EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 6
279-295
Year: 2021

THE CHEMISTRY BEHIND ALZHEIMER'S DISEASE & TWO DECADES OF NEW DRUG DISCOVERY AND DEVELOPMENT FOR ALZHEIMER'S DISEASE

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Article Received on 15/04/2021 **Article Revised on 05/05/2021** **Article Accepted on 25/05/2021**

ABSTRACT
Alzheimer's disease (AD) is a devastating, fatal, neurological disorder with no known cause and no cure. It is primarily a disease of old age, and it has become a very serious problem with the general life-expectancy gradually decreasing. In the US, 13% of people over the age of 65 are afflicted, the figure rising rapidly to 40% of those over 85 years of age.[1] The average period of survival is 8 years after diagnosis. The afflicted person suffers progressive loss of memory and thinking ability, mood swings, personality changes, and loss of independence. Physically, AD is characterized by massive loss of neurons and disruption of synaptic function throughout the brain, beginning in the hippocampus, an area of the cortex that plays a key role in formation of new memories. Genetics plays a small role. A few cases, about 5% of the total, are called familial AD. Due to its complex pathophysiological characteristics, complicated interactions with a large number of genes and proteins, there is still no effective drug treatment of the disease. Amyloid cascade aggregation of senile plaques and hyper-phosphorylation of Tau protein to form neurofibrillary tangles are the main pathological features of Alzheimer's disease, other mechanisms, such as oxidative stress, lack of central cholinergic neurotransmitters, inflammatory reaction and toxic metal ions have also been involved. The purpose of this review is to briefly introduce the progress of the development of the therapeutic agents based on their main mechanisms of action.

KEYWORDS: The average period of survival is 8 years after diagnosis.

INTRODUCTION

Main molecular features of Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population accounting for 50 to 80 percent of dementia cases (www.alz.org). The worldwide prevalence of AD is approximately 30 million, a number that is expected to quadruple in the next 40 years. As a direct consequence, AD represents a global public health problem that will become even more important in the next few years. An early symptom is the difficulty to remember newly learned information. Later on, more severe symptoms are encountered including mood and behaviour changes, confusion, serious memory loss, judgment alteration, and difficulties in speaking, writing and walking. Post-mortem neuro-histological hallmarks are extracellular amyloid plaques and intracellular neurofibrillary tangles of hyper-phosphorylated Tau protein. It has been proposed that the apparition of the amyloid plaques (or senile plaques) is an early event that precedes, and thus likely induces, the hyper-phosphorylation of Tau protein and the associated neuronal degeneration.[2] This is in line with the so-called amyloid cascade pictured in Fig. 1. According to this hypothesis, aggregation of the amyloid-b (Ab) peptide is linked to the aetiology of the disease, since soluble monomeric forms are found in the healthy brain while amyloid plaques are

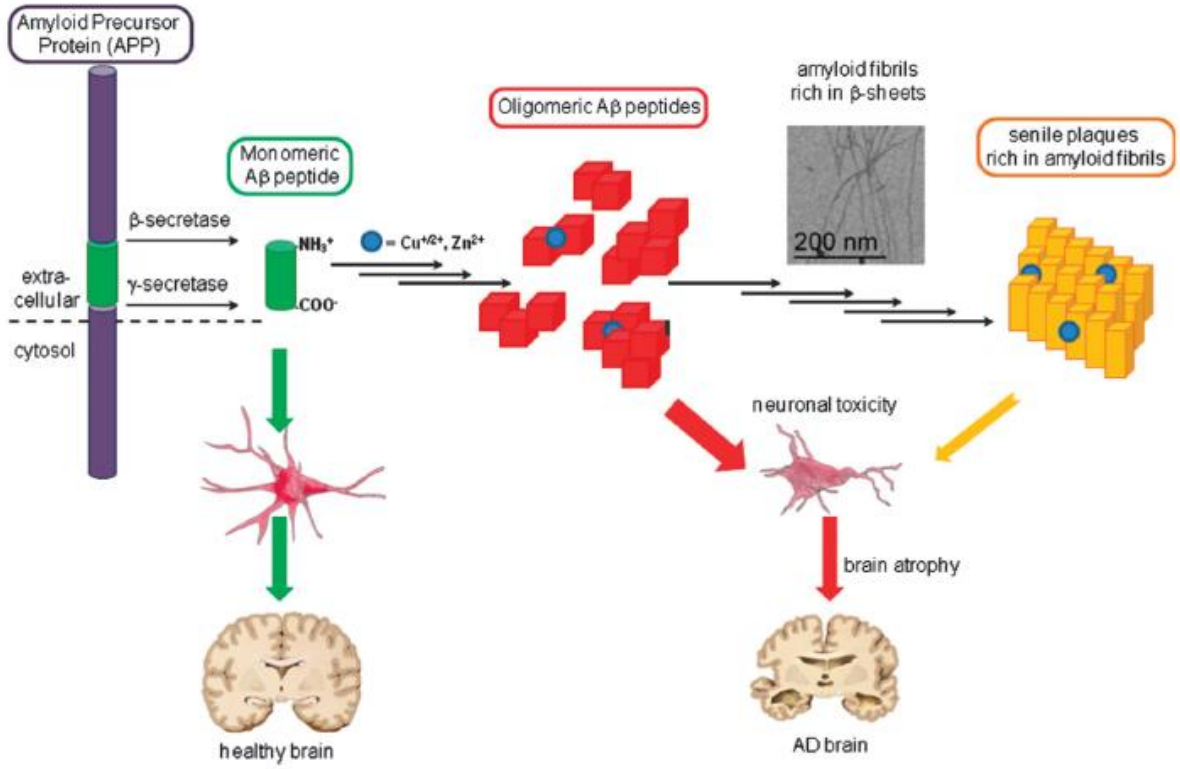


Fig. 1 The amyloid cascade process enhancing the role of metal ions. The Ab peptide is obtained by cleavage of the Amyloid Precursor Protein (APP) by the b and g secretases. The monomeric soluble Ab is not neurotoxic (green arrow) and is found in healthy brains. The senile plaques are a post-mortem hallmark of the disease detected in AD brains. Intermediate species between the monomeric Ab and the amyloid plaques are considered to be more toxic (red arrow) than the plaques themselves (orange arrow) leading to neuronal death and brain atrophy. Metal ions (blue circles) are considered as triggering agents of the aggregation process. Note that only the reactions resulting to the amyloid plaques are shown. However, all the reactions involved in this process are equilibrated ones.

detected in an AD patient's brain.[3,4] These plaques contain amyloid fibrils of several microns in length and 7–12 nm in diameter, rich in b-sheets. Hence they can be imaged by PET/ SPECT techniques associated with the use of radiolabelled molecules able to bind the b-sheet structure.[5] Actually, intermediate species of the aggregation process, i.e. the oligomeric soluble forms of the Ab peptides, are considered as the most toxic species, which disrupt synaptic function and the integrity of the membrane bilayers and lead to production of Reactive Oxygen Species (ROS).[4] But, there is currently no efficient tool to detect oligomeric intermediates. In addition, metal ions were found in high concentrations in the amyloid plaques, leading to a modified amyloid cascade hypothesis in which metal ions modulate the aggregation of the Ab peptide (Fig. 1). The Ab peptide is a 39 to 43 residues polypeptide incorporating an N-terminal weakly structured hydrophilic part (residues 1–16) and a C-terminal hydrophobic part, which contains two b strand sequences (residues 17–21, known as the central hydrophobic core and residues 29–39/43). The N-terminal part is responsible for the binding of metal ions, mostly Cu and Zn, involved in modulation of Ab aggregation properties and, for Cu, in ROS production.[6] At present, the slowdown of the disease progression is hampered by the absence of curative molecules, i.e. drugs able to stop the neurodegenerative process. All the medications so far are symptomatic treatments tackling the consequences of the disease rather than the disease itself. In addition, the lack of physiological markers for the early diagnosis of the disease precludes both the administration of the symptomatic treatments at the right time and the development of curative drugs. Last but not least, a thorough characterisation of the amyloid cascade process and in particular of the Ab aggregation can help in the design of diagnostic and curative tools to fight AD. Interestingly, the 2-arylbenzothiazole (ABT) molecular scaffold is a very valuable tool used in these three aspects: (i) understanding of the amyloid cascade, (ii) developing new markers for the early detection and (iii) bi-functional curative molecules incorporating an ABT unit to target the Ab aggregates and/or to impact Ab aggregation. This explains why the use of the ABT scaffold as a building moiety has underwent a great development over the last few years in the AD field. This is illustrated by the constant increase of the citation numbers from almost zero in 2000 to more than 500 in 2012 (data from the ISI web of knowledge with ''benzothiazole'' and ''amyloid'' as topic key words).

Biological implications of Alzheimer's disease

Pathologically, AD arises mainly due to the formation of two types of lesions in the brain, neuritic plaques and neurofibrillary tangles. Neurofibrillary tangles are insoluble bundles of fibers that locate in the perinuclear cytoplasm and are generally composed of phosphorylated tau protein. These tangles can also be found in other neurodegenerative disorders such as Kuf's disease and subacute sclerosing panencephalitis.[7] What is lacking in these alternate forms of neurodegeneration, however, is the formation of neuritic plaques. While neurofibrillary tangles and neuritic plaques can arise independently[8] neuritic plaques seem to be the primary lesion in AD.4 It has been suggested that the appearance of tangles in the AD brain could be due to neuronal responses to the formation of plaques.[7,10] Neuritic plaques are spherical lesions that contain extracellular aggregates of amyloid-b protein (Ab).[11] Surrounding these plaques are an array of abnormal dendrites and axons.[12] Ab comes from the processing of b-amyloid precursor protein (APP) via a pair of proteases, b-secretase (BACE1) and g-secretase.

Two main species of Ab are produced, Ab40, which ends at residue 40 of the preceding APP, and Ab42, which ends at residue 42 of the preceding APP. Ab42 seems to favor aggregation more so than Ab40; however, both species have been found in senile plaques. Increases in both Ab40 and Ab42 are seen early on in AD and overall levels of Ab in the brain have been shown to correlate with the degree of dementia in AD patients.[13] The less aggregative Ab40 is much more abundantly produced in normal cells and accounts for about 90% of the Ab produced.[8] Once these plaques are formed they are quite stable.[14,15] Ab has been shown to be neurotoxic and lead to neuron death.11 In contrast to the insoluble deposition of neuritic Ab plaques, diffuse plaques of Ab, lacking the compact nature of neuritic plaques, have also been found. Diffuse plaques are generally more amorphous and granular, made almost entirely of Ab42, and contain few amyloidogenic filaments and fibers that are found in neuritic plaques.3,7 These plaques are usually found in areas of the brain that do not have any implications in the symptoms of AD. This, in addition to the appearance of diffuse plaques in identical areas of the brains of healthy patients, leads to the assumption that diffuse plaques do not play a significant role in the progression of AD.[7] The production of Ab40 and Ab42 comes from the processing of a much larger peptide, APP. APP is a 695–770 residue peptide that is expressed in many tissues throughout the body, with higher concentrations being found in the kidneys and brain.[17]

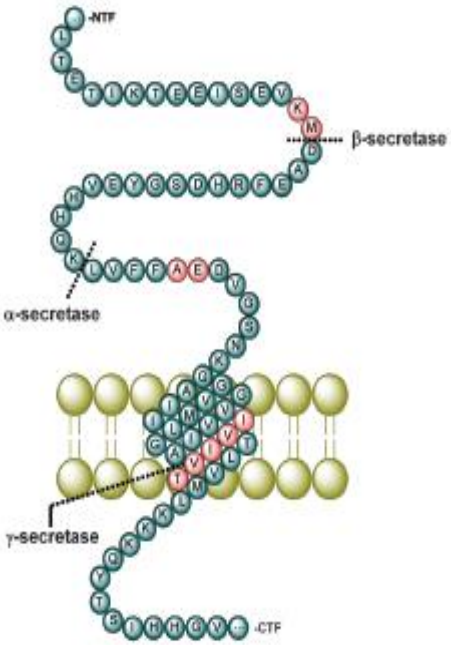
Cellularly, it is found mostly in the late endosomes; however some cycling from the cell surface through the endocytic system does occur.[18] The main form expressed in neuronal cells is APP695, which lacks a 56-amino acid sequence similar to the Kunitz serine protease inhibitors that is present in the longer isoforms of APP, APP751, and APP770.[1] While the exact physiological function of APP is not entirely clear, APP and its derivatives have broad functions in cell–cell14 or cell–matrix interaction and synapse localization and metabolism.[20] Further functions include roles in serine protease inhibition, in the case of APP751 or APP770, as well as cell adhesion,[21,22] growth promotion, neuroprotection via regulation of intracellular calcium levels[7,12] and synapse formation and maintenance.[22]

[A] Proteolytic processing of APP by a-secretase, b-secretase, and c-secretase

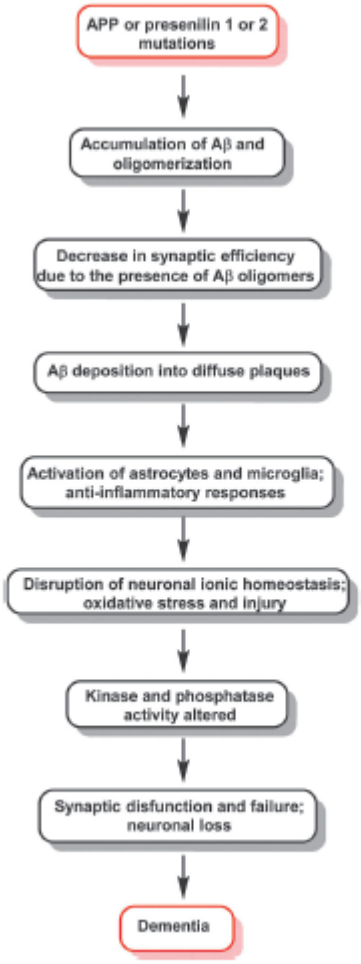
APP is a type I transmembrane protein consisting of an N-terminal 17 residue signaling peptide, a large ectodomain, a 23 residue hydrophobic transmembrane domain, and a 47 residue cytoplasmic domain.[11,12] The Ab region accounts for only a small portion of APP, 28 residues in the lumenal domain plus the first 12–14 residues of the transmembrane domain. The signal peptide translocates APP to the endoplasmic reticulum (ER), where it is bound to the membrane via the 23 residue hydrophobic stretch. It is then posttranslationally modified via N- and O glycosylation, sulfation, and phosphorylation[12] as it is moved through the secretory pathway by way of the Golgi apparatus and endosomes. Along the way, APP is subjected to different proteolytic events that can release a variety of soluble and membrane bound fragments. Only fully modified and glycosylated APP undergoes proteolytic processing.[20] These proteolytic cleavages are performed by enzymes initially called a-secretase, b-secretase (BACE1), and g-secretase. a-Secretase acts on full length APP. It cleaves in the lumenal region of APP releasing a soluble ectodomain fragments APPa) and a membrane bound, 83 residue C-terminal fragment (C83). Interestingly, a-secretase cleavage takes place between residues 16 and 17 of the Ab region. Therefore, the proteolytic cleavage performed by a-secretase precludes the formation of Ab, thus eliminating the possibility of the formation of aggregates and plaques and causing a-secretase activity to be considered nonamyloidogenic.[12,23] Further, sAPPa has been suggested to have some neuroprotective properties.[24] It seems that the specificity of a-secretase does not come from the identity of the amino acids adjacent to the scissile bond, as a-secretase has shown activity on a variety of peptidic bonds. Specificity seems to arise from the proximity of the enzyme to the membrane, as it has been demonstrated that a-secretase consistently cleaves 12–13 residues N-terminal to the membrane.7 BACE1 also cleaves full length APP. It cleaves at the N-terminus of Ab.[25] 16 residues down from the a-secretase cleavage site, which results in a smaller soluble ectodomain fragment (sAPPb), but a larger C-terminal fragment (C99).[7] C99 starts at residue 1 of Ab, whereas C83 from a-secretase cleavage starts at residue 17 of Ab. BACE1 cleavage between methionine 671 and aspartic acid 672 is common in neuronal cells, but is observed far less in peripheral cells such as HEK-293 cells.[23] Further processing of both C-terminal fragments, C83 and C99, takes place via g-secretase activity. g-Secretase cleaves at the C-terminus of the Ab

region in both C83 and C99. In the case of C83, g-secretase activity releases a peptide fragment called p3. In the case of C99, g-secretase activity releases Ab. The cleavage to release both Ab and p3 occurs within the transmembrane region of APP indicating that this cleavage must occur within the membrane rather than the luminal cleavage of both a- and b-secretase.[11] This sequential proteolytic cleavage of APP by b-secretase and g-secretase accounts for the formation of Ab in the brain.

[B] Amyloid hypothesis of Alzheimer's disease



COMMON APP MUTATIONS		
Mutation	Type	Effect
K670N/M671L	Swedish	Increase in β-secretase processing
A692G	Flemish	Increased Aβ aggregation
E693Q	Dutch	Increased Aβ aggregation
T714I	Austrian	Increase in Aβ42 levels
V715M	French	Increase in Aβ42 levels
I716V	Florida	Increase in Aβ42 levels
V717L/V717F	Indiana/London	Increase in Aβ42 levels
L723P	Australian	Increase in Aβ42 levels



The amyloid hypothesis of the progression of Alzheimer's disease.

While many Alzheimer's patients seem to have no genetic predisposition to the disease, as in sporadic AD, many genetic mutations can lead to forms of familial AD. Phenotypically, these forms of AD are very similar or identical; however, familial AD often occurs as early-onset AD.[7] Missense mutations in both APP and the presenilins, with presenilin mutations being more common, lead to these familial cases of AD[7]. There are multiple known mutations in the APP sequence that lead to the onset of familial AD. The mutation of the two residues near the N-terminus of the Ab sequence of APP, K670N and M671L, is known as the Swedish mutation.21,22 Located near the b-secretase site, these mutations make APP a better substrate for b-secretase, thus generating more C99 to be further processed into increased levels of Ab.[12] about three to six times higher than normal APP.[25] Swedish Ab production is thought to occur only in the secretory vesicles, as Swedish Ab has been found to be excreted from the apical surface asopposed to wild-type Ab which is excreted from the basolateral surface.[25] As Ab is a normal outcome of cellular metabolism, people carrying the Swedish mutation will have elevated levels of Ab throughout their entire life, even before AD symptoms are present.[27] There are five different mutation sites that may appear just after the g-secretase cleavage site, T714I (Austrian type),[28] V715M (French type),[29] I716V (Florida type),[30] V717I/G/F/L (London type and Indiana type),[31-34] and L723P (Australian type).[35] These each affect the cleavage by g-secretase slightly differently; however the overall effect is that more Ab42 is produced. The last two mutations occur within the Ab sequence. While they do not affect the cleavage activity of either b- or g-secretase, they seemto have an effect on the aggregative properties of Ab. E693Q is associated with Dutch type hereditary cerebral hemorrhage with amyloidosis (HCHWA-D)[36] which shows Ab aggregation in the meningeal and cerebral microvessels,

while A692G (Flemish type)[37] affects both microvascular b-amyloidosis similar to HCHWA-D and neuritic plaque formation[7,12] As knowledge of this disease increases, more and more APP mutations are being linked to familial AD. Much more common are the presenilin mutations. There are as many as 75 missense mutations in presenilin 1 (PS1) and three in presenilin 2 (PS2) which lead to a much earlier onset of familial AD, with some cases showing clinical signs of AD as early as age 30. These mutations lead to an increased level of Ab42, as much as 3-fold, which subsequently leads to an increase in Ab plaques. Overproduction of Ab is also seen in Down's syndrome. Since APP in encoded on chromosome 21, AD-type symptoms often appear in Down's syndrome patients due to the duplication of chromosome 21.[7] This is not caused by a mutation of the APP or presenilins, as discussed above, but by overexpression of normal APP.

This results in an overproduction of Ab, both Ab40 and Ab42, which can cause diffuse plaques to be seen in Down's syndrome patients as early as age 12.[7] This accumulation of Ab starts from birth, with plaque formation and cognitive loss associated with AD found in patients in their 30s. The discovery of these mutations, along with the formation of Ab aggregates in sporadic AD, has led to the development of the amyloid hypothesis of the progression of Alzheimer's disease. The amyloid hypothesis states that the formation of Ab plaques begins a cascade of events ultimately leading to dementia in AD patients.[38] It seems that the accumulation of Ab may initially affect the efficiency and function of synapses, leading up to neuronal loss and dementia.[39] Many research groups have focused on the amyloid hypothesis and there are significant findings that support this hypothesis. However, the subject is very complex and there are questions that remain to be answered. It has been found that frontotemporal dementia with Parkinson's disease is caused by mutations in the gene for the tau protein, which in turn cause neurofibrillary tangles in the brain. The lack of AD-like plaques in the brains of these patients, even in the most severe cases, leads to the suggestion that Ab plaques are formed first and tangles in the brains of AD patients are perhaps a result of this.[38] Further, mice that overexpress mutated human APP and tau show increased formation of tangles, while plaques remain about the same, which suggests that the APP processing occurs prior to the tau tangle formation.[38] The genetic mutations of familial AD seem to be a strong proponent of the amyloid hypothesis.[9] However, the amyloid hypothesis does not adequately explain all observations. For instance, it has histologically been argued that the number of Ab plaques in the brain does not correspond to the degree of dementia in AD patients. However, when investigated biochemically rather than histologically, Ab count does correlate with the impaired cognitive state.[38] Some studies have shown that there is, in fact, a quantitative correlation between histologically visible plaques and the degree of cognitive decline.4,8 However, since the exact effects of Ab on neuronal toxicity are not known in vivo, the amyloid hypothesis is drawn into question.[38] Still, the amyloid hypothesis seems to be a broadly accepted general scheme of the pathophysiological events in AD.

[C] Role of b-secretase in Ab production

The proteolytic cleavage activities of APP were deemed a-, b-, and g-secretase prior to the identity of any specific enzyme being known to have these functions. The ambiguity of these enzymes made some studies difficult to perform and conclusions difficult to draw. However, since the identity of the secretases responsible for APP processing has now been discovered, much knowledge has been obtained on the role of these enzymes in both AD and other physiological functions. Several proteins have been found to have a-secretase activity.

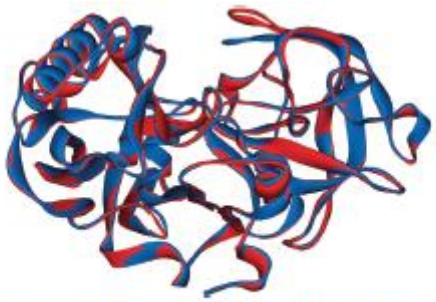
They are all a part of the A disintegrin and metalloprotease (ADAM) family of enzymes. While some, like ADAM[22] (tumor necrosis factor-a converting enzyme, TACE), are believed to have shown a-secretase activity,[40] ADAM[15] is generally regarded as the a-secretase most active in the brain.[41] Overexpression of ADAM[15] in HEK cells resulting in increased levels of sAPPa and C83, led to this conclusion.[41] It is speculated that g-secretase activity is carried out through a complex of proteins rather than being attributed to one enzyme. The presenilins (PSs) are thought to be a part of that complex, either by having g-secretase activity themselves, or by modulating g-secretase activity. PS1 has two transmembrane aspartates that are aligned with the transmembrane domain of APP making g-secretase cleavage by this enzyme feasible.[42]

Mutations in PS1 and PS2 that support the formation of Ab42, the form of Ab more susceptible to aggregation, solidify the influence of PSs on g-secretase activity.[43] It has also been found that PS1 knockout reduces Ab formation, further supporting the role of PSs in g-secretase activity.[44,45] Other proteins thought to be a part of the g-secretase complex are nicastrin (Nct), anterior pharynx-defective phenotype (APH-1), and PS-enhancer (PEN-2). In fact, the expression of all of the above components together in Saccharomyces cerevisiae, which normally lacks any form of g-secretase activity, results in fully active g-secretase, suggesting that the above-mentioned four components account for full g-secretase activity[44] Many different enzymes have been proposed as b-secretase throughout the years. Many, including cathepsin D,[46-49] a chymotrypsin-like protease (CLIP) isolated from the rat brain,[50] cathepsin G,[51] BACE2,[52] metalloendopeptidase MP78,[48] and metalloprotease MP100,[53] have been ruled out for various reasons. At about the same time, five independent groups correctly identified the protein responsible for b-secretase activity. It has been named Asp2,[49-54] b-site APP cleaving enzyme 1 (BACE1),[55] b-secretase,[56] and memapsin 2.52 Herein, b-secretase will primarily be referred to as BACE1. BACE1 is

formed in the endoplasmic reticulum as an immature, glycosylated propeptide, pro-BACE1.[58] Pro-BACE1 is then processed and cleaved into mature BACE1 in the Golgi apparatus.[59] The propeptide has two conformations, both an open and closed conformation. When in the open conformation, pro-BACE1 can exhibit some enzymatic activity. However, when in the closed conformation, with the pro-domain

covering the active site, the activity of pro-BACE1 is diminished which allows the pro-domain to serve as a weak inhibitor of BACE1 activity.[60] This pro-domain has also been shown to play a role in the folding of mature BACE1.[60] Cleavage of the pro-domain allows BACE1 to have full enzymatic activity by allowing the catalytic active site to be fully accessible to the substrate.[61] BACE1 is a monomeric protein that is shuttled between the endosomes and the cell surface. It presents four potential N-glycosylation sites as well as six cysteine residues that form three disulfide bonds.[62] These disulfide bonds are important for proper folding and activity of the enzyme. In particular, Cys330/Cys380 is found inside the active site of BACE1 and is especially important for stability and activity.[63] As BACE1 ismatured through the endoplasmic reticulum and Golgi apparatus, three of the four potential N-glycosylation sites are utilized. BACE1 is then trafficked through the endosomal system to the cell surface where it is then reinternalized and recycled. There is a dileucine motif in the cytoplasmic domain that aids in the localization of BACE1 to the endosomes.[62] While BACE1 is cycled from the endosomes to the cell surface, it has been shown that BACE1 is most active at a mildly acidic pH of about 5.5. This suggests that BACE1 acts in the endosomes on the way to the cell surface and not at the cell surface.[62-64]

Similarly to pro-BACE1, mature BACE1 also has two major conformations, a flap open conformation and a flap closed conformation. Free BACE1 is found in the flap open conformation. This conformation is energetically stable and is held by optimal hydrogen bonds.[65] However, when bound to the substrate, BACE1 adopts a flap closed conformation. To make this conformational shift, there is breakage of hydrogen bonds between the oxygen of Tyr71 and the nitrogen of Gly74, the nitrogen of Lys75 and the oxygen of Glu77, and the hydroxyl of Tyr71 and the oxygen of Lys107. This destabilization is justified by the interaction of the enzyme with the substrate.[65] Also a new interaction of the Tyr71 side chain with the indole nitrogen of Trp76 is formed. While in the open conformation, substrates can enter through a cleft in the enzyme. However, due to a bottleneck being formed by Thr72, Arg235, Ser328, and Thr329, some flexibility in the substrate is needed.[65] It has been suggested that this bottleneck may serve as a specificity mechanism.

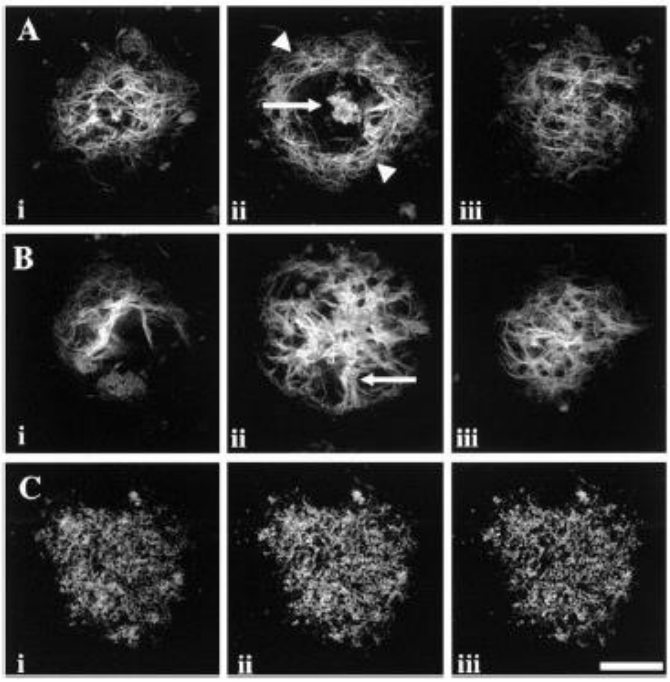


Open flap (red, PDB: 1SGZ) and closed flap (blue, PDB: 1M4H) conformations of mature BACE1.

[D] Structural morphology of amyloid plaques

The relationship between soluble Ab monomers, oligomers Ab, protofibrils, fibrils and plaques is complicated and several aspects of the dynamics remain uncertain.[66,67] Amyloid-b plaques are classified into two morphological types: (i) neuritic and (ii) diffuse (‘‘pre-amyloid’’) plaques. Neuritic plaques, observed after staining, have a dense compact spherical appearance with a diameter ranging from 10 mm to greater than 120 mm. Neuritic plaques can be further sub-classified into ‘fibrillar’ or ‘dense-cored’ plaque forms. Fibrillar plaques show dense accumulations of Ab throughout the plaque structure (Fig.B) whereas cored plaques have a distinct central core of Ab encircled by a void or clearing surrounded by an outer spherical rimof Ab (Fig.A). These Ab deposits are referred to as neuritic plaques as

they are spatially localized with dystrophic neuritis (clusters of abnormal neuronal processes) both inside and immediately surrounding the deposits.[68-70] Following staining, diffuse plaques display a finely granular pattern of amorphous shape that lacks a fibrillar, compacted centre (Fig.C). These non-fibrillar plaques are the only Ab deposits found in regions of the brain not clearly associated with the typical symptoms of AD. They also form within the same regions of the brain as neuritic plaques, however, very little or no detectable dystrophic neurites are associated with diffuse plaques. Studies in a transgenic AD mousemodel, expressing mutant human APP, show that the mice develop diffuse plaques before fibrillar plaques supporting the hypothesis that diffuse plaques are immature or precursor lesions to neuritic plaques and are therefore also termed ‘‘pre-amyloid’’ plaques.[68]



Serial sections of human brain tissue displaying Ab plaques of different morphologies. (A, i–iii) Optical sections of a dense core plaque, observed after staining with a histological dye for amyloid. Images were captured at B14.5 mm intervals and show the dense Ab core of the plaque (arrow) (A, ii) surrounded by a void defined by an outer rim of Ab (arrow heads). (B, i–iii) Similar images of a fibrillar plaque with images captured at intervals of B17.5 mm. Spoke-like Ab accumulations (arrow) radiate from the dense central accumulation. (C, i–iii) Confocal images of a diffuse plaque after treatment with an Ab antibody. The images were captured at intervals of B2.5 mm and show the punctate accumulation of Ab giving a granular pattern. Scale bar = 20 mm (A and B), 50 mm (C). Reproduced with permission from T. C. Dickson and J. C. Vickers, Neuroscience, 2001, 105, 99–107. Copyright 2001 Elsevier.

The pathogenesis

[A] Amyloid cascade hypothesis

One of the main pathological features of AD is the formation of senile plaques (SP), which caused by amyloid beta (Ab) deposition. Normally, Ab is soluble small peptides, which is produced by cleavage of the amyloid precursor protein (APP) by the action of asecretase, b-secretase and g-secretase. The imbalance between b-amyloid (Ab) production and clearance leads to various types of toxic oligomeric, namely protofibrils, fibrils and plaques depending upon the extent of oligomerization. The reason of the formation of Ab is still unknown, but the sequence, concentration and conditions of stability of Ab are important factors. Some studies suggested that neurotoxicity required assembly of the peptide into oligomers,and other evidences suggested that soluble oligomers forms of Ab could produce more neurotoxicity. A thorough study shows that amyloid toxicity associated with both protein-specific and conditional-determined by the function of vascular endothelial growth factor receptor 2 (VEGFR2) loss, which is essential for target protein in a biological context. A recent work systematic reviewed of the 25 years of the development and latest findings of the amyloid hypothesis, and elaborated the features of its cell biology and genetics. Suggesting that amyloid dyshomeostasis has emerged as the most extensively validated and compelling therapeutic target.[71]

[B] Hyperphosphorylated Tau protein

The neurofibrillary tangles (NFTs) formed by hyperphosphorylated Tau protein are another major pathologicalfeature of AD. Tau protein belongs to the

family of microtubuleassociated proteins, mainly existing in the axon, and its main function is to maintain the stability of microtubules. There are several phosphorylation sites on Tau protein, compared with the mature nerve cells, the Tau protein in the growing nerve cells is more likely to be phosphorylated. Normally, the phosphorylation and dephosphorylation of Tau protein maintain a dynamic balance, but when the hyperphosphorylated Tau protein aggregates to form a double-helix fiber, it loses the function of connecting and stabilizingmicrotubules, which leads to the death of neurons. There has been a long-standing debate over the temporal mechanistic relationship between the two major pathological features of AD, and evidence reports that soluble amyloid beta protein dimers induces Tau protein hyperphosphorylation and neurodegeneration.[72]

[C] Oxidative stress hypothesis

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in many normal and abnormal processes in humans, they play a dual role as both beneficial function of numbers of cellular signaling pathways and deleterious process that can lead to damage of cellular structures (including cell membrane, lipid, protein, and DNA). The high oxygen consumption of the brain, which utilizes 20% more oxygen than other mitochondrial respiratory tissues, means that the brain is more vulnerable to oxidative stress. Neuron is the basic function unit of the brain, which contains a large number of polyunsaturated fatty acids. It can interact with ROS, leading to the lipid peroxidation reaction and molecular apoptosis, in addition, less glutathione in neurons is also one of the causes of oxidative stress injury.[73]

[D] Cholinergic hypothesis

Bartus et al. proposed the cholinergic hypothesis that dysfunction of cholinergic activity in the brain of healthy elderly and dementia patients may play a role in the loss of memory and related cognitive impairment, so reconstruction of cholinergic function may be able to reduce the serious lack of cognitive function. The activity of the cholinergic system was evaluated by choline acetyl transferase (ChAT) and acetylcholinesterase (AChE) etc. The study showed that the Ach, ChAT and AChE in the brain of AD patients showed a continuous decline.

Acetylcholine (ACh) is neurotransmitters regulating cognitive performance and learning and memory process, synthesized by acetyl CoA and choline under the catalysis of ChAT, ACh and its receptor (AChR) combined with transfer nerve impulses, AChE hydrolyzed into acetic acid and choline. Acetylcholine established synaptic contacts in networks of brain cells to remodelling of the cerebral cortical circuits, which will subserve complex cognitive functions.[74]

[E] Inflammatory hypothesis

Inflammatory responses in the brain is another pathological characteristic in AD, usually chronic inflammation, main characteristics for a large numbers of mononuclear leucocytes and macrophages in the central nervous system, such as small glial cells. Compared with normal subjects, acute phase proteins and pro-inflammatory cytokines over expression in AD patients brain tissues. Microglia and astrocytes are the main causes of the inflammatory response, the activated cells produce pro-inflammatory mediators, such as interleukin 1 beta (IL-1) and interleukin 6 (IL-6) and tumor necrosis factor (TNF alpha), chemotactic factor interleukin 8 (IL-8), macrophage inflammatory protein-1, prostaglandins, leukotrienes, coagulation factor, protease, protease inhibitors; The production of these substances can kill the neighboring neurons.[75]

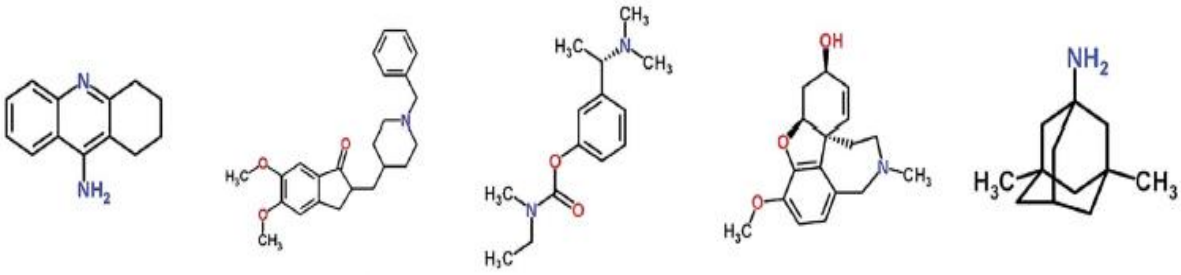
[F] Metal ion hypothesis

Metal ions play an important role in the maintenance of homeostasis, and the relationship between metal ions and neurodegenerative diseases has attracted much attention in recent years. The brain is rich in metals that act as essential cofactors in metallo-proteins to participates in the process of metabolism, the concentration of metal ions in the brain is tightly regulated through the blood brain barrier, when the blood brain barrier of metal ion regulation system degradation, metal ion transport dysfunction, metal ions (iron, copper, manganese, aluminum, zinc, etc.) begin to affect the oxidative stress response of mitochondria and the wrong folding proteins, and ultimately lead to neurodegeneration, Studies have indicated that aluminum, zinc, copper and iron can lead to changes in the conformation of the Ab protein. Aluminum can lead to the accumulation of Ab and Tau protein, aluminum and copper are involved in the process of the development of nerve inflammation. The increased levels of iron, aluminum and copper in the aged human brain may reflect the relationship between age and neurodegenerative diseases.[76]

Cholinergic drugs

Drachman and Leavitt suggested that memory was associated with the cholinergic system and was age dependent, and Bartus proposed Alzheimer's cholinergic hypothesis on which the development of cholinergic inhibitors is mainly based. Study suggested that the acetylcholine (ACh), neurotransmitters regulating cognitive performance and learning and memory process, in the brain of AD patients showed a continuous decline. The loss of cholinergic function is related to cognitive impairment and behavioral disorder, and these symptoms can be improved by acetylcholinesterase (AChE) inhibitors or by modulating other cholinergic receptors, such as muscarinic and nicotinic ACh receptors. In 1993, tacrine was first approved by FDA to treat with mild to moderate AD, in addition three cholinesterase inhibitors were followed: donepezil (1996)[77] rivastigmine (2000)[78] and galantamine (2001)[79] However, other drugs have not been approved yet, including AChE inhibitors velnacrine, physostigmine, eptastigmine, metrifonate etc. muscarinic receptor agonists cevimeline (AF102B), milameline, sabcomeline (SB 202026),

talsaclidine, xanomeline and alvameline (LU 25-109). In addition to the cholinesterase inhibitor, memantine, a N-methyl D-aspartate (NMDA) receptor



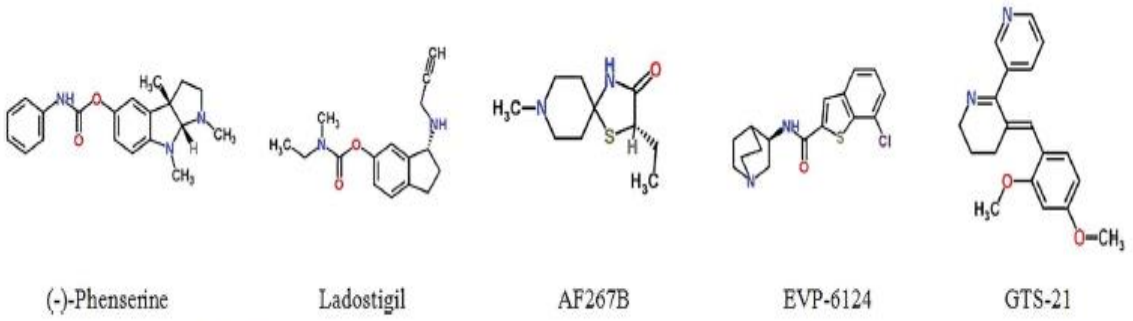
Five medicines approved by the US FDA for treatment in Alzheimer's disease.

antagonist which acts on the glutamatergic system, is another FDA approved for treatment of moderate-to-severe AD drugs. Besides the drugs approved by FDA, there has still been progress in development of cholinergic drugs in clinical trials as well as patented lead compounds and show poistive drug approved by the US FDA for treatment in AD.

Huperzine A, an alkaloid derived from the Chinese herb Huperzia serrata, acts as a selective inhibitor of acetylcholinesterase, which has a mechanism of action similar to donepezil. Huperzine A has shown promising effects on the treatment of Alzheimer's disease, including improvement of cognitive function, daily living activity, and global clinical assessment.[82]

However, one trial demonstrated no significant change in cognitive function as measured by Alzheimer's disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and clinical data are limited by poor methodological quality. Pro-drug of huperzine A, named ZT-1, derived from natural product, is a potent and selective AChE inhibitor. The results from the Phase I clinical trials showed that ZT-1 has an admirable pharmacokinetic with a rapid absorption and a wide distribution in human. Physostigmine, originally having been extracted from calabar beans, is an AChE inhibitor, but it has limited treatment effects and serious side effects. The (_)-phenserine, a derivative of physostigmine, is an AChE inhibitor that has an effect on cognitive improvement. It also can reduce the translation of APP to reduce Ab concentrations, suggesting (_)-phenserine may be a promising multitarget drug of AD.[83] Memogain (Gln-1062), an inactive pro-drug of galantamine, liberates galantamine on cleavage by a carboxylesterase in the brain. Memogain has more than 15-fold higher bioavailability in the brain than the same doses of galantamine due to the more hydrophobic characteristics. Memogain may represent a valuable drug with higher potency in enhancing cognition for AD treatment, a significantly lower plaque density in the brain, and much lesser gastrointestinal side effects.

Ladostigil is a novel multitarget drug combined with acetylcholine–butyrylcholinesterase cholinesterase inhibitor and brain selective monoamine oxidase A and B inhibitor. It can relieve scopolamine-induced impairment in spatial memory, and increase brain cholinergic activity in rat. Furthermore, it was proved to possess anti-apoptotic and neuroprotective including the regulation of APP process, activation of protein kinase C and mitogen-activated protein kinase signaling pathways. NGX267 (AF267B), as M1-selective muscarinic agonists, can enhance the cognitive ability.[84] In AD transgenic mice, it also reduced Ab1-42 and Tau hyperphosphorylation in the cortex and hippocampus, presenting an unique beneficial effects on therapy in AD. EVP-6124 is a partial, selective agonist of the a-7 nicotinic acetylcholine receptor (a7-nAChR) with highly CNS-penetrant. It can improve cognitive deficits by boosting the ACh response of a7 nAChRs. EVP-6124 moved into Phase III for AD, supporting a new therapeutic strategy for the treatment of cognitive impairment. Additionally, GTS-21 is a selective agonist of the a7 nicotinic receptor, showed promising characteristics during Phase II clinical trial. And show cholinergic inhibitors in clinical trials.



(-)-Phenserine Ladostigil AF267B EVP-6124 GTS-21

Cholinergic inhibitors in clinical trials.

Amyloid-targeted therapies

Decreasing Ab production

[1] b-Secretase inhibitors

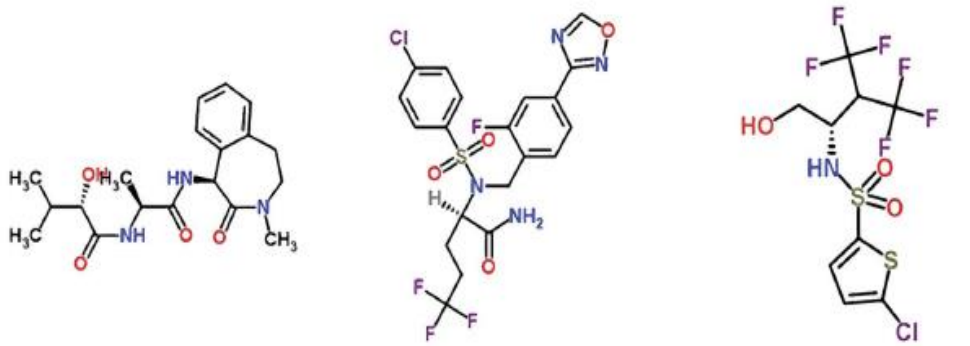
LY2811376 is the first orally non-peptidic small-molecule BACE1 inhibitor with satisfactory pharmacokinetic and pharmacodynamic properties from preclinical animal models to man. It can penetrate the blood brain barrier, and showed long-lasting effect on reducing the level of Ab in healthy volunteers. However, clinical development was stopped according to a chronic non-target-associated toxicology. LY2886721, next-generation orally available BACE1 inhibitor with agreeable drug properties, reduce the concentrations of cerebral Ab40, Ab42 and sAPP-b with safety and good tolerability. Unfortunately, it was terminated because of abnormal liver biochemical tests. MK-8931 developed by the pharmaceutical company Merck, is a BACE1 inhibitor tested for the treatment of AD in Phase I clinical trial. It can significantly reduce the levels of CSF Ab in a dose-dependent and sustained way. MK-8931 also reduces the concentration of CSF Ab in patients with mild-to-moderate AD. And further research shows that MK-8931 elicits few adverse effects previously ascribed to BACE inhibition, different doses are well tolerated and reduce CNS b-amyloid in both healthy human subjects and AD patients. The human data are suitable for the amyloid pathway model and provide a meaningful guidance for further experiments. E2609, an orally available BACE1 inhibitor, showed dose-dependent reductions of Ab concentrations in CSF and/or plasma in a single oral ascending dose study and multiple oral ascending dose study respectively. Phase 2 clinical trial of E2609 is planned by Eisai.[85,86] and show b-secretase inhibitors in clinical trials.

[2] g-Secretase inhibitors and modulators

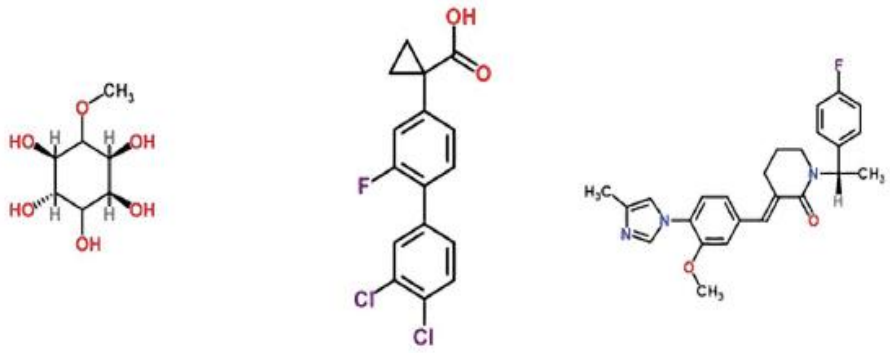
Semagacestat (LY-450139) is a g-secretase inhibitor in the aim to treat AD. It can lower Ab concentrations in the plasma and cerebrospinal fluid with a dose-dependent manner. However, the trial was terminated owning to severe adverse effects and worsen cognition performance compared to placebo group. It is believed that inhibiting g-secretase may disturb Notch signaling proteins and other cell surface receptors.[87] Avagacestat (BMS-708163) is also a g-secretase inhibitor with Notch-sparing effect. Nevertheless, avagacestat did not demonstrate obvious efficacy from Phase II trials in MIC.[88] Begacestat (GSI-953) is a thiophene sulfonamide g-secretase selectively inhibitor which inhibits cleavage of APP over Notch.[89] The compound has shown promise in recent Phase I clinical trials. NIC5-15 is a natural compound acted as a Notch-sparing gsecretase inhibitor and an insulin sensitizer. The compound can improve cognitive function through multiple mechanisms including reduce Ab production by modulating g-secretase. The result shows that NIC5-15 is safe and has good tolerability and further feasibility trials are needed. CHF5074, a new microglial modulator, reduces brain Ab burden to enhance spatial memory cognitive in transgenic mice of AD model. CHF5074 shows dose-dependent effects in central nervous system and well tolerated and safety in mild-to-moderate patients. E2012 is also a novel g-secretase modulator which decreases the concentration of Ab40 and Ab42 in rat in a dosedependent manner, and without affecting Notch cleavage.[90] and show g-secretase inhibitors and modulators in clinical trials.

LY2811376 LY2886721 MK-8931

β-Secretase inhibitors in clinical trials.



Semagacestat (LY-450139) Avagacestat (BMS-708163) Begacestat (GSI-953)



NIC5-15 CHF5074 E2012

γ-Secretase inhibitors and modulators in clinical trials.

Promoting Ab clearance

[1] Active AD immunotherapy

AN-1792 was the first fulllength Ab1-42 active vaccine used in clinical trial. Although the trial was terminated after 6% of the participants developed severe side effect, it provided an important proof of concept. CAD106 is the second-generation active immunotherapy vaccine that comprises Ab1–6 peptide. The study suggests that CAD106 can reduce Ab accumulation and lead to acceptable antibody response with a safe and tolerance manner in Phase II trials. ACI-24 is a Ab1–15 liposome-based vaccine that can restore the memory defect and reduce plaque in transgenic mice. A phase I/II clinical trial is currently ongoing for investigating the safety and efficacy of mild-to-moderate AD patients. UB-311 is synthetic peptides, consisting of UBITh helper T-cell epitopes and coupled to the Ab1–14 peptide. A phase 1 clinical trial of UB-311 has successfully completed, illustrating safety and tolerability.[91] In addition, Phase 2 clinical trials are being prepared. Other ongoing trails include ACC-001, V950, Lu AF20513 and AD02 as well. ACC-001 (Vanutide cridi□car), an Ab1–7/Qs21 adjuvant immunotherapeutic vaccine, was evaluated in phase 2a and results came out with safety profile in mild-to-moderate AD patients.102 V950, multivalent Ab peptide/ISCOMATRIX™ adjuvant, aimed to produce Ab antibodies to recognize pyroglutamate-modified and other N-terminally truncated Ab fragments. A phase 1 has been completed and further studies were discontinued. Lu AF20513 was developed by Lundbeck A/S (Valby, Denmark) according to Ab1–12 peptide replaced with two foreign T-helper epitopes from tetanus toxoid. It is currently being tested in preclinical trail.104 Additionally, AD02 is an amyloid-beta (Ab)-targeting vaccine to elicit anti-Ab antibodies, and phase II study was finished. DNA amyloid-beta protein immunotherapy is currently being investigated in preclinical studies. Tau immunotherapy AADvac1 and ACI-35 are also under preclinical.[92]

[2] Passive AD immunotherapy

AAB-001 (Bapineuzumab) is the first humanized monoclonal antibody targeting the Ab N-terminus (Ab1–5). The antibody binds strongly to deposite amyloid plaques to reduce the Ab plaque burden and induces Fcmediated microglial phagocytosis of Ab plaques inmouse. Two phase 3 trials involved patients with mild-to-moderate Alzheimer disease were conducted, however, bapineuzumab failed to improve primary clinical outcomes. LY-2062430 (Solanezumab) is a humanized monoclonal antibody to the middomain of Ab16–24, which binds to the soluble Ab. The compound has been proved safety in Phase 2 findings, nevertheless, neither clinical trial in phase 3, showing significant cognition improvement nor functional ability in patients with mild-to- moderate AD.111 Two previously phase 3

clinical trial results were renewed in some mild patients, and the secondary outcomes suggested that Solanezumab can slow the cognitive decline of 34% according to the ADAS-Cog andMini-Mental State Examination (MMSE) ($P < 0.05$).112 PF-04360365 (Ponezumab) is a humanized IgG2dA monoclonal antibody aiming to reduce immune effector. PF-04360365 showed accepted safety and well tolerated findings without antibody-induced side effects. And another clinical trial also showed similar results. GSK-933776 is a humanized Fc-attenuated/inactivated anti-Ab monoclonal antibody. GSK933776 showed pharmacological activity and engaged target in plasma and CSF without causing brain amyloid-related imaging abnormalities-edema (ARIA-E/H) in patients with mild AD or MCI. MABT5102A was a humanized Ab1–15 monoclonal antibody with IgG4 isotype. It can inhibit Ab aggregation and promote its disaggregation without vasogenic edema and cerebral microhemorrhage induced by overactivation of microglial cells.117 Aducanumab (BIIB037) is a human monoclonal antibody that selectively targets misfolded Ab peptides. Aducanumab restores calcium homeostasis in Tg2576 mice, and also reduce soluble and insoluble Ab in a dose-dependent manner. In a recent study, Sevigny et al. reports beneficial effects on the amyloid pathology and the cognitive status in patients with prodromal or mild AD.[93] The phase 1b clinical test had revitalizes the "amyloid cascade hypothesis" and bring mononuclear phagocytes to the center stage of AD treatment.

[3] Preventing Ab aggregation

Tramiprosate (3-amino-1-propanesulfonic acid, 3APS) is an orally-administered amyloid antagonist, which binds to soluble Ab peptide and designs to reduce Ab aggregation and prevent fibril formation. Tramiprosate produces cytoprotective effects against Ab-induced neurotoxicity, and exerts significant reduction of soluble and insoluble Ab in the brain of transgenic mice. Clinical trials show that tramiprosate can slow hippocampal atrophy and have some benefit on cognition. However, the further Phase III trial has been terminated due to its unsuccessful in demonstrating efficacy. Scyllo-inositol, an endogenous inositol stereoisomer, is another antiaggregation compound, exerting specific health-promoting effects for Alzheimer disease.[94] It stabilized a small conformer of Ab42 in vitro, and neutralized cell-derived Ab oligomers in vivo. Moreover, scyllo-inositol can decrease neuronal toxicity and abate cognitive deficits in multiple mouse models of AD. A Phase II clinical trial demonstrated acceptable safety, however, primary clinical efficacy outcomes were not significant. Epigallo-catechin-3-gallate (EGCG), a natural fiavanol derived from green tea which shows multiple neuroprotetive activities, bind to unfolded peptide to prevent the formation of Ab toxic oligomers. It can also modulate cell signalling and reverse superoxide dismutase activity and the damage effects of AlCl3 neurotoxicity, which improves mitochondrial and cholinergic synaptic functions. PBT1 is a metal chelator that promotes the solubilisation by disturbing the chelation between Ab and metal ions in vitro or mouse model studies. However, Phase II clinical suggested that there was no significant positive clinical benefit for patients with AD. Unfortunately, phase III trial was abandoned. The second-generation metal–protein attenuating compound PBT2, was developed as a metal chaperone which affected the metal-induced Ab oligomerisation. It has greater blood–brain barrier permeability. PBT2 can obstruct Ab oligomerization, decrease soluble and insoluble Ab and promote the clearance of Ab oligomer. Phase II study showed that PBT2 can reduce the concentrations of Ab42 in CSF and improved cognitive function with safety and tolerance. TTP488 (PF-04494700) is a smallmolecule oral antagonist of the receptor for advanced glycation end products. The low dose (5 mg) shows good safety profile but associated with conclusive results in Phase II trial with mild to moderate AD. Another clinical trial demonstrates that low-dose (5 mg) could be a benefit dose in further Phase 3 trials in patients with mild AD.

A recent study provided a new framework for the rational identification of a series of drug candidates for neurodegenerative diseases, chemical kinetics approach was applied to study the effect of small molecules on the deposition rate of Ab42 which was quantitatively analyzed. An anticancer drug, bexarotene, was reported to suppress Ab42 deposition by targeting the primary nucleation step in the aggregation of Ab42 and delaying the formation of toxic species in neuroblastoma cells.[95] and show drugs in clinical trials to prevent Ab aggregation.

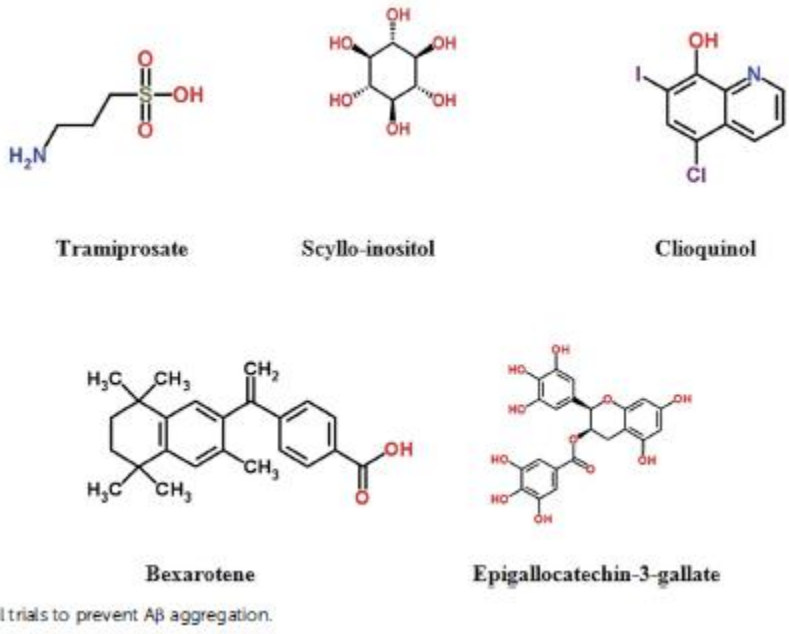
Other potential therapeutic strategies in AD

[1] Drugs to target Tau protein

Leuco-methylthioninium (LMTX), a selective Tau aggregation inhibitor by preventing the formation and spread of NFTs and reducing the Tau pathology in transgenic mouse models, is the reduced form of methylthioninium (MT), which is the first substance that can degrade Tau protein. An exploratory phase 2 study in mild or moderate Alzheimer's disease was conducted, safety and effectiveness was determined as well.142 The main results of a broad participation of academic centres in 15 months of randomised, controlled double-blind, parallel-group clinical trial is negative, the results do not suggest the benefit of LMTX as an additional treatment of mild to moderate Alzheimer's disease.

Glycogen synthase kinase 3 b (GSK3b) is a serine/threonine kinase which plays an important role in regulating Tau protein phosphorylation and involves in processing of amyloidbeta peptides. Tideglusib (NP-031112) is a small non-ATP competitive GSK-3 inhibitor, for the treatment of Alzheimer's disease in clinical trials. It can lower Tau protein hyperphosphorylation, reduce brain amyloid plaque levels, improve learning and memory and prevent the loss of neurons in some animal models. Studies

demonstrated valuable safety in clinical trials in AD patients.[96,97]



Drugs in clinical trials to prevent Aβ aggregation.

[2] Neurotrophins

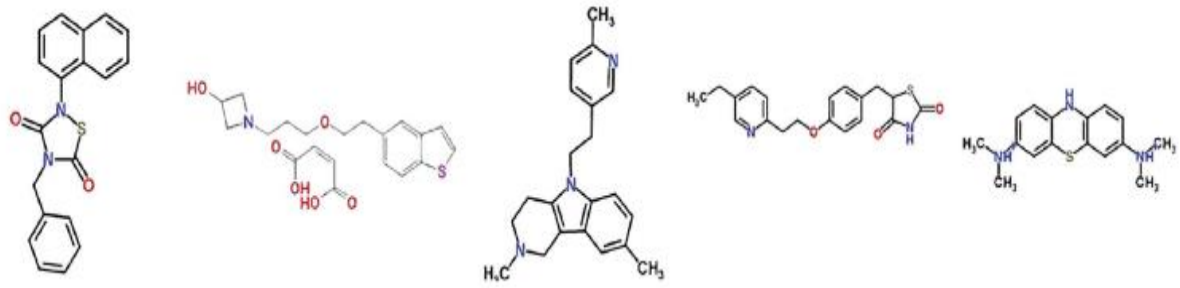
Neurotrophins are dimeric peptide hormones. Nerve growth factor (NGF), the first neurotrophin, regulates many aspects of neuronal development and function, plays an important role in the survival and differentiation of neurons. Recently, the study shows that BDNF exerts substantial protective effects on crucial neuronal circuitry involved in Alzheimer's disease, revealed the correlation between the decreased NGF and AD. Thus, neurotrophins have been acted as an attractive target for treatment of AD. AAV2-NGF (CERE-110) is designed to deliver NGF by gene to cross the blood–brain barrier, which increases production of acetylcholine and enhances the function basal forebrain cholinergic neurons. A Phase I study has been completed and a multi-center, placebo-controlled Phase II clinical trial in the observation phase. T-817MA [1-{3-[2-(1-benzothiophen-5-yl)ethoxy]propyl}azetidin-3-olmaleate] has both neuroprotective and neurotrophic effects and also has the ability to improve the cognitive impairment in transgenic mice, The Phase II trial has been completed for its evaluations on safety and tolerability.[98]

[3] Targeting mitochondrial dysfunction

There is a growing body of evidence supporting that mitochondrial dysfunction has a significant influence on the process of AD. Mitochondrial dysfunction also has connection with oxidative stress and Tau pathology. Due to the correlation between mitochondrial alterations and AD, strategies targeting decreases the related oxidative stress and removal of damaged mitochondria possess great promise in AD treatment. Latrepirdine (Dimebon®), a small molecule compound, is used for the treatment of AD. Dimebon can modify hippocampal APP/Ab pathology and ameliorate mitochondrial membrane potential and ATP production, indicated the potential treatment for neurodegenerative diseases.[99]

[4] PPAR-g agonists

Pioglitazone is an insulin sensitizer of the thiazolidinedione class of peroxisome-proliferator activated receptor g (PPAR-g) agonists. Takeda developed pioglitazone as a once-daily treatment of type 2 diabetes. The PPAR-g agonist improves cognition in AD mice, and mixed results in prior human trials. In August 2013, Takeda and Zinfandel Pharmaceuticals began ‘Tomorrow’, a Phase III trial that is to enroll 5800 cognitivelynormal participants and run for 4 years. The study has two separate goals; one is to evaluate how accurately a diagnostic algorithm based on the genes ApoE and TOMM40, developed by Zinfandel, predicts a person's risk of developing mild cognitive impairment due to AD within 5 years. The other is to evaluate pioglitazone's ability to delay this diagnosis.



Tideglusib(NP-031112) T-817MA Latrepirdine Pioglitazone LMTX

Other potential therapeutic strategies in AD.

[5] Computer simulation

Studies showed that molecular dynamics simulation can be used to design Ab aggregation inhibitor and a structure-based drug discovery procedure has been explored to identify the binding pockets between small-molecule and Ab peptide. In a study, a total 11 compounds were identified which reduce Ab cytotoxicity by shining the equilibrium of Ab from oligomers to fibers by comprehensive performing these methods. NQTrp (1,4-naphthoquinon-2-yl-L-tryptophan), a small molecular which has been reported to inhibit aggregation of Ab. NQ-trp was found to be the best binders of small-molecule drugs with Ab17-42 by using a hierarchical computational procedure, further more, an extensive atomistic replica exchange molecular dynamics simulations was used to explain the beneficial effect of NQTrp in reducing both the level of Ab1-42 aggregation and toxicity. Aiming to investigate the molecular mechanism of NQ-Trp combined experimental and simulation studies were performed. The converging results explained its low inhibitory efficiency which due to the lack of specific "binding site"-type between NQ-Trp and Ab, and suggested that another mechanism was involved in anti-AD activity of NQ-Trptype molecules models in vivo.[101] Yang Z. et al. found that graphite can inhibit Ab peptide monomer fibrils and can clear the mature amyloid fibers through penetration and extraction of peptides. Experimental evidence such as molecular dynamics simulations, atomic force microscopy images, thiofiavin fluorescence assays, and cell viability and ROS assays confirmed the prediction results of computer simulation. The molecular mechanism and molecular dynamics of (_)-epigallocatechin gallate (EGCG) and CQ1-3 inhibiting Ab aggregation were studied respectively by computer simulation. Computer simulation provides new insights into the underlying molecular mechanisms that define drug–amyloid interaction and suggests the directions of further AD drug development.[102] and show other potential therapeutic strategies in AD.

Conclusion and future perspective

So far, the development of AD drugs has achieved some success in the improvement of symptoms, whereas it also has several failed aspects of disease modification. While many clinical and drug design studies are undergoing, we must recognize that it is quite difficult to successfully cure AD with single therapy, which is attributed to the complex pathophysiology of AD. It is believed not to be caused by single gene defects, but rather by a large number of genes, proteins and their complex interactions that ultimately lead to the change of this disease. Multi target drug discovery may be a more promising treatment strategy for AD. It could overcome the deficiency of the poor development effects of one-target-one-compound. Several multi target compounds already have been designed, such as dual binding AChE and BACE1 inhibitors, AChE inhibitors and antioxidants, which provide better therapeutic effects on both symptomatic and disease modifying in AD. At this point, multiple pharmacology natural products can be used as prototypes for the drugs design of AD treatment. Herbal formulae such as Kai-Xin-San (consisting of Ginseng Radix, Poria, Polygalae Radix, and Acori Tatarinowii Rhizoma) has been used in the treatment of Parkinson's disease and Alzheimer's disease also provide new insight for the treatment of AD. New methods, such as quantitative systems pharmacology, chemo genomics knowledgebase, metabolomics and chinmedomics strategy will meet the challenge and provide a promising avenue for the discovery and clinical development of new generation drugs for AD.

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