

New Strategies for the Treatment of Alzheimer's Disease

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One of the most interesting discoveries in the last decade has been the finding that many neurodegenerative disorders share a common biochemical feature, namely, the presence of abnormal protein deposits, which can cause neuronal dysfunction or degeneration (**Table 1**). The protein deposits can be extracellular (amyloid), as occurs in Alzheimer's disease, British dementia or prion diseases such as Bovine spongiform encephalopathy (mad cow disease). In other cases, the deposits are intracellular, as in frontotemporal dementia, Pick's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis. The discovery that this common biochemical feature is associated with many neurodegenerative disorders has led to a flurry of activity in recent years aimed at targeting the production, deposition or toxicities of these protein deposits (1).

Alzheimer's disease – the prototypic amyloidosis

Alzheimer's disease (AD) is by far the most common form of dementia in the elderly. Pathologically, the disease is characterised by the presence of amyloid plaques, cerebral amyloid angiopathy and neurofibrillary tangles (NFTs). The NFTs are comprised principally of a hyperphosphorylated form of the cytoskeletal protein tau whereas the amyloid plaques contain a 4-kDa polypeptide known as the β -amyloid protein or A β , which is derived from a much larger β -amyloid protein precursor (APP) (**Fig. 1**). APP is cleaved by enzymes known as secretases, which cut on the N- and C-

terminal sides of the A β sequence. The free A β is then secreted from cells. The major form of A β contains 40 amino acid residues (A β 40). However a minor form of the protein contains 42 residues (A β 42). In most individuals, the level of A β 42 comprises less than 10% of the total A β ; however, in AD the proportion of A β 42 is elevated (2).

Because of its propensity to aggregate, A β builds up in the brain of AD patients, ultimately forming amyloid (**Fig. 2, Fig. 3**). There is now very strong evidence that this aggregation is a key event in the pathogenesis of AD (2). Familial AD mutations have been found to cause increased production of A β 42 (3). This is significant because A β 42 aggregates more readily than A β 40. Aggregated A β has also been found to be toxic to cells grown in culture (2, 4) (**Fig. 2**).

Currently, acetylcholinesterase inhibitors are used for the treatment of AD. Loss of basal forebrain cholinergic neurons is a prominent feature of AD and acetylcholinesterase inhibitors boost cholinergic neurotransmission in the brain by inhibiting acetylcholinesterase, the key enzyme involved in the degradation of the neurotransmitter acetylcholine (4). However, acetylcholinesterase inhibitors provide only limited symptomatic relief, without retarding the underlying neurodegeneration. Therefore there is a great need for new drugs. As all forms of AD involve a single biochemical

Table 1. Neurodegenerative disorders involving abnormal protein aggregation and deposition in the central nervous system

Disease	Pathologic feature	Protein	Symptoms
Alzheimer's disease	Amyloid plaques	A β	Dementia
	Neurofibrillary tangles	tau	
Prion diseases	Prion plaques	PrP ^{Sc}	Dementia, movement disorders
British/Danish dementia	Amyloid plaques	ABri/ADan	Dementia
	Neurofibrillary tangles	tau	Movement disorders
Parkinson's disease/Lewy body dementia	Lewy bodies	α -Synuclein	Movement disorder, dementia
Huntington's disease	Nuclear and cytoplasmic inclusions	Huntingtin	Movement disorder
Frontotemporal dementia, Pick's disease, Corticobasal degeneration, Progressive supranuclear palsy	Neurofibrillary tangles	tau	Diverse behavioural disorders, movement disorders
Amyotrophic lateral sclerosis	Lewy body-like hyaline inclusion	SOD1	Limb weakness

pathway which causes toxic amyloid accumulation, it should be possible to develop therapeutic agents which block one or more steps in this pathway. In this article, three new therapeutic strategies are described.

Therapeutic strategies targeting A β production

A β is produced through the combined actions of two enzymes. Initially, β -secretase cleaves APP on the N-terminal side of the A β sequence producing a 99-residue C-terminal fragment (APP-CTF β), which is then cleaved by γ -secretase to produce the free A β (Fig. 3). β -Secretase has now been identified as a transmembrane aspartyl protease known as the beta-site APP cleaving enzyme-1 or BACE1. The crystal structure of BACE1 has been determined and several inhibitors have been identified (5). The identification of the γ -secretase has been more problematic. Like BACE1, the γ -secretase is an aspartyl protease. A number of high affinity γ -secretase inhibitors have now been identified (6).

Although treatment of patients with either a β - or γ -secretase inhibitor might be expected to reduce A β and thereby stop the progression of AD, the development of a successful therapy may not be so easy. Both β - and γ -secretase probably cleave several physiological substrates. So far, it is unclear what effect inhibition of the secretases would have on these normal physiological processes. For example, γ -secretase cleaves Notch, an important protein which regulates cellular differentiation (5). Although BACE1 has a more restricted specificity than γ -secretase and BACE1 knockout mice are phenotypically normal, it is too early to say whether inhibition of BACE1 will have any toxic consequences.

It may also be possible to block A β production through indirect means. Our own studies have shown that APP-CTF β can also be cleaved within the cytoplasmic domain by the proteasome (7). Interestingly, the proteasome cleaves in a region which contains a consensus motif (YENPTY) that is known to bind a number of proteins (Fig. 1). Mutagenesis studies confirm that removal of this region of the protein decreases trafficking of APP-CTF β to the γ -secretase (8). Therefore, the binding of one or more of these proteins to the cytoplasmic region of APP may be necessary for production of A β . It follows that inhibition of this binding should block A β production. Our studies are now aimed at identifying the proteins responsible for this trafficking of APP-CTF β to the γ -secretase.

An Alzheimer vaccine?

In 1999, Dale Schenk and his colleagues at Elan Corporation reported that when transgenic mice engineered to express high levels of the amyloid protein were immunised with A β , anti-A β antibodies were detected in the brains of the mice and the amyloid plaque load in their brain was decreased substantially (9). This result was surprising because the mice were apparently generating an immune response against an endogenous protein. However, the result

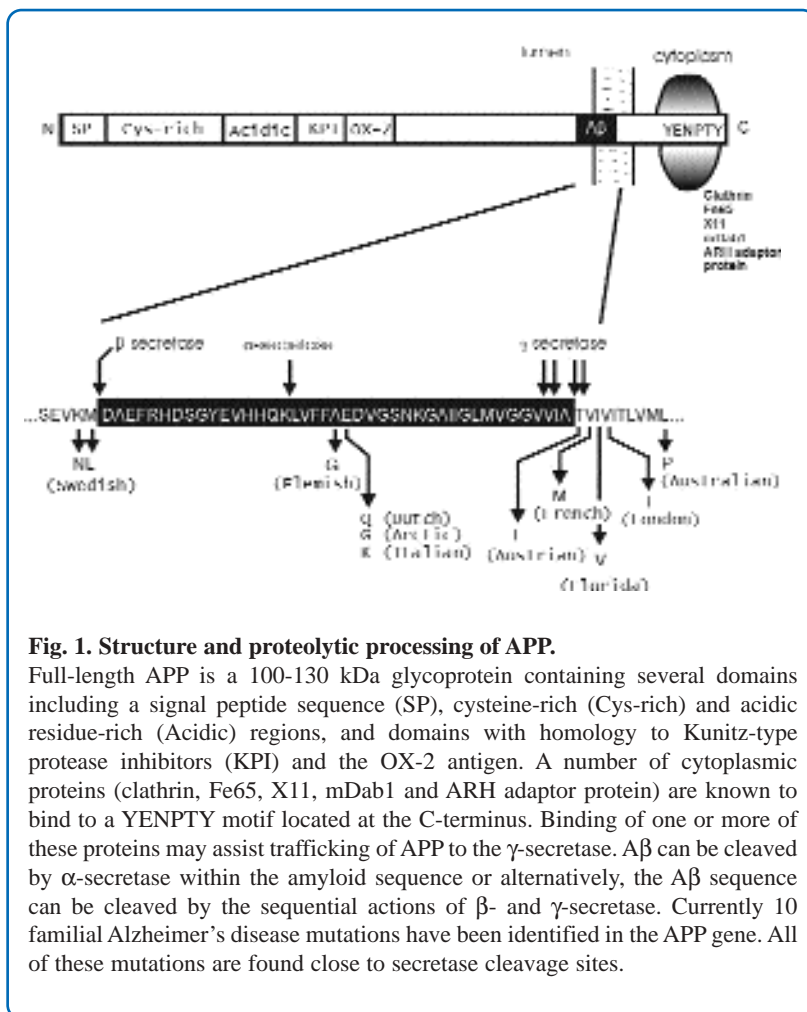


Fig. 1. Structure and proteolytic processing of APP.

Full-length APP is a 100-130 kDa glycoprotein containing several domains including a signal peptide sequence (SP), cysteine-rich (Cys-rich) and acidic residue-rich (Acidic) regions, and domains with homology to Kunitz-type protease inhibitors (KPI) and the OX-2 antigen. A number of cytoplasmic proteins (clathrin, Fe65, X11, mDab1 and ARH adaptor protein) are known to bind to a YENPTY motif located at the C-terminus. Binding of one or more of these proteins may assist trafficking of APP to the γ -secretase. A β can be cleaved by α -secretase within the amyloid sequence or alternatively, the A β sequence can be cleaved by the sequential actions of β - and γ -secretase. Currently 10 familial Alzheimer's disease mutations have been identified in the APP gene. All of these mutations are found close to secretase cleavage sites.

was confirmed in several other laboratories very soon after, and it was also reported that the immunisation procedure slowed down cognitive decline in the mice (10).

Elan Corporation in collaboration with American Home Products/Wyeth Pharmaceuticals initiated phase II clinical trials to test the Alzheimer vaccine, known as AN1792. However, very soon after the trial began a small percentage of the patients who had received the vaccine were found to have developed brain inflammation. A single pathology report on one of the patients who died from unrelated causes confirmed the presence of a meningoencephalitis probably caused by an immune reaction to amyloid deposits in the meningeal blood vessels (11). The vaccination trial was discontinued immediately. However, notwithstanding these adverse findings, the majority of the patients who received the Alzheimer vaccine have shown no clinical signs of inflammation. Furthermore, a recent report suggests that those patients who produced a strong immune response to the vaccine showed much less cognitive decline than patients who did not develop a strong immune response (12). Therefore, there is reason to believe that a vaccination strategy may work. However, the challenge will be to develop a vaccine without risk of meningoencephalitis. Testing this vaccine will be difficult because of the inherent safety concerns of any clinical trial in the future.

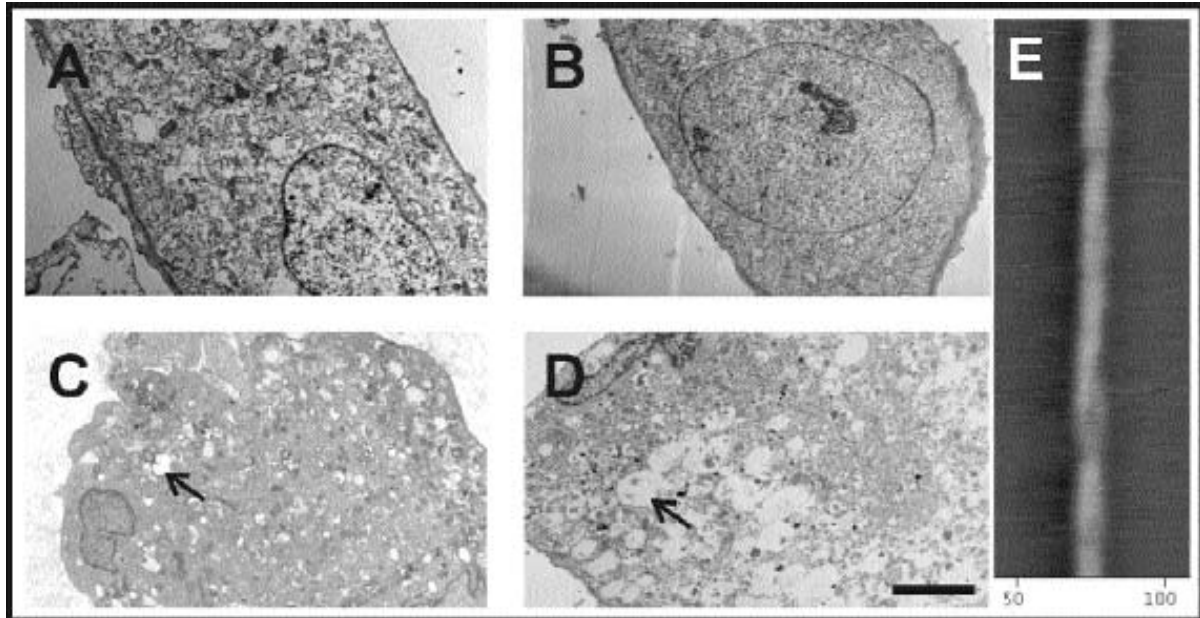


Fig. 2. Cytotoxicity of β -amyloid.

Figure shows electron micrographs of vascular smooth muscle cells (VSMCs) grown on plastic tissue culture dish (A) or on a commercial basement membrane preparation (Matrigel) (B). VSMCs treated with A β 40 (C) or A β 42 (D) show degenerative changes including vacuolation (arrow). Panel E shows the structure of an amyloid fibril as seen by atomic force microscopy. Fibrils are typically 5-10 nm in diameter. Scale bar for panels A-D is 5 μ m. Figure in panels A-D from B. Turner, Honours thesis, University of Melbourne.

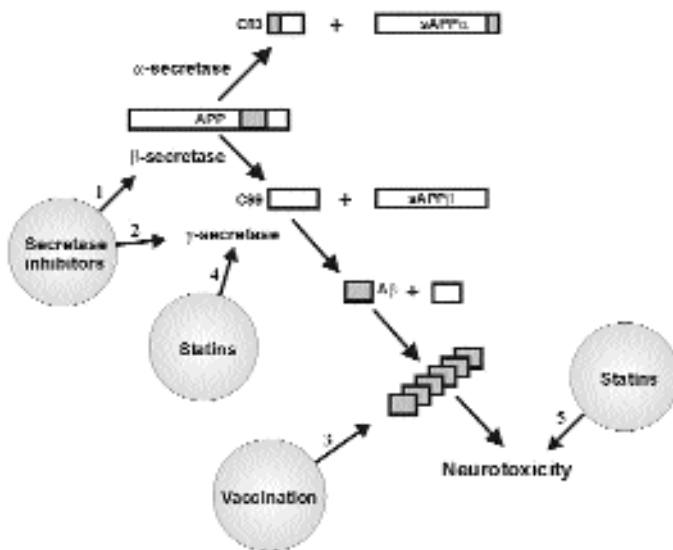


Fig. 3. Potential therapies for the treatment of AD block various steps in the biochemical pathway.

Normally APP is cleaved by α -secretase to produce two fragments, C83 and sAPP α . This pathway is non-amyloidogenic. Cleavage by β -secretase produces sAPP β and C99 (also known as APP-CTF β), which is then cleaved by γ -secretase to yield A β . The aggregation of A β forms toxic species which affect neuronal function. Potential drugs for AD therapy include β -secretase inhibitors (1) and γ -secretase inhibitors (2). Vaccination with A β has been suggested as a therapy as it may cause an immune response which eliminates amyloid fibrils from the brain (3). Finally cholesterol-lowering drugs or statins may help by decreasing γ -secretase activity (4) and reducing neurotoxicity (5).

Can statins help?

There is some epidemiological evidence that lowering cholesterol may be of benefit for AD. In one population-based study, the incidence of AD was found to be higher in individuals with higher cholesterol levels (13) and two retrospective studies have reported that use of the cholesterol-lowering drugs known as statins is correlated with a drastic decrease in the risk of developing AD (14, 15). Statins inhibit HMG-CoA reductase, an important step in the biosynthetic pathway which produces cholesterol. The idea that cholesterol may be involved in the pathogenesis of AD is supported by the observation that AD-like pathology is less severe in APP transgenic mice that have been treated with a cholesterol-lowering drug (16).

Cholesterol may be involved in the regulation of A β production. High cholesterol uptake can increase A β deposition in transgenic mice and cholesterol depletion can inhibit the production of A β from APP (17). Cholesterol may also be important for the toxic effects of A β . A β can bind to lipid membranes and it has been suggested that A β -membrane binding may be important for A β 's toxic effects. We have found that the cholesterol content of the plasma membrane can influence the binding of A β to cells; in particular, A β binds better to cholesterol-containing phospholipid membranes than to pure phospholipid membranes (18). Furthermore, when cells in culture are treated with statins, the toxic effects of A β are substantially reduced (18). These studies suggest that depletion of cellular cholesterol can decrease A β -membrane binding and thereby decrease cellular toxicity (Fig. 3). Statins have few major side effects and, if the results of the retrospective studies on their use are substantiated in prospective studies, they could become part of the standard treatment of AD within a few years.

Summary

There are a number of exciting new therapeutic strategies on the horizon for the treatment of AD. All of these strategies are based on targeting A β production or toxicity. At present, it is difficult to determine which strategy will be the best. The vaccination approach is a high-risk method with enormous potential, whereas treatment with statins is a very low-risk method with an unclear action. Secretase inhibitors may be of value if they are not toxic. Ultimately, a combination therapy may be needed, as is used in the treatment of AIDS. Despite the challenges ahead, there is every reason to be optimistic that an effective treatment for AD will be found in the near future. In addition, what we learn about the pathogenesis and therapy of AD may have application to other neurodegenerative diseases which also involve abnormal protein deposition.

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