiss chemistry [320, 321]. The resulting radicals, such as hydrogen peroxide (H_2O_2) and superoxide (OH^-), induce oxidative stress damage of lipids, proteins and DNA, ultimately leading to synaptic and neuronal loss [230, 231, 320–326]. Based on this hypothesis, pharmacotherapeutics that aim to restore metal homeostasis, inhibit Aβ-metal interactions and/or inhibit metallated Aβ-catalysed oxidation are being developed.

AD pharmacotherapies targeting metal ions

The equilibrium (concentrations, distribution, stability and bio-availability) of metal ions is critical for many physiological functions. This is particularly true for the CNS, where metals are essential for development and maintenance of enzymatic activities, mitochondrial function [327, 328], myelination [329], neurotransmission [330], learning and memory [331, 332]. Due to their importance, cells have evolved complex machinery for controlling metal-ion homeostasis. However, when these mechanisms fail, the altered homeostasis of metal ions can result in a disease state, including several neurodegenerative disorders [333, 334]. Understanding the complex structural and functional interactions of metal ions with the various intracellular and extracellular components of the CNS, under normal conditions and during neurodegeneration, is essential for the development of effective therapies [335]. Accordingly, modulation of metal ions has been proposed as a disease-modifying therapeutic strategy for AD [336–338] and other neurodegenerative diseases [339, 340]. Antioxidants and metal-modulators represent two such therapeutic strategies.

Antioxidants

Antioxidant molecules are capable of neutralizing free or incorrectly bound metals, thereby interfering with the 'down-stream' generation of ROS and other radicals. Therefore, antioxidants may be used mainly as a preventative approach [341]. Numerous molecules with antioxidant properties, such as oestrogen, melatonin, vitamin C and E (L-ascorbate and α- topopherol, respectively), ginkgo bilboa extract, curcumin and flavonoids, have been shown to have neuroprotective effects against Aβ-induced toxicity in cell-based experiments [342, 343] and animal models [344–348], but have had conflicting results in a clinical setting [349–351].

Metal chelators

By definition, metal chelators bind strongly to two or more metal ions and form a cyclic ring, which converts the metal ions into an inert form and depletes the total pool of bioavailable metals. Desferrioxamine (DFO), an Fe chelator with high binding affinities for Zn, Cu and aluminium (Al) [352], was the first such agent to enter clinical investigations for the treatment of AD. Results of a 2-year-long, blinded phase II trial with a cohort of 48 AD patients demonstrated that 125 mg intramuscular injections twice daily for 5 days a week significantly slowed down the decline of some cognitive functions, compared to the two control arms (an oral placebo or no treatment) [353]. DFO, however, is a large hydrophilic molecule, which is not orally bio-available and does not normally penetrate the BBB. Hence, it is unknown whether the beneficial effect seen with the DFO treatment was due to the drug's interaction and/or chelation of metals, or due to a different mechanism all together [354]. Another hexadentate chelator, DP-109 (DPharm, Rehovot, Israel), is a large synthetic pro-drug that becomes activated following the cleavage of its two long-chain esters. Daily administration of DP-109 by oral gavage to female Tg2576 mice over a 3-month period reduced the formation and deposition of CAA and APs, as well as re-solubilized Aβ [355]. Like DFO, DP-109 is not expected to cross the BBB, therefore the way it exerts its anti-amyloidogenic effect is still not clear. Recently, DP-109 and DP-460 (another Ca, Cu and Zn lipophilic chelator) were reported to have neuroprotective effects in a G93A transgenic mouse model of amyotrophic lateral sclerosis [356], another neurodegenerative disease associated with metal imbalance [357, 358]. Other chelating agents have been reported to have different effects *in vitro*, including reduced $Aβ_{42}$-induced oxidative stress [359], and the solubilization of hypophosphorylated τ [360] and Aβ from AD brain [361]. Further *in vivo* studies with these chelators is required to further advance this therapeutic route and to rule out any systemic effects.

An alternative approach to chelation is to modulate metals with metallo-complexes. Such an approach serves to remove metals from biologically deleterious sites and potentially deliver them to areas of deficiency, thereby maintaining overall metal homeostasis.

Metal complexes

Metallo-complexes are emerging as a new potential therapeutic for AD. The rational guiding this strategy is the delivery of Cu, for example, to cellular compartment which are Cu-deficient, using metallo-complexes of pyrrolidine dithiocarbamate (M^{2+}-PDTC) or *bis*(thiosemicarbazone) (M^{2+}-BTSC), or preventing the harmful binding of Cu to Aβ, using platinum (Pt) complexed to 1,10 phenanthroline derivatives (L-PtCl$_2$).

PDTC is traditionally considered an inhibitor of the transcription-factor regulator nuclear factor-κB (NF-κB) with anti-inflammatory, antioxidant and anti-apoptotic properties [362–364] – all of which have been attributed to the synergistic interaction

between PDTC, Cu and/or Zn [365–371]. As well as preventing the nuclear translocation of NF-κB in a neonatal hypoxia-ischaemia model, PDTC also activates Akt and inhibits GSK-3β [372]. *In vivo*, oral PDTC treatment of APP/PS1 double transgenic mice resulted in increased cerebral Cu levels, as compared to non-treated APP/PS1 mice, as well as down-regulation of the GSK-3β signalling cascade, which lead to a decrease in τ-phosphorylation and an improvement in spatial memory, but had no effect on amyloid burden, glial activation or oxidative stress [238]. The latest data to emerge indicate that PDTC complexed to either Cu^{2+} or Zn^{2+} can act as proteasome inhibitors to induce apoptosis in numerous human cancer cells [373–375]. It would be of interest to examine if the same effects occur in cellular and/or animal models of AD.

The metallo-complexes of diacetyl*bis*(N^4-methylthiosemicarbazone) (M^{2+}-ATSM) and glyoxal*bis*(N^4- methylthiosemicarbazone) (M^{2+}-GTSM) have both been shown to have anti-bacterial, anti-fungal and anti-neoplastic/cytotoxic activities, by selectively delivering exogenous metal ions into metal-deficient cells [376, 377]. Cu^{2+}-ATSM is membrane permeable, selective for oxygen-deprived (hypoxic) cells, and is redox inactive therefore the ligand retains its Cu molecule [378, 379]. These properties are being exploited for its development as a radiotherapeutic agent [380–382] and as a radiopharmaceutical for positron emission tomography imaging [383, 384]. Cu^{2+}-GTSM can also cross the BBB; however, once inside the cell it is reduced by various cellular reductants and releases its Cu molecule, which is made available for the cell [378, 385, 386]. Treatment of hAPP$_{695}$-overexpressing CHO cells with Cu^{2+}/Zn^{2+}-BTSC ligands resulted in increased intracellular metal levels that, in turn, activated Akt/PI3K, c-Jun N-terminal kinase and GSK-3 [387]. Phosphorylation of the above kinases lead to the up-regulation of MMPs, which reduced extracellular levels of Aβ [387]. Examination of the effects of Cu^{2+}/Zn^{2+}-BTSC ligands on τ and translation of these studies to animal models of AD is currently underway.

Other radiopharmaceutical-based compounds being evaluated for treatment of AD are 1,10 phenanthroline derivatives complexed to platinum (Pt^{2+}). These ligand-PtCl$_2$ complexes have been designed to bind and alkylate the imidazole side chains on histidine residues 6, 13 and 14 on Aβ, thereby preventing the detrimental binding of Cu^{2+} to this Aβ metal binding site and subsequent Aβ-Cu^{2+} binding to the cell membrane [388]. This study identified the Pt(4,7-diphenyl-[1,10] phenanthroline)Cl$_2$ as a compound that binds to Aβ, changes the conformation of Aβ and inhibits Aβ aggregation [388]. In addition, this complex is able to inhibit Aβ-related neurotoxicity (restore the cell viability of primary mouse cortical neurons and suppresses the Cu^{2+}-Aβ-dependent H$_2$O$_2$ generation), and reverse Aβ-inhibited long-term potentiation (LTP) of mouse hippocampal slices as a measure of synaptotoxicity [388]. Future evaluation of the compound's ability to cross the BBB and exert beneficial effects in animal models for AD need to be performed prior to its advanced development as an AD pharmacotherapeutic.

The Aβ-metal interaction can be targeted not only to the Aβ sequence that binds metals, but also to the metals themselves.

Metal-protein attenuating compounds (MPACs)

MPACs have weak, reversible affinity towards metals, which enables them to compete with endogenous ligands for metal ions, target the harmful 'up stream' metal-protein reactions and restore normal metal levels in specific cellular compartments [389]. The first-generation series of MPACs were based on clioquinol (CQ; 5-chloro-7-iodo-8-hydroxyquinoline). CQ is highly lipophilic, absorbed quickly, can convert to glucuronated and sulphate metabolites, is able to cross the BBB and is excreted in urine and faeces [390–395]. CQ had been used as a therapeutic in cattle and human beings with Zn-deficiency diseases and for many decades was prescribed as an oral anti-amebic in addition to being used for the treatment of dysentery and diarrhoea [396, 397]. However, its oral preparation was withdrawn from the market during the 1960s to 1970s, as it was suspected to be involved in the development of subacute myelo-optico-neuropathy (SMON) [398–401]. SMON is characterized by sensory and motor disorders in the lower limbs, peripheral neuropathy and visual impairment due to demyelation of the spinal cord, optic nerve and peripheral nerves [402]. SMON affected people worldwide; however, it reached near-epidemic proportions in Japan, where a few related deaths were reported [403]. At the time, a mechanistic link between CQ and SMON was not established [404]. Later, it was suggested that CQ may transport metals into the CNS, which leads to neurotoxicity. Early studies demonstrated that CQ-Fe^{3+}, but not CQ or Fe^{3+} alone, induced degeneration of cultured retinal neuroblasts [405] by increasing cellular Fe concentrations and promoting lipid peroxidation [406]. However, it is now believed that intake of CQ at doses far exceeding the recommended ones and for prolonged periods, together with a post-World War II iron-deficient diet, are the reasons for a vitamin B12 deficiency that presented as SMON in Japan [407, 408].

CQ binds Cu^{2+} and Zn^{2+} (2:1 ratio) in a square, planar arrangement [409, 410] and exerts different effects on Cu and Zn, depending on its route of administration and the system in which it is tested [411–413]. The known interaction of CQ with Cu^{2+} and Zn^{2+} thus prompted an investigation into the effects of CQ on AD-related pathology. CQ was initially shown to dissolve synthetic Aβ-Cu^{2+}/Zn^{2+} aggregates and amyloid deposits from post-mortem AD brain [414]. This then prompted a study of the oral administration of CQ to Tg2576 mice over 9 weeks, which resulted in the normalization of cerebral Cu and Zn levels, a reduction in H$_2$O$_2$ synthesis, and a significant decrease in cortical amyloid deposition by ~49%, compared to control littermates [415]. Subsequently, CQ was shown to reverse Cu-suppressed, but not Zn-suppressed Aβ$_{1–40}$ fibril formation [416], and to rescue Ca^{2+}-mediated Aβ toxicity in neuronal cell culture [417]. Other studies, however, have suggested that CQ increases oxidative neurotoxicity [418]. As previously mentioned, CQ treatment caused a reduction in Aβ levels in CHO-APP cells, accompanied by increased phosphorylation of GSK-3 and MMP2/3 activity [225]. The cumulative data led to CQ being entered into clinical trials for the treatment of AD (PBT-1, Prana Biotechnology, Melbourne, Victoria, Australia), in which CQ slowed the cognitive decline of moderate to severe AD patients, with no signs of

severe adverse effects. It also influenced CSF-τ, lowered plasma Aβ_{1-42} with no change to CSF-Aβ_{1-42} levels [419]. Subsequent phase II/III studies, however, were stalled by the difficulties encountered in preventing di-iodo-8-hydroxy quinoline contamination during the required larger scale chemical synthesis for such trials [420]. The subsequent drug discovery program identified PBT2 (Prana Biotechnology) as an 8-hydroxy quinoline that lacks iodine, thereby enabling easier chemical synthesis, and which also has higher solubility and increased BBB permeability than CQ. This compound was then extensively screened in a variety of pre-clinical assays. In APP/PS1 Tg mice, PBT2 was shown to decrease soluble interstitial Aβ within hours, and to improve cognitive performance to levels equivalent to or greater than wild-type controls within days of treatment [421]. In addition, there was a significant decrease in insoluble Aβ load and the phosphorylation of τ, as well as a significant increase in synaptophysin levels – suggesting that a number of primary indices that characterize the AD brain had been successfully modulated by this orally bioavailable MPAC [421]. PBT2 then progressed into human clinical trial, and following a successful phase I study, it entered into a randomized, double blind, placebo-controlled, multi-centred, 12-week-long phase IIa trial with 78 mild AD patients (Prana Biotechnology). This study demonstrated safety and tolerability, reduced CSF levels of Aβ_{1-42} and improved cognition in patients taking PBT2 as compared to placebo [422]. Taken together, these data support the notion that the modulation of metals may be sufficient to significantly alter the onset and progression of AD, and that targeting metals may represent a more potent disease intervention than systemically targeting the production or degradation of the Aβ protein; however, these concepts need to be further explored in a larger phase III trial.

While CQ is continuing to be examined as a therapeutic for other diseases, such as Parkinson's disease, Prion diseases, Huntington's disease, diabetes and cancer [373, 411, 423–431], a finer dissection of the mechanism of action of drugs such as CQ and PBT2 will enable researchers to better design additional pharmacotherapies for the treatment of AD and other diseases.

Conclusion

It is evident that AD pathogenesis is a complex process involving both genetic and environmental factors; therefore development of effective disease-modifying drugs is proving to be a difficult task. Aβ, τ and metals are some of the therapeutic targets identified and compounds that modulate them represent promising drug candidates. With ongoing basic science and clinical research, we look forward to a greater understanding of the pathogenesis of AD, the completion of several comprehensive clinical trials and the development of new potential pharmacotherapeutic agents for the treatment and/or prevention of AD.

Disclosers and Acknowledgements

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