The Development of New Therapeutics for Alzheimer's Disease

MD Carter[1], GA Simms[1] and DF Weaver[1,2,3]

Existing treatments for Alzheimer's disease (AD) fail to address the underlying pathology of the disease; they merely provide short-lived symptomatic relief. Consequently, the progression of AD is unrelenting, leading to a continual decrease in cognitive abilities. Recent advances in understanding the genetic factors that predispose to AD, as well as in biomarker development, have brought with them the promise of earlier and more reliable diagnosis of this disease. As improvements continue to be made in these areas, the shortcomings of current AD treatments appear all the more acute because opportunities for early intervention are hindered by a lack of "curative" or even disease-modifying drugs. This State of the Art report reviews existing AD therapeutics and highlights recent progress made in the design and development of drugs that are aimed at disrupting AD disease progression by inhibition of the protein misfolding of β-amyloid (Aβ) into neurotoxic oligomeric aggregates.

Alzheimer's disease (AD) is the most common form of dementia and is therefore a fundamental disorder of cognitive awareness—one of the defining components of human consciousness.[1] The neuropathology of AD manifests as an immunoinflammatory reaction involving activation of both the innate and adaptive immune systems in the brain, thereby invoking a neurochemical cascade in which aggregation of β-amyloid (Aβ) and tau peptides sequentially leads to complement activation, microglial activation, cytokine/chemokine release, and ultimately diffuse neurotoxicity. Accordingly, current hypotheses suggest that the neuropathology of AD is initiated by the aberrant deposition within the brain of misfolded proteins, namely extracellular amyloid-based plaques and intracellular tau-based neurofibrillary tangles (NFTs).[2]

The clinical symptoms that are concomitant with these pathological changes include an erosion of reasoning, abstraction, memory, and cognitive capacities. Consequently, the onset of the disease is inevitably followed by increasing mental and physical incapacitation, including the loss of ability to look after one's own daily needs, followed by institutionalization and death. AD is the most common cause (60–80%) of dementia worldwide. The estimated rates of occurrence in various age groups are as follows: 65–74 years, 2.5%; 75–79 years, 4%; 80–84 years, 11%; and 85–93 years, 24%. AD is fourth in the list of leading causes of death in industrialized societies, preceded only by heart disease, cancer, and stroke; AD affects individuals of all races and ethnic groups, occurring slightly more commonly in women than in men.[1,2] Although AD is emerging as the most prevalent and socially disruptive illness of aging populations, its cause and cure remain enigmas.

The immense social and economic ramifications of AD have generated major efforts toward obtaining a better understanding of the disease and toward developing therapeutic agents for its treatment. Regrettably, however, there is no remission in the progression of AD, nor are there any disease-stabilizing drugs currently available.[3] An improved understanding of the underlying etiopathogenesis of AD and the discovery of novel disease-modifying agents for the treatment of AD are now neuropharmacologic research priorities.[4–6]

MOLECULAR NEUROPATHOLOGY OF AD

AD is characterized by two neuropathological hallmarks: extracellular deposits known as amyloid or "senile" plaques and intracellular NFTs.[7] In addition to these deposits, the AD brain is characterized by a dramatic loss of neurons and synapses in many areas of the central nervous system (CNS), particularly in areas involving higher-order cognitive functions such as the basal forebrain and hippocampus. Also, the levels of many neurotransmitters are greatly reduced, including serotonin, noradrenaline, dopamine, glutamate, and especially acetylcholine. These reduced neurotransmitter levels are thought to be responsible for the broad and profound clinical symptoms of

[1]Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada; [2]Division of Neurology, Department of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; [3]Department of Biomedical Engineering, Dalhousie University, Halifax, Nova Scotia, Canada. Correspondence: DF Weaver (donald.weaver@dal.ca)

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AD, which consist of memory impairment, cognitive deficits, and an impaired capacity for abstract thought.[1]

Amyloid plaques

Amyloid plaque is primarily composed of a 4.3-kDa peptide, first isolated from the brains of patients with AD in 1984. The Aβ peptide is a 39–43 amino acid protein that forms extraneuronal aggregates with a fibrillar, β-pleated structure. Aβ is cleaved from the amyloid precursor protein (APP), a 695–770 amino acid transmembrane protein found in virtually all peripheral and brain cells.[3] Although there is no conclusive evidence of the function of APP, an increasing body of data suggests that it is involved in regulating neurite outgrowth, synaptic plasticity, and cell adhesion.[8] The sequence of $A\beta_{1-42}$ is shown in **Figure 1**; the region contained within the transmembrane sequence of APP is identified, as are the sites of action of three APP-processing enzymes: α-, β-, and γ-secretase.

APP is normally cleaved within the Aβ domain by α-secretase (**Figure 2**), liberating a soluble N-terminal fragment (s-APPα) and a membrane-bound C-terminal fragment (C83). Alternatively, APP can be cleaved by β-secretase at the N-terminus of the Aβ domain, yielding s-APPβ and C99. The latter membrane-bound fragment then undergoes intramembrane cleavage by γ-secretase at the C-terminus of Aβ, resulting in the liberation of Aβ into the cell.[9] The secretion of Aβ follows, allowing the peptide to participate in extracellular aggregation and to become incorporated into growing plaques.

Numerous mutations have been identified in the gene encoding APP, which alter its expression or processing.[10] These mutations can cause early onset of AD and are implicated in the autosomal dominant form of the disease, which constitutes ~5% of cases. Patients with Down's syndrome, who have three copies of the *APP* gene (as a result of localization on chromosome 21), show diffuse Aβ deposits as early as in the second decade of life. These eventually develop into mature neuritic plaques that are indistinguishable from those found in the brains of patients with AD.

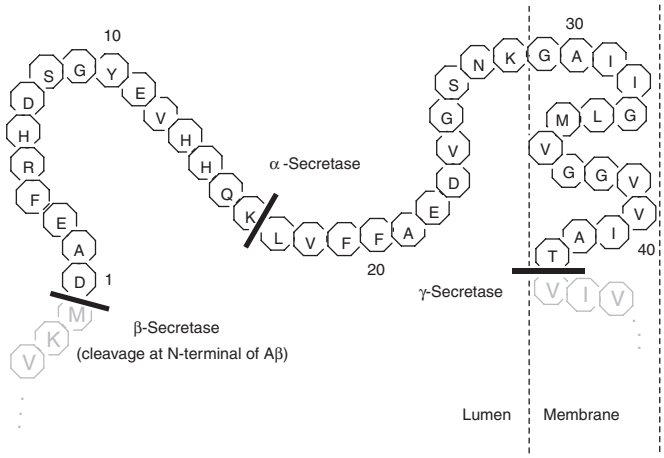

Figure 1 Sequence of $A\beta_{1-42}$ and sites of secretase cleavage on amyloid precursor protein (APP). γ-Secretase has low specificity, cleaving the APP anywhere between residues 39 and 43 of Aβ.

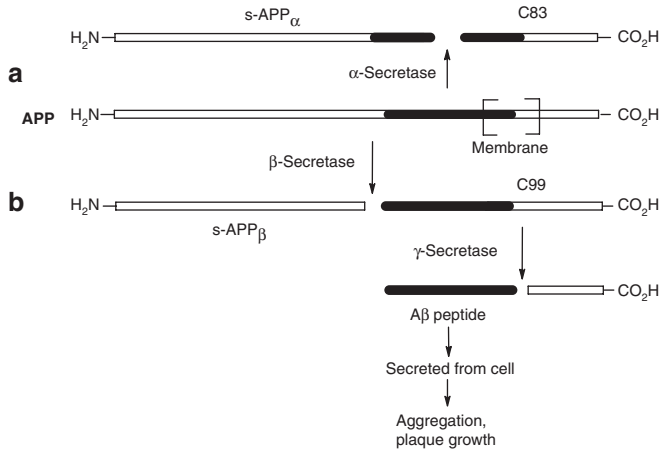

Figure 2 Processing of amyloid precursor protein (APP). (**a**) "Normal" cleavage within the Aβ region by α-secretase; (**b**) pathogenic cleavage of APP by β- and γ-secretase, liberating Aβ, which can self-assemble into neurotoxic aggregates and become incorporated into growing plaques.

NFTs

The chief component of intracellular NFTs is tau, a microtubule-associated protein abundant in six different isoforms in the adult brain. In AD, highly phosphorylated and glycosylated tau protein forms paired, helically wound fragments (paired helical filaments) ~10 nm in diameter, which associate to give insoluble tangles in nerve cell bodies and dendrites.[7] Tangles are a hallmark of many dementia-related diseases, including corticobasal ganglionic degeneration, frontotemporal dementia, myotonic dystrophy, Niemann–Pick disease, and progressive supranuclear palsy. The wide variety of neurological disorders in which NFTs occur suggests that paired helical filament formation is a nonspecific marker of certain types of neuronal injury.

Aβ AS A CAUSE OF AD

There are several findings to support the hypothesis that aggregation of Aβ is causally related to AD.[2,3] (i) In peripheral (non-CNS) amyloidoses, amyloid deposits have been shown to correlate strongly with tissue damage and organ dysfunction. (ii) The deposition of Aβ is one of the earliest neuropathological occurrences in AD; in related disorders, such as Down's syndrome, Aβ deposition can precede tau misfolding by many years. (iii) Aβ is toxic to cultured neurons. (iv) Unlike NFTs, which are common to a number of types of dementia, Aβ plaques are a relatively unique feature of AD and of the aging process (although other amyloidogenic plaques are involved in the pathology of other neurodegenerative diseases, e.g., prions in Creutzfeldt–Jacob disease). (v) Missense mutations in the APP gene cause early-onset familial AD; one mutation causes dramatic Aβ overproduction, and another causes an overproduction of the 42-residue, highly amyloidogenic form of Aβ. (vi) Transgenic mouse models in which Aβ is overexpressed show evidence of abnormalities of memory and behavior.[11,12] Collectively, these findings strongly implicate Aβ in the pathogenesis of AD. This link has motivated the search for therapies based on inhibition or reversal of Aβ aggregate formation.

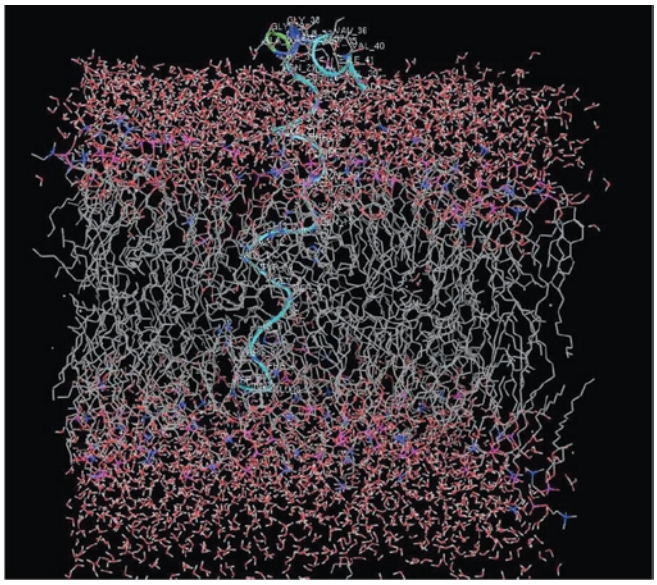

Figure 3 An atom-level exploration of the pathogenesis of Alzheimer's disease, by means of *in silico* studies using molecular dynamics and force-field calculations and employing dipalmitoylphosphatidylcholine bilayers as model membranes, demonstrated attachment of helical Aβ to the membrane through its cationic N-terminal face ($Aβ_{1–16}$), specifically anchored by the $Aβ_{13–16}$ HHQK domain, with subsequent insertion producing destructive disruption of the neuronal membrane. This insertion was greatly enhanced by the inclusion of cholesterol rafts that reduced the hydrophobic barrier to insertion while interfering with close packing of intramembrane fatty acid tails. Figure courtesy of V. Fermo, C. Barden, and D. Weaver, Dalhousie University.

Although there is substantial evidence that implicates Aβ in AD, there is debate as to the level of Aβ aggregation that is responsible for the observed neurotoxicity. Traditionally, large aggregates, present as senile plaques in the brains of patients with AD, were suspected of being the pathogenic species. Recent evidence, however, has suggested that smaller, diffusible Aβ oligomers may be the chief mediators of neurotoxicity in AD. Research into these assemblies has alternatively identified Aβ trimers and dodecamers as the principal toxic species.[13] The mechanisms whereby Aβ, or an oligomeric aggregate thereof, causes cellular death of neurons remain incompletely understood. It is currently thought that Aβ penetrates the neuronal membrane, where it aggregates and causes mechanically destructive changes to the cell membrane, culminating in cell death. Extensive *in silico* simulations of Aβ penetrating the neuronal membrane have been performed by Weaver and co-workers and are shown in **Figure 3**.

Whereas Aβ oligomers may be the pathologic species in AD, insoluble Aβ fibrils may contribute to neurotoxicity by supplying Aβ for continued formation of toxic oligomers; alternatively, the fibrils may constitute a neuroprotective safe reservoir of Aβ, preventing further formation of toxic oligomers. Analogously, Aβ monomers may likewise be neuroprotective, protecting mature neurons from excitotoxic damage and becoming neurotoxic only when they aggregate into oligomers.[14] Regardless of whether oligomeric Aβ are the cause of the greatest damage to neurons, preventing the self-assembly of Aβ is expected to mitigate neuronal damage. The $Aβ_{1–42}$ isoform has been the subject of much study because it aggregates more readily than $Aβ_{1–40}$ does, and its production is enhanced in familial, autosomal dominant cases of AD.[8] The concentration of free $Aβ_{1–42}$ in cerebrospinal fluid (CSF) is lower in patients with AD, while $Aβ_{1–40}$ levels are unchanged, a finding that could be explained by preferential incorporation of $Aβ_{1–42}$ into growing plaques. Supporting this explanation for depletion of $Aβ_{1–42}$ in CSF is the observation that this isoform binds twice as often to Aβ plaques taken from AD brain as does the shorter $Aβ_{1–40}$.

In addition to the uncertainty about the specific identities of toxic Aβ species, it is also not clear whether it is the extracellular or the intracellular Aβ aggregates that are the primary causative agents in AD.[15] Although extracellular plaques caused initial studies to focus on Aβ aggregation taking place outside the cell, much of the recent evidence has strongly implicated intraneuronal Aβ assemblies in AD disease progression.

Finally, there is the issue of whether Aβ aggregation alone is sufficient to account for the complex pathology of AD. Given that AD is a disorder of protein misfolding, it is also possible that Aβ and tau are coconspirators in the neuropathology of AD—if one is the bullet, the other is the trigger (or vice versa!).

STRATEGIES FOR THERAPEUTIC INTERVENTION IN AD

As currently conceptualized, AD is a chronic neurodegenerative disorder in which protein misfolding of Aβ (plus tau) leads to neuronal damage and destruction; as neurons die, they reduce their biosynthesis of multiple neurotransmitters (particularly acetylcholine), leading to a multiplicity of behavioral symptoms, including decreased memory and cognition. In line with this amyloid hypothesis of AD, there exists a cascade to neurotoxicity, commencing with protein misfolding and ultimately culminating in behavioral symptoms. If a therapeutic target is "high" in this cascade (e.g., protein misfolding), the resulting agent may be disease-modifying or even curative; if a therapeutic target is "low" is this cascade (e.g., neurotransmitter deficit replacement), the resulting agent is more likely to offer "symptomatic" improvement rather than disease modification.

Drug design and development for AD are focused on the identification of small-molecule therapeutics, i.e., new chemical entity organic molecules with drug-like properties (as determined by Lipinski's rules), with the ability to cross the blood–brain barrier (BBB) by either (most commonly) passive diffusion or active transport. In order to cross the BBB by passive diffusion, the drug molecule should be relatively small (molecular weight <400 Da) and have the right balance of lipophilicity and hydrophilicity ($1.0 < \log P < 3.5$, where P is the octanol–water partition coefficient). The druggable targets currently being exploited for the discovery of such agents may be summarized as follows:

1. Inhibitors of Aβ aggregation
   (a) Nonpeptidic small-molecule antiaggregants (synthetic or natural)
   Glycosaminoglycan-mimetics (tramiprosate-like agents)
   Scyllo-inositol
   Tricyclic pyrones and pyridinones

Monocyclic indoles
Peptidic antiaggregants

2. Inhibitors of Aβ production
   (a) β-Secretase inhibitors
   Hydroxyethylamine inhibitors
   Amine inhibitors
   Acylguanidine and related heterocyclic inhibitors
   Macrocyclic inhibitors
   (b) γ-Secretase inhibitors
   Azepinones
   Sulfonamide and sulfones
   Peptidic isostere-based inhibitors
   (c) γ-Secretase modulators
   Nonsteroidal anti-inflammatory drugs (NSAIDs)
   NSAID-like
   (d) α-Secretase activators
3. Inhibitors of Aβ-induced neurotoxic effects
   (a) Anti-inflammatory agents
   (b) Antioxidant agents
4. Inhibitors of Aβ-induced neurotransmitter effects
   (a) Cholinesterase enzyme inhibitors
   (b) *N*-methyl-D-aspartate antagonists
5. Inhibitors of tau-induced neurotoxicity
   (a) Tau antiaggregants
   Phenylthiazolyl hydrazides
   Rhodanine-based inhibitors
   Thiacarbocyanines
   Anthraquinones
   Phenothiazines
   (b) Glycogen synthase kinase-3 enzyme inhibitors
   Indirubins
   Maleimides
   Thiadiazolidinones
   Organometallic inhibitors

INHIBITORS OF Aβ AGGREGATION

Many molecules have been shown to inhibit *in vitro* aggregation of Aβ. Included in this diverse group of compounds are the natural products nicotine, melatonin, *scyllo*-inositol, and 3-aminopropane-1-sulfonic acid (tramiprosate); the surfactants sodium dodecyl sulfate and hexadecyl-*N*-methyl piperidinium bromide; NSAIDs; the antibiotic rifampicin; and polyanionic sulfonates and sulfates, including the histological dye Congo red (**Figure 4**).[16–18] More recently, a number of antihypertensive agents, most notably sartan angiotensin receptor blockers (e.g., candesartan), have also been shown to inhibit Aβ aggregation.[19]

Among the Aβ antiaggregants, *scyllo*-inositol and 3-aminopropane-1-sulfonic acid have been studied the most and have been shown to reduce Aβ plaque load and circulating Aβ levels

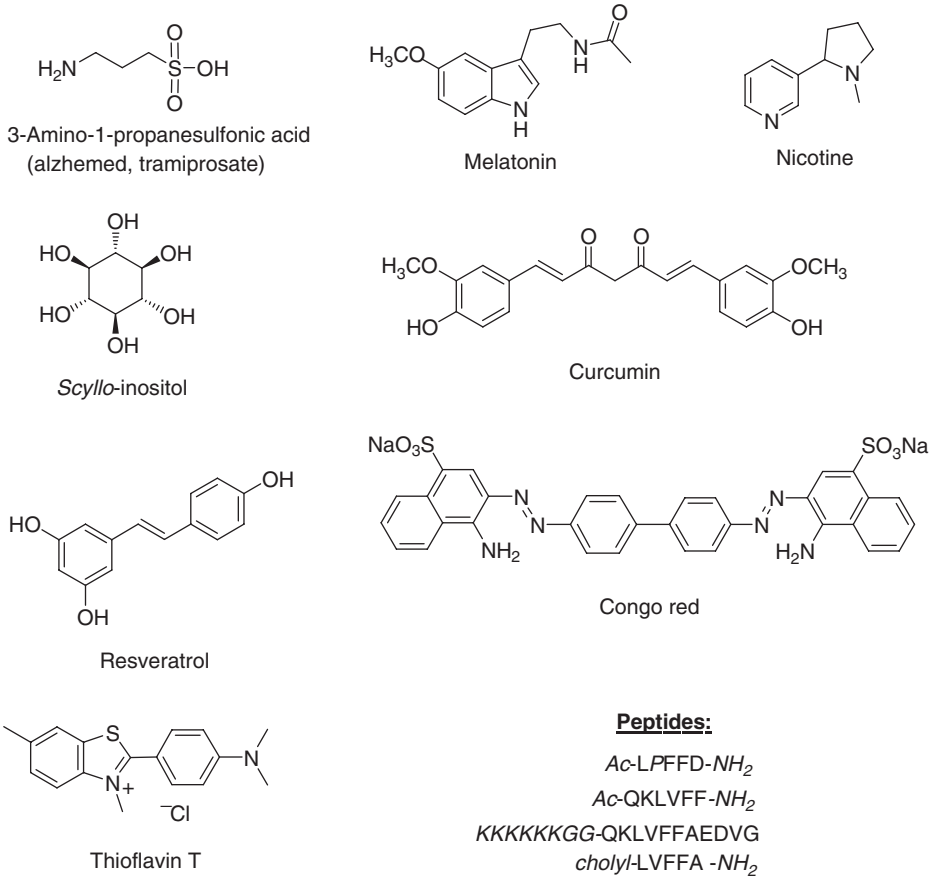

Figure 4 Some inhibitors of Aβ aggregation. Single-letter amino acid abbreviations are used for peptidic inhibitors, with modifications to Aβ sequence shown in italics. Ac, acetylated N-terminal; NH_2, amidated C-terminal.

in transgenic AD mice expressing human Aβ.[20,21] *Scyllo*-inositol has been further shown to rescue memory deficits in transgenic mice and in wild-type mice after cerebroventricular injection of neurotoxic Aβ oligomers. Clinical trials of *scyllo*-inositol in humans are under way, but Neurochem announced in November 2007 that tramiprosate would not be developed as a pharmaceutical because of insufficient proven efficacy in a phase III human clinical trial.

Recently, dietary polyphenols such as those found in red wine (e.g., resveratrol) and curry (e.g., curcumin) have also garnered much interest for their ability to disrupt Aβ aggregation and block the peptide's toxicity.[22,23] However, it is unclear how much of their activity is due to their antiaggregant properties and how much to the antioxidant and anti-inflammatory activities the two molecules are known to possess.

The type and size of Aβ aggregates that are affected by antiaggregant compounds have not yet been completely elucidated. Given that Aβ species are believed to exist *in vitro* and *in vivo* as a dynamic mix of monomers, small oligomers (dimers, trimers, etc.), larger oligomers (10-mers, 12-mers, etc.), protofibrils, and fibrils, compounds may bind to only a subset of these structures or possibly to all of them. It was recently shown, using surface plasmon resonance spectroscopy, that the dyes Congo red and Thioflavin T (**Figure 4**), already known to bind large fibrillar Aβ aggregates, also bind to soluble oligomers with high affinity. This raises the hope that small drug-like molecules may be found that bind to toxic Aβ oligomers and neutralize them, either by causing them to disassemble/assemble into smaller/larger nontoxic species or by binding to and thereby inactivating the toxicophore. Analysis of transgenic AD mice treated with *scyllo*-inositol suggested that the molecule may act through one such "oligomer-modulating" pathway; the mice receiving treatment had increased levels of Aβ monomer and trimer and decreased levels of an Aβ ~40-mer, a finding that could be explained by *scyllo*-inositol either binding to and breaking down the ~40-mer or binding to Aβ monomer and trimer and inhibiting their aggregation into larger oligomers.[20]

In addition to small molecules that inhibit Aβ aggregation, other efforts to develop molecules with this mechanism of action have focused on peptides with Aβ sequence homology.[24–26] These are designed to bind to Aβ and disrupt its normal assembly into toxic aggregates. This strategy generally involves synthesizing peptides of 5–11 residues in length that contain a "recognition" sequence, usually a region homologous to the hydrophobic LVFFA region at residues 17–21 of Aβ and a "disrupter" group attached to either the N- or C-terminal. Alternatively, the disrupter group can occasionally lie within the recognition sequence, as is the case when proline is used as a disrupter. Some peptidic Aβ aggregation inhibitors are shown in **Figure 5**.

The recognition sequence of peptidic Aβ aggregation inhibitors allows the peptide to become adopted into growing β-sheet Aβ assemblies. Once it is there, the bulky disrupter group (e.g., cholic acid, poly-lysine, and proline) acts to prevent any further Aβ strands from becoming incorporated in the aggregates, thereby arresting their growth.

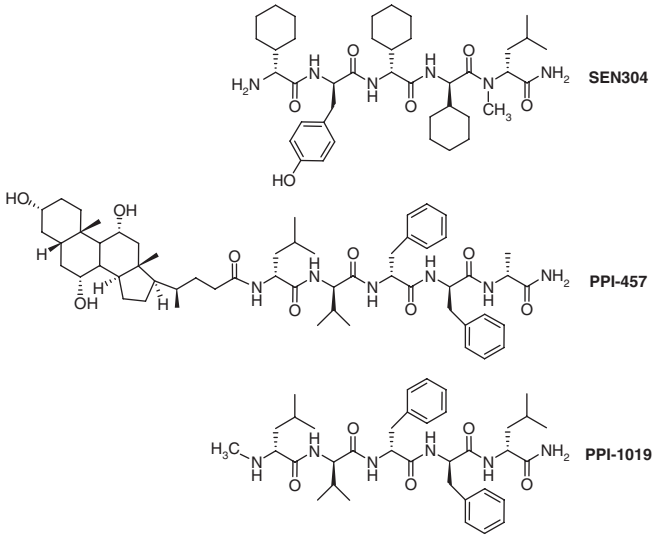

Figure 5 Structures of peptidic inhibitors of Aβ aggregation: SEN304, PPI-457, and PPI-1019. The peptide sequence of SEN304 is D-chGly-D-Tyr-D-chGly-D-chGly-D-mLeu-NH_2, where "chGly" = cyclohexylglycine and "mLeu" = *N*-methylleucine; that of PPI-457 is D-Leu-D-Val-D-Phe-D-Phe-D-Ala-NH_2, with a cholyl group attached to the N-terminal; and that of PPI-1019 is CH_3-D-Leu-D-Val-D-Phe-D-Phe-D-Leu-NH_2.

In one approach to designing peptidic inhibitors of Aβ aggregation, disruption was achieved by methylating several of the amide nitrogens on the peptide backbone.[26] These *N*-methyl peptides, or "meptides," bind to Aβ with their free face and block any further assembly because of their inability to form β-sheet N–H hydrogen bonds on the other (methylated) face. Although every second amide nitrogen should in theory be methylated for maximum effect, the lead meptide, SEN304, was found to be highly active despite bearing an *N*-methyl group at only one of its four peptide bonds.[26] Originally generated as part of a meptide analog library of the KLVFF region of Aβ, SEN304, having three unnatural cyclohexylglycine residues, bears little resemblance to the parent pentapeptide, unlike most peptidic Aβ aggregation inhibitors.

In the case of the peptidic inhibitor cholyl-LVFFA-NH_2, the peptidomimetic strategy of substituting D-enantiomers in the place of the naturally occurring L-amino acids has been explored. It was found that the all-D form of the peptide, termed PPI-457 and depicted in **Figure 5**, completely inhibits Aβ aggregation at peptide:Aβ ratios as low as 1:3. Unlike its all-L stereoisomer counterpart, however, PPI-457 was found to be stable in rhesus monkey CSF, its unnatural stereochemistry enabling it to avoid enzymatic degradation. Substitution of the bulky N-terminal cholyl group with a methyl group and the change of the C-terminal residue from D-alanine to D-leucine yields PPI-1019, a compound with reduced size but comparable activity.

PPI-1019, also known as Apan, completed phase I and II human clinical trials.[27,28] It was found to be safe, well tolerated, and able to cross the BBB. Furthermore, administration of the compound led to increased levels of $A\beta_{1-40}$ in CSF, suggesting that it may enhance clearance of Aβ from the brain into the CSF.[28]

The peptide Ac-LPFFD-NH_2 is a similar Aβ aggregation inhibitor; it was shown to elicit a reduction in aggregated amyloid and an increase in neuronal survival in transgenic mice.[29] It was found to be stable to proteolytic degradation despite being composed of naturally occurring L-amino acids, and it is also able to cross the BBB.

An additional strategy is to focus less on the specific aggregation of Aβ in particular and to generalize the therapeutic to the global process of protein misfolding that results in aggregates of both Aβ and tau. A class of synthetic bi-aromatics that prevents the neurotoxic aggregation of both Aβ and tau has recently been described.[30]

In addition to the promising results seen for inhibitors of Aβ aggregation, this strategy of drug discovery may also benefit from allowing normal Aβ production, a characteristic not shared by the β- and γ-secretase inhibitors described below. Although a definitive role for Aβ has not been discovered, some research groups have suggested that the monomeric form of Aβ is neurotrophic. If monomeric Aβ does indeed have a beneficial neurological function, it is possible that elimination or reduction of its production by secretase inhibitors could have damaging side effects. Inhibitors of Aβ aggregation, by contrast, would be less likely to suffer from this drawback.

INHIBITORS OF Aβ PRODUCTION

An alternative strategy to the direct blocking of Aβ inhibition is to preemptively prevent the production of the Aβ peptide. This goal can optimally be achieved by inhibiting the various secretase enzymes implicated in the production of Aβ from its APP.[31] The structures of several early secretase inhibitors are shown in **Figure 6**.

β-Secretase inhibitors

β-Secretase—also known as BACE1, or β-site of APP cleaving enzyme—is a novel 501-amino acid aspartyl protease that selectively cleaves APP between residues 671 and 672 to form APPs-β and membrane-bound C99 (the precursor to Aβ).[32] β-Secretase is composed of an N-terminal signal peptide (residues 1–21), a preprotein domain (residues 22–45), a catalytic domain (residues 45–459), a transmembrane domain (residues 460–477), and a cytoplasmic tail. Central to the protease are two conserved aspartyl protease active sites (DTGS and DSGT, at residues 93–96 and 289–292, respectively).

The mechanism of the aspartyl proteases has been thoroughly investigated using kinetic methods, affinity labeling, and x-ray crystallography. These investigations are consistent with a general acid–base mechanism in which a nucleophilic water molecule is held between the two catalytic aspartic acid residues. For activation of the water molecule, one of the two aspartic acids must be deprotonated; this is congruent with the optimal pH range for aspartyl proteases—approximately 4–4.5. On deprotonation (activation) by the aspartate anion, the water molecule is able to attack the carbonyl of the scissile amide bond, resulting in the formation of an oxyanion tetrahedral intermediate. Subsequent protonation of the amide nitrogen atom, and the resultant rearrangement about the tetrahedral center, leads

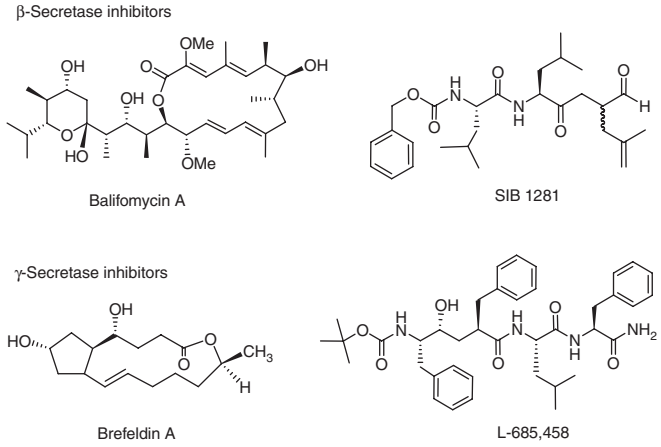

Figure 6 Some compounds that reduce Aβ production by inhibiting β- or γ-secretase.

to the formation of the hydrolysis products and cleavage of the amide bond. β-Secretase cleavage of APP is highly sequence specific, with the scission occurring at the N-terminus of Aβ via recognition of the sequence VKM*DA.

Aspartyl proteases are a well-characterized class of protein that also includes pepsin, renin, cathepsins D and E, and napsin A. β-Secretase shares significant sequence homology with these enzymes, and therefore its crystal structure displays the conserved general folding of aspartyl proteases. However, co-crystallization studies with peptidomimetic inhibitors show that β-secretase also has some very definite differences in structure as compared with these other aspartyl proteases. For instance, the β-secretase active site is more open and solvent accessible and has S2 and S4 subsites that are relatively hydrophilic as compared with other aspartyl proteases. These differences could be exploited in the design of selective β-secretase inhibitors.

β-Secretase is therefore an attractive therapeutic target for the prevention and treatment of AD, being the catalyst of the initial, rate-limiting step of Aβ production.[33] Further supporting its validity as a druggable target is the observation that BACE-1-knockout mice do not generate Aβ. These mice appear healthy and do not show developmental, neurological, or behavioral abnormalities; however, some of these animals exhibit hypomyelination of peripheral nerves and aberrant axonal segregation of small-diameter afferent fibers. Nevertheless, the observation that these mice are generally healthy lends support to the notion that inhibition of β-secretase could in fact lower Aβ production and alter the course of AD progression, with minimal side effects.

During the past decade, several β-secretase inhibitors have been described. The majority of these are peptidomimetic and are based on the amino acid sequence at the cleavage site of APP by BACE1. There have been very few reports of synthetic, small-molecule inhibitors. Naturally occurring small-molecule noncompetitive inhibitors such as hispidin and the catechins have only micromolar potency and poor specificity. To date, it has been difficult to design small-molecule inhibitors that avidly bind the rather large catalytic site domain of BACE1.

First-generation β-secretase inhibitors such as OM99-1 and OM99-2 were designed as transition-state mimetics by using β-secretase residue preferences in eight subsites and incorporating a hydroxyethylene isostere. Although these were very potent (having low-nanomolar affinities), they were essentially peptidic in nature and lacked sufficient drug-like properties. In addition, neither compound was specific to β-secretase, and therefore both inhibited other aspartic proteases. Further refinement of OM99-2 has led to reductions in size that maintain nanomolar potency. However, with a molecular weight around 700 Da, these molecules are still too large to cross the BBB. Unfortunately, tight binding of a transition-state mimetic of β-secretase requires at least six residues, which makes it difficult to reduce the size of peptidomimetic inhibitors.

Numerous crystal structures of BACE1 have been reported; accordingly, the active site of BACE1 has been rigorously characterized, which has allowed for the development of brain-permeable peptidomimetic inhibitors via structure-based design cycles. The recently developed GRL-8234 has been shown to be a potent and highly selective inhibitor of Aβ production both *in vitro* and *in vivo*. It has shown excellent inhibitory activity in Chinese hamster ovary cells, and intraperitoneal administration to Tg2575 mice (an AD murine model) has shown a 65% reduction of $A\beta_{1-40}$ formation. GRL-8234 has demonstrated that β-secretase inhibition may be a viable target for the treatment of AD. This notion is further bolstered by reports that CTS-21166, a highly potent, orally bioavailable, and highly selective brain-penetrating β-secretase inhibitor had recently completed a phase I clinical study.

γ-Secretase inhibitors

The proteolytic scission that ultimately generates Aβ is mediated by γ-secretase; moreover, it is γ-secretase that determines the ratio of $A\beta_{1-40}$ to $A\beta_{1-42}$; the latter is highly prone to aggregation and has a higher propensity to form amyloid plaques. Although γ-secretase displays the pharmacologic characteristics of an aspartyl protease, it shares little or no sequence homology with most known members of the aspartyl protease family.[34]

During the past decade, a concerted effort has been centered around determining the structure and function of γ-secretase. γ-Secretase is a multiprotein complex with at least four membrane-spanning constituents: presenilin, nicastrin, anterior pharynx-1, and presenilin enhancer-2. The catalytic component of the γ-secretase complex is presenilin, with two aspartate residues forming the active site. There are two presenilin genes—PS1 (located on chromosome 14) and PS2 (located on chromosome 1)—mutations of which are associated with early-onset forms of AD. To date, over 100 missense mutations have been identified in PS1 alone. The majority of these mutations skew the proportion of Aβ toward the more aggregation-prone $A\beta_{1-42}$ and account for >50% of cases of familial early-onset AD. Mutations around the γ-secretase cleavage site of APP have been shown to have a similar effect, increasing the proportion of $A\beta_{1-42}$ generated.

Inhibition of γ-secretase may be a feasible strategy for altering the course of AD progression. Neuronal Aβ production is markedly decreased in PS1-knockout mice. In these studies, Aβ production was lowered to 20% of the levels seen in primary neuronal cultures from wild-type littermates. Subsequent studies showed that cells from PS1/PS2-double-knockout mice were completely devoid of γ-secretase activity. However, the deletion of PS1 in these mice was lethal *in utero* or shortly after birth. Indeed, γ-secretase inhibition may have significant safety drawbacks. γ-Secretase inhibitors affect Notch signaling by blocking proteolysis of Notch-1 (another γ-secretase substrate) through inhibition of cleavage at site 3 of the Notch receptor. The Notch signaling pathway plays a significant role in cell differentiation, both during development and in adulthood. To address this concern, long-term studies for monitoring the effect of chronic γ-secretase inhibition in AD patients will be needed.[35]

Despite these potential safety concerns, γ-secretase inhibition is a viable druggable target, and several nonpeptidic, orally available γ-secretase inhibitors have been synthesized, some of which have been shown to lower levels of CSF-soluble Aβ. The first γ-secretase inhibitor to be tested *in vivo*, DAFT (*N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine-*t*-butyl ester), yielded a 30% decrease in both $A\beta_{1-40}$ and $A\beta_{1-42}$. γ-Secretase inhibitors have had success in terms of progression to clinical human trials. Several γ-secretase inhibitors are currently in clinical trials. MK-0752 and E2012 have completed phase I trials; BMS-708163 and GSI-953 have proceeded to phase II trials; and LY-450139 is currently in phase III trials. Two other compounds, BMS-299897 and PF-3084014, did proceed to clinical testing but have since been abandoned. Of all these γ-secretase inhibitors, LY-450139 is the best documented, and the results of its trials have been published. In phase I and phase II studies, patients given LY-450139 showed a transient period of plasma Aβ reduction, followed by a period of significantly elevated plasma Aβ, which eventually dropped to baseline. However, CNS levels of Aβ remained unaltered in both studies. A subsequent study of the effects of LY-450139 on CNS Aβ synthesis and clearance has recently been reported and has shown that LY-450139 does decrease CNS Aβ concentrations. LY-450139 has since progressed to phase III trials.

γ-Secretase modulators

Although a number of highly potent γ-secretase inhibitors have been identified, interference with Notch signaling is a potential impediment to their ultimate successful implementation as therapeutic agents for the treatment of AD. Consequently, γ-secretase "modulators" (rather than inhibitors) are also being evaluated as potential therapeutic agents. Selective $A\beta_{1-42}$-lowering agents, or SALAs, are being developed as potential disease-modifying therapeutic agents. SALAs do not inhibit γ-secretase outright; rather, they allosterically modulate γ-secretase to change the site of action on the APP membrane. In doing this, they cause a reduction in the proportion of the longer, more toxic $A\beta_{1-42}$ relative to $A\beta_{1-40}$.

Nonsteroidal anti-inflammatory drugs are the first class of molecules that were identified as having potential for use as SALAs.[34] Treatment of Aβ-secreting cells with sulindac sulfate,

ibuprofen, or indomethacin selectively reduced $A\beta_{1-42}$ production and increased Aβ38 production without altering the levels of $A\beta_{1-40}$. The ability of these NSAIDs to modulate Aβ production is not related to their inhibition of cyclo-oxygenase. Interestingly, epidemiological studies have shown that chronic use of some NSAIDs is associated with a significantly lower risk of developing AD. However, in humans (especially in the elderly), the toxicity of high-dose NSAIDs may limit the feasibility of this approach. More recently, the NSAID-related compound (R)-flurbiprofen was the first SALA to undergo extensive clinical study as an AD therapy. (R)-Flurbiprofen reduced the concentrations of $A\beta_{1-42}$ in the brain in a murine model of AD, and long-term dosing of this compound prevented defects in memory and learning. Although the results from a phase II study were encouraging, a large phase III study with a duration of 18 months yielded negative results.

Several other γ-secretase modulators are being developed and have reached or are approaching clinical testing. E2012, a diarylcinnamide derivative, has been shown to lower Aβ by γ-secretase modulation without interfering with Notch processing. Another promising SALA in development is CHF5074, which has been shown to preferentially lower $A\beta_{1-42}$ secretion in human neuroglioma cells overexpressing the Swedish-mutated APP (half-maximal inhibitor concentration, $IC_{50} = 3.6\,\mu mol/l$). Further, long-term dosing of CHF5074 in an aggressive murine AD model attenuated brain Aβ pathology and the associated behavioral deficits. At concentrations of 100 μmol/l, CHF5074 does not alter the intercellular cleavage of Notch in human embryonic kidney 293swe cells.

By not completely inhibiting γ-secretase, SALAs may exert a beneficial effect without affecting Notch processing *in vivo*. This is an attractive alternative to γ-secretase inhibitors, which have a tendency to produce gastrointestinal and immunological side effects. Although selective lowering of $A\beta_{1-42}$ has been shown to attenuate plaque formation and behavioral deficits in AD murine models, it remains to be seen whether these beneficial effects will be translated to humans. This question will undoubtedly be answered as SALAs progress through clinical trials.

α-Secretase activators

Compounds that potentiate the α-secretase pathway of APP processing may be putative AD therapeutics. α-Secretase processing occurs within the Aβ domain of APP and thereby precludes the formation of neurotoxic Aβ. Furthermore, processing of APP by α-secretase results in the formation of membrane-retained C83 and APPs-α. The latter confers neuroprotection and may even have memory-enhancing effects. α-Secretase activation may therefore serve as an alternative to therapeutics that inhibit the amyloidogenic enzymes β- and γ-secretase.

Many stumbling blocks still impede the rational design of therapeutic agents that act by α-secretase activation, and it is generally thought that potentiating α-secretase cleavage is more challenging than inhibiting β- and γ-secretase.[36] The identity of α-secretase is still somewhat elusive, although the general consensus is that the enzyme is a member of the ADAM (a disintegrin and metalloprotease) family of proteases. It has been suggested that ADAM10 is in fact α-secretase, given that overexpression of ADAM10 in transgenic murine models of AD has been shown to lead to a decrease in amyloid pathology. Conversely, expression of a catalytic inactive form of ADAM10 results in an increase in amyloid pathology. It has been suggested that, in addition to ADAM10, ADAM9 and ADAM17 may also possess α-secretase activity. Direct stimulation of ADAM 9/10/17 activity through activation of G protein–coupled receptors is a possible strategy for stimulating α-secretase processing of APP, and indeed such α-secretase activators exist. However, most of these drugs are intended for other pharmacological actions, and this lack of specificity represents a major limitation. An alternative approach to enhancing α-secretase activity may be through activation of protein kinase C (PKC). Indeed, the activation of ADAM proteases is controlled by the protein phosphorylation signal transduction pathway of PKC.

As mentioned, there are several G protein–coupled receptors whose activation has been reported to increase the nonamyloidogenic processing of APP; however, it is important to note that the molecular mechanisms of α-secretase activation via G protein–coupled receptors are still unclear. Activation of the M1 (predominately expressed in brain) subtype of muscarinic acetylcholine receptors has been shown to upregulate the nonamyloidogenic processing of APP. Treatment of rats with the M1 agonist RS86 increased APPs-α levels, and the M1 agonist AF102B reduced Aβ levels in CSF. NGX267 is an M1 agonist that inhibited the formation of Aβ in a triple transgenic mouse model of AD. Stimulation of other G protein–coupled receptors may be an alternative avenue of α-secretase-mediated cleavage and would bypass cholinergic-mediated side effects. PRX-0314 and prucalopride are partial serotonin 5-HT4 receptor agonists and have been shown to increase APPs-α levels in various cell lines.

Activation of α-secretase is also mediated by the protein phosphorylation signal transduction pathway of PKC; activators of PKC have been shown to upregulate nonamyloidogenic APP processing. In particular, phorbol esters are PKC activators that have been shown to significantly reduce Aβ production both *in vitro* and *in vivo*. However, their tumor-promoting characteristics preclude their development as AD therapeutics. The design of other agents has been successful and includes benzolactam-based compounds and linoleic acid derivatives that alter Aβ processing. Bryostatin 1, a molecule that was initially being investigated because of its promising anticancer activity, has sub-nanomolar affinity for PKC. At these concentrations, it is able to promote APPs-α secretion in fibroblasts taken from patients with AD.

Statins are a class of approved agents that have been shown to increase APPs-α via α-secretase activation. This shift from amyloidogenic to nonamyloidogenic APP processing may account for the finding in epidemiological studies of an association between statin use and reduced risk of developing AD.[37,38] It also suggests a mechanism by which the ε4 allele of apolipoprotein E—the main genetic risk factor for sporadic AD—predisposes individuals to AD; although all forms of apolipoprotein E distribute cholesterol throughout the CNS, the ε4 allele is associated with increased plasma concentrations of cholesterol, which

may increase amyloidogenic processing of APP. *In vitro* studies have shown that lovastatin, atorvastatin, simvastatin, and rosuvastatin stimulate APPs-α shedding in human cell lines. Although statin use has been epidemiologically associated with a reduction in the risk of developing AD, the mechanism(s) by which these effects occur are poorly understood; increased α-secretase activation may play a role, but other pathways have been investigated.[39] Prospective clinical trials of statins for the treatment of patients who already have AD have been negative thus far, but the trials are ongoing.

Inhibitors of APP processing have been shown to be highly effective in reducing Aβ production *in vitro*. Although research in this area is promising, many hurdles must be overcome before studies in humans can be attempted for drugs of this general class.[40] These hurdles include finding a molecule with high specificity for the targeted secretase, adequate absorption and pharmacokinetic profile, low toxicity, and sufficient BBB permeability. Another caveat in developing secretase inhibitors for AD is the finding that the C99 fragment of APP generated by β-secretase cleavage may be more toxic than Aβ. This suggests that blocking γ-secretase could be detrimental, leading to the worsening of AD symptoms. Finally, inhibitors of γ-secretase may also disrupt processing of the Notch receptor, a molecule that has a number of functions, including involvement in cell differentiation of immune, mucosal, and skin cells.[3] The fact that the Notch receptor, like APP, is processed by presenilins (two components of the γ-secretase complex), suggests that inhibitors of γ-secretase may be associated with toxicity, which could preclude their clinical application.[31]

INHIBITORS OF THE NEUROTOXIC EFFECTS OF Aβ

It has been recognized for many years that an Aβ plaque-induced inflammatory response is part of the pathology of AD, as demonstrated by the presence of activated microglia and several markers of severe inflammation in brains of people with AD.[41] Consistent with this phenomenon, there is a delay in onset of AD in individuals who have received anti-inflammatory therapy for rheumatoid arthritis. Furthermore, in a large epidemiological study, the prevalence of AD in long-term users of NSAIDs was found to be only one-fifth of that in controls.[42] Presumably, the later onset results from the neuroprotective effect of the therapy and is not related to the pathology of arthritis, the condition for which the NSAIDs were being used. Adding further support to this line of exploration is the observation that patients with leprosy who have been treated with the anti-inflammatory drug dapsone develop AD at lower rates and have fewer Aβ plaques at autopsy as compared with controls.

It is not yet clear how much of the protection of NSAIDs against the development of AD is attributable to anti-inflammatory activity and how much to their aforementioned ability to modulate γ-secretase.[34] A further complicating factor is that many NSAIDs have also been shown to inhibit aggregation of Aβ, suggesting a third mechanism through which the drugs may be of benefit in AD.[43]

Although some clinical trials of anti-inflammatory drugs such as indomethacin and aspirin have suggested a beneficial therapeutic effect in with AD, other trials have shown no benefit from the drugs. This has led to the suggestion that NSAIDs may work in preclinical AD or mild cognitive impairment, but not in established AD.[35]

Aβ-mediated oxidative stress is another important part of the etiology of AD. It has been reported that oxidation of Aβ promotes its aggregation, that free radicals are generated by Aβ, and that radical-forming peroxides mediate Aβ neurotoxicity. The protective effects of the antioxidant vitamin E against Aβ-mediated toxicity have been demonstrated in cultured PC12 cells, and supplementation of the vitamin was initially shown to be of benefit to AD patients in some trials; in 1997, a study showed an increased median survival of 230 days with vitamin E supplementation relative to placebo recipients.[44] All these findings support the role of free radicals in the mechanism of Aβ toxicity and initially led some to recommend high-dose vitamin E supplementation, in addition to cholinesterase inhibitor therapy, as the standard of care for treating AD.[45] A large subsequent clinical trial, however, showed that vitamin E did not affect the rate of AD progression, casting doubt on its usefulness in AD treatment.[46] Consequently, current treatment guidelines no longer recommend vitamin E supplementation in view of risk–benefit considerations.

Although antioxidants may offer some benefit to patients with AD, they have the same drawback as neurotransmitter therapy (discussed below): i.e., they merely slow cognitive decline rather than targeting its cause. In contrast, anti-inflammatory drugs may inhibit Aβ pathology in addition to attenuating the inflammation caused by Aβ toxicity.

INHIBITORS OF Aβ-INDUCED NEUROTRANSMITTER EFFECTS

It has long been appreciated that, as neurons die during the disease progression of AD, they stop producing neurotransmitters, and the symptoms of the disease emerge. Accordingly, neurotransmitter augmentation was the mechanism of action of tacrine, the first drug developed for treating AD, and for three of the four drugs currently prescribed for AD: donepezil, rivastigmine, and galantamine (**Figure 7**).[2,3] These agents act by inhibiting acetylcholinesterase (AChE), thereby raising levels of acetylcholine, a neurotransmitter that is deficient in the brains of patients with AD. The other commonly prescribed AD drug, memantine, also acts by modulating neurotransmitter activity, but as an uncompetitive antagonist of the *N*-methyl-D-aspartate receptor.[47,48]

Although improvements in cognitive ability can result from neurotransmitter therapy, such improvements are often modest and temporary—a consequence anticipated for drugs that address the symptoms of AD rather than the underlying disease progression. Recently, however, it has been suggested that AChE inhibitors may also slow disease progression in addition to providing symptomatic benefits; nevertheless, clinical evidence to support this suggestion remains inconclusive. Given that AChE appears to act as an Aβ chaperone, the mechanism underlying the action of cholinesterase inhibitors in bringing about disease modification may involve the interruption of Aβ-AChE interactions and subsequent disruption of Aβ pathology.

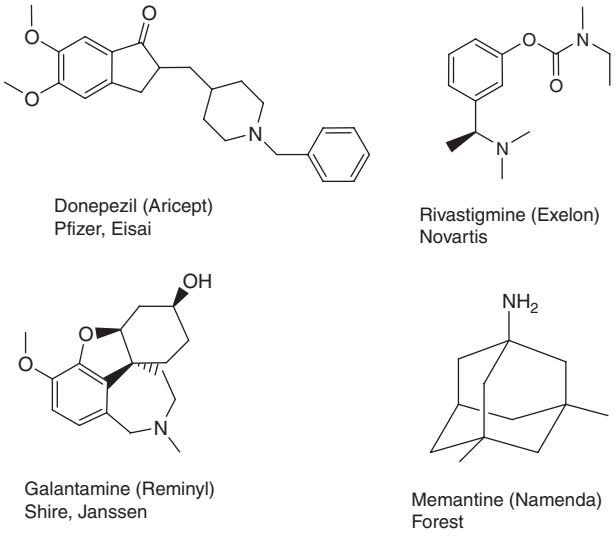

Figure 7 Drugs commonly prescribed for Alzheimer's disease. Generic names are given first, followed by brand names in brackets. Companies involved in the development of each drug are given below. Donepezil, rivastigmine, and galantamine are acetylcholinesterase inhibitors; memantine is primarily an antagonist of the *N*-methyl-D-aspartate (NMDA) receptor.

INHIBITORS OF TAU-INDUCED NEUROTOXICITY

Given that AD is a disorder of protein misfolding, Aβ's molecular coconspirator, tau, must also be considered. In AD and other dementias, tau, a microtubule-stabilizing protein, becomes hyperphosphorylated at serine and threonine residues, causing it to detach from microtubules and aggregate as paired helical filament. This is thought to destabilize the neuronal skeleton, leading to impaired transport and contributing to neuronal death in AD.

Analogous with the approach to Aβ, approaches to tau-based therapeutics design can be focused either on antiaggregants or on enzyme inhibitors. With respect to antiaggregants, the well-known compound methylene blue has recently attracted considerable attention as a potential tau antiaggregant. The other approach to countering tau-based toxicity is through inhibiting the tau-phosphorylating enzyme glycogen synthase kinase 3 (GSK-3).[49] Inhibition of the α isoform (GSK-3α) of the enzyme appears to lead to reduced Aβ production, whereas GSK-3β is the isoform primarily responsible for tau hyperphosphorylation and therefore the one whose inhibition should be targeted for tau-based therapies. Although this approach to treating AD appears promising, no GSK-3β-specific inhibitors have yet reached human efficacy trials. One trial, however, is currently evaluating the efficacies of lithium and valproic acid, two existing neurological drugs, in the treatment of AD; alongside other proposed or confirmed mechanisms of action, these two drugs are known to nonselectively inhibit GSK-3 (ref. 50).

HURDLES TO THE DEVELOPMENT OF SMALL-MOLECULE THERAPEUTIC AGENTS FOR AD

As discussed in this review, multiple viable druggable targets are available for the development of therapies for AD. However, the development of such small-molecule therapeutics, especially disease-modifying ones, will continue to confront a diverse array of problems. The following 10 problems are key hurdles, either conceptual or technical, to the successful development of a useful drug for AD:

1. Is AD a disease or a syndrome? AD can manifest differently from person to person. In some individuals, AD occurs secondary to genetic predisposition, in others, it arises as a long-term consequence of head trauma, and in others, it is completely sporadic. Are these all the same disease, or are they merely subsets of a common syndrome? Failure to differentiate distinct clinical subtypes could constitute a major impediment to successful drug design and development. Moreover, the clinical presentation for AD is too heterogeneous. AD frequently presents in people who also have comorbidities such as widespread ischemic changes within the brain. The concomitant occurrence of AD and vascular dementia is a well-appreciated problem. Designing a drug for "pure" AD, when the clinical reality suggests a complex clinical heterogeneity, is a definite hurdle to successful drug discovery.
2. Are the pathogenesis and etiology of AD sufficiently well understood to permit drug design? Nearly all current drug design approaches are based firmly on the amyloid hypothesis, which postulates that by removing, preventing, or reducing the amyloid deposits, AD will somehow be "cured." The weakness of this approach is that amyloid deposition may be only another symptom rather than a cause of degenerative processes, and therefore disrupting it will leave the underlying disease processes intact.
3. What is the normal physiological role of Aβ and APP? Conventional drug design for AD relies on targeting the pathological processes of Aβ and APP, but their physiological roles remain unelucidated, and the consequences of interfering with these macromolecules are unclear. Designing a drug to inactivate a normally occurring endogenous macromolecule may have unforeseen consequences.
4. Should drug design focus on one target or on more than one target? Aβ is just one target for AD drug design. Is it sufficient to address a single target or will truly meaningful therapeutic approaches have to target multiple key steps in the neuropathological cascade of AD?
5. Are there sufficient experimental structural data to enable rational drug design? Conventional approaches to AD drug development are focused on Aβ, but there is no crystal structure available for this all important peptide. It is difficult to successfully achieve rational drug design if the three-dimensional structure of a key molecular player remains unsolved.
6. Are there sufficiently robust *in silico* data to enable meaningful virtual high-throughput screening? Given that the three-dimensional structure of Aβ and indeed the entire protein-misfolding process is not well understood, advanced computer modeling methods are at a distinct disadvantage when applied to the task of drug discovery for AD.
7. Are the animal models good enough? In humans, mutations in APP and PS trigger frank AD, with plaques, tangles,

inflammation, and severe atrophy. In the analogous mouse models, these same mutations typically lead only to plaques. The currently available animal models, although much better than a decade ago, remain plagued with some limitations, especially in terms of the ability to fully represent the human clinical spectrum of this disease.

8. How will a definitive clinical trial be performed? Even if a disease-modifying agent can be discovered, will it be possible to perform a clinical trial to demonstrate its efficacy? Most clinical trials for AD merely address the symptoms of the disease, using conventional neuropsychology assessments such as the mini-mental status examination. Proving that a drug is truly disease-modifying will require appropriate study design, prolonged clinical trials, improved assessment measures, and innovative means of quantifying disease progression, such as sequential magnetic resonance imaging.
9. Are diagnostic methods for AD acceptable? Even if one develops clinical trial methods for determining whether a drug is disease modifying, there is still the problem of identifying patients who have AD for the purposes of the clinical trial. There are no biomarkers for AD. The diagnosis of AD remains mired in the realms of "the possible" and "the probable," with definitive diagnoses requiring autopsy. This remains an impediment to the successful execution of clinical trials.
10. Will the drugs being designed be "disease modifying", "curative," or "preventive"—or some combination of these three end points? Many of the therapeutics currently under development may be better suited to disease prevention. The design and conduct of prevention trials, as well as the practical considerations about how to use such drugs for prevention (i.e., when to initiate and how long to treat), will be extremely challenging.

ALTERNATIVES TO THE DEVELOPMENT OF SMALL-MOLECULE THERAPEUTICS FOR AD: BIOLOGICS

Given that the development of small-molecule therapeutics for AD is proving to be quite challenging, other approaches are understandably being actively pursued. Another approach to eliminating Aβ aggregates is vaccination (to stimulate an autoimmune response against the peptide). This method was proven effective in transgenic mice expressing human Aβ. Mice that were "immunized" with fibrillar Aβ1–42 showed not only an inhibition of amyloid formation but also some clearance of pre-formed amyloid plaques. Significantly, the immune response mounted by the mice was reduced if the Aβ was not incubated prior to inoculation, suggesting that the β-fibrillar form of the peptide is a more effective immunogen. In addition to forming fewer Aβ plaques, immunized mice also performed better than their littermates in maze tests, suggesting that increased cognition results from the diminished Aβ levels. No adverse effects of $A\beta_{1-42}$ immunization were observed in the treated mice.

Human clinical trials of Aβ vaccination followed the promising results seen in transgenic mouse models. Two phase I studies found that vaccination with Aβ1–42 and an adjuvant, the pair termed AN-1792 or Betabloc, was well tolerated in individuals with moderate AD, with a subset of patients developing an immunological response. This prompted phase IIa studies in the United States and Europe, in which, of 375 AD patients enrolled, 300 were to receive the highest dose of Aβ1–42 combined with the lowest dose of the adjuvant tested in the previous trials. The program was suspended in January 2002, after 18 of the 298 treated patients (6%) developed aseptic meningoencephalitis. This inflammation of the brain was unexpected, given the safety profile of the drug during phase I testing and considering that none of the five different animal species used for testing developed encephalitis. It has been suggested that the adverse reaction may have been a result of excessive inflammation from an autoimmune attack directed at APP rather than at Aβ, a scenario that would explain why animals injected with the human form of Aβ did not experience similar autoimmune toxicity.

Given the numerous positive *in vivo* mouse studies and the reduced Aβ burden found in the brains of three trial participants at autopsy, this approach remains a viable and important direction for continuing research. However, the development of biological therapeutic agents will share most if not all of the same developmental hurdles as the small-molecule approach.

CONCLUSION

The many advances made in AD research over the past decade have brought us closer to the goal of supplementing current symptomatic therapies, which typically bestow modest and temporary benefit, with effective disease-modifying drugs. These second-generation AD therapies target various steps in the progression of the disease (e.g., aggregation, production and clearance of Aβ; tau phosphorylation; and paired helical filament formation), raising the hope that one or more of these drug design approaches will prove successful at stabilizing or even reversing the cognitive deficits of AD. As improvements are continually made to diagnostic techniques, the importance of having an effective drug to treat AD will become ever greater. The administration of such a drug might be started even in presymptomatic individuals in order to delay or eliminate the onset of AD. This scenario is particularly relevant to cases of early-onset AD in middle-aged individuals, in whom disease progression is typically very rapid—an outcome made particularly tragic by the young age of those afflicted. Efficacious intervention in AD, early-onset or otherwise, holds the promise of sparing patients with AD and their families the terrible emotional and financial burdens of the disease.

CONFLICT OF INTEREST

D.F.W. is a codiscoverer of tramiprosate. D.F.W. and M.D.C. are cofounders of Treventis Corp., a biotech company involved in the discovery of therapeutics for AD. G.A.S. declared no conflict of interest.

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