

Review paper

Alzheimer's: A Disease Fraught with Syndromes, Hypotheses and Theories with attendant intractable Pharmacological Management

ABSTRACT

Alzheimer's disease is an insidious disease of the brain. It is a degenerative and intractable neurological disorder that causes deterioration of memory, judgment, and reasoning in the elderly. About 46.8 million people worldwide are suffering from AD; prevalence is expected to affect 131.5 million by 2050. It is also characterized by myriad of syndrome, diverse theories and multifactorial causes. It is a syndrome rather than a disease. Pathological hallmarks include; deposition of GPR3 protein in the brain which augment toxic amyloid plaques, abnormal deposition of tau proteins in neurons and its hyperphosphorylation into NFTs, increased levels of toxic amyloid β ($A\beta$) oligomers, neuronal and vascular death due to oxidative stress, inflammation or excessive action of the brain's immune cells-glial, among others. Currently there is no cure for it and much of the treatments have only been able to delay the progression of the disease. However, this paper attempt to provide update information on available pharmacological regimens and their effectiveness in curtailing AD as well as future therapeutic target.

Keywords: *Alzheimer's disease, Pharmacological regimen, effectiveness, future therapeutic target*

1.0 INTRODUCTION

The etiology and pathogenesis of many neurodegenerative diseases are mostly vague and shrouded in mystery, so also is Alzheimer's disease (AD). AD is a progressive, neurodegenerative disorder that mostly affects elderly people and is considered to be responsible for about 65% of all dementia in older people. About 46.8 million people worldwide are now suffering from AD, and disease prevalence is expected to affect 131.5 million by 2050 [1]. The annual incidence worldwide is estimated at 4.6 million cases which is the equivalent of one new case every seven seconds. It is a disorder whose syndromes, hypotheses and theories are multifactorial with debilitating effects and recalcitrant pharmacological management. The significance of AD is further compounded as the number of identified cases is estimated to double or triple in 2050 [1]. AD is an intractable disorder and currently there is no cure for it and much of the available treatments have only been able to delay the progression of the disease. This article is aimed to holistically review the current pharmacological interventions in ameliorating the debilitating monster called AD.

2.0 ALZHEIMER'S DISEASE AND ITS PHARMACOLOGICAL INTERVENTIONS.

2.1 CURRENT PHARMACOLOGICAL REGIMENS

2.1.1 Cholinesterase inhibitors (ChEI)

The selective degeneration of cholinergic neurons that originate in the basal forebrain projects to the cortex and hippocampus results in the loss of all known cholinergic markers, such as choline acetyltransferase (ChAT), acetyl-choline (ACh) levels and acetylcholinesterase (AChE). ACh is associated with cognition and it is the deficit of this neurotransmitter which contributes to cognitive dysfunction. The degeneration of these cholinergic neurons is attributing to amyloid fibril-induced neuronal injury, tangle formation, astrocyte phagocytic activity [2].

On the other hand, ACh is known to promote non-amyloidogenic processing and reduce tau phosphorylation by reducing the activity of protein kinase which phosphorylates tau. Therefore, disruption of cholinergic signalling may lead to a feedback loop that increases production of A β through altered APP processing, increasing phosphorylation of tau protein, thereby contributing to the progression of AD pathology [3].

Based on what has been mentioned above, restoration of the central cholinergic function via cholinesterase inhibitors significantly improved cognitive impairment and may inhibit AD progression in patients. Cholinesterase inhibitors (ChEI) act by inhibiting the enzymes (acetyl- and butyryl-cholinesterase) responsible for the breakdown of acetylcholine hence increasing its availability at the synaptic cleft. Three ChEI's are widely available and include; donepezil, rivastigmine, and galantamine. They are considered symptomatic treatments as they improve symptoms without modifying the course of the disease.

- I. Donepezil: Donepezil is a piperidine derivative that reversibly inhibits acetylcholinesterase [4].
- II. Rivastigmine: Rivastigmine is a carbamate derivative that reversibly inhibits both acetyl- (AChE) and butyryl- (BuChE) cholinesterase [5].
- III. Galantamine: Galantamine is a tertiary alkaloid agent that reversibly inhibits AChE [6].
- IV. Memantine: Memantine is a N-methyl-D-aspartate (NMDA) non-competitive glutamate receptor antagonist [7].

2.1.2 Beneficial of Anti-diabetic drugs in Alzheimer's disease state

With several studies suggesting a possible association between diabetes mellitus and Alzheimer's disease, various clinical trials are underway to establish an effective treatment strategy for Alzheimer's disease. There are controversial reports on the effects of insulin therapy on the cognitive functions of dementia patients. While some studies report that insulin therapy increases the risk of dementia in diabetes patients other studies suggest that insulin therapy helps in slowing down the cognitive decline in Alzheimer's disease patients. However, with regards to pathological

findings, a combination therapy (insulin and oral hypoglycaemic medications) was found to have positive therapeutic effects. This was evidenced by the decreased amount of the neuritic plaques in diabetes patients who received both insulin and anti-diabetic therapies.

With a possible intimate connection between the two disorders, and with a good number of studies suggesting a positive effect of anti-diabetic medications on cognitive functions, a common pharmacotherapy is expected to be effective in controlling the course and progression of these two disorders. However, researchers could not exclude the possibility that the beneficial effects of some anti-diabetic medications could be due to their intrinsic properties that are unrelated to their sugar-controlling effects.

I. Insulin Therapy for AD patients

Insulin can be administered through subcutaneous, transdermal, oral, sub-lingual, buccal, rectal, vaginal, intramuscular, intraperitoneal, and intranasal routes [8]. Intranasal delivery of insulin, as well as other compounds, is enabled by the direct neuroanatomical connections between the olfactory nerves and brain. This portal of access to the brain is advantageous because structural modifications of existing therapeutic compounds may not be required, particularly when they are small molecules. Another point is that drugs which could be used to treat neurological and psychiatric diseases may have limited therapeutic responses due to poor penetrance across the blood-brain barrier and therefore restricted ability to access the CNS. Intranasal therapy circumvents these problems while simultaneously reducing unwanted systemic responses. The discovery that nasal mucosa expresses and can deliver insulin to the brain suggest that nasal-to-brain transfer of insulin is physiological. Altogether, intranasal therapy provides a practical and noninvasive approach for treating cerebrovascular, behavioral, neoplastic, and neurophysiologic diseases, and may also enable rapid and safe delivery of neuroprotective agents. Since normal neuroanatomical connections between the olfactory and limbic systems mediate memory, cognitive function, and behavior, intranasal therapy could be used to target neurodegenerative and psychiatric diseases.

Intranasal insulin therapy provides an excellent means of supplying the brain with insulin to overcome deficits in production, transport, and responsiveness, reproducing the effects of systemic insulin but with avoidance of adverse effects. Intranasal insulin improves cognitive function with respect to attention, and verbal and hippocampal declarative memory [9, 10,11].

In addition, intranasal insulin was demonstrated to alter activity in the hypothalamus, which regulates homeostatic and reward-related functions [12]. These findings suggest that brain insulin resistance can differentially affect cognitive and metabolic signaling pathways. Intranasal insulin administration results in direct transport of insulin from the nasal cavity to the CNS via intra-neuronal and extra-neuronal pathways that bypass the blood-brain barrier and minimize systemic side effects [9]. Therefore, a major advantage of intranasal delivery is that it offers the potential

for unrestricted transfer of therapeutic compounds to the brain or CSF, enabling higher local levels to be achieved for disease-specific targeting [13].

II. Incretins

As an alternative to insulin, another promising approach is therapeutic administration of incretins, such as glucagon-like peptide-1 (GLP-1). GLP-1 is an insulinotropic peptide that is generated by cleavage of proglucagon protein, and secreted by small intestinal L cells following food intake. GLP-1 has a half-life of only a few minutes, and is rapidly degraded by dipeptidyl peptidase-4. GLP-1 stimulates insulin gene expression and secretion, and suppresses glucagon. GLP-1 lowers blood glucose in individuals with T2DM [14,15] and it restores insulin sensitivity. The latter attribute is perhaps one of the most important considerations for the potential use of GLP-1 and related molecules for the treatment of brain insulin resistance in AD.

GLP-1 stimulates neuritic growth in CNS neurons and exerts neuroprotective actions against glutamate-mediated excitotoxicity, oxidative stress, trophic factor withdrawal, and cell death [16,17]. In addition, inhibition of dipeptidyl peptidase-4, which degrades GLP-1, reduced oxidative and nitrosative stress, inflammation, memory impairment, and A β PP-A β deposits in an AD transgenic mouse model [18]. GLP-1 can cross the blood-brain barrier, and may effectively reduce brain A β PP-A β burden in AD [14,15,19]. With the realization that GLP-1 has a short half-life and therefore limited practical use for long-term therapy, synthetic long-lasting analogues of GLP-1 have been generated and proven to be effective in preserving cholinergic neuron function [20]. The development of GLP-1 receptor agonists, such as Geniposide or Exendin-4, which harbor the same neuro-protective and neuro-stimulatory properties as GLP-1 [21], but have longer half-lives [16,19,22,23], may provide effective and standardized long-term options for treating brain insulin resistance diseases such as AD.

III. Anti-hyperglycemic agents

Metformin is a biguanide anti-hyperglycemic drug that is used to treat T2DM. Metformin functions by suppressing gluconeogenesis and enhancing glucose uptake and insulin sensitivity. Metformin treatment is protective against neurological complications of T2DM, including cognitive impairment and cerebral vascular disease [24]. Although metformin treatment was found to increase the generation of both intra- and extracellular A β PP-A β due to increased expression of BACE1, suggesting that metformin and insulin may have opposing effects on A β PP-A β accumulation, the administration of both insulin and metformin provided significant neuroprotection. Importantly, the combined treatments reduced A β PP-A β levels, the severity of AD pathology, including A β PP-A β neuritic plaques, and oligomeric A β PP-A β -mediated down-regulation of the insulin receptor. These findings suggest that metformin mono-therapy may be harmful due to exacerbation of AD-type neurodegeneration [25], whereas the combined use of insulin and hypoglycemic drugs may benefit elderly patients in the early stages of AD by significantly improving cognitive performance and slowing the rate of neurodegeneration.

IV. Alpha lipoic acid (ALA)

ALA is a natural compound that supports mitochondrial function, serving as a cofactor for pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase. Importantly, ALA enhances production of acetylcholine by activating choline acetyltransferase and increasing glucose uptake [26]. Therefore, the potential benefits of ALA are mediated by the supportive actions of ALA on insulin and GLP-1. However, beyond those effects, ALA has anti-oxidant effects, since it serves as an inhibitor of hydroxyl radical formation and can scavenge reactive oxygen species and lipid peroxidation products such as 4-hydroxy-2-nonenal [26], which are increased in AD brains [27,28]. In addition, ALA inhibits expression of pro-inflammatory cytokines and inflammation-associated nitric oxide synthase, which have important roles in mediating neuro-inflammation in the early stages of AD [29, 30,31,32].

V. Familial/Genetic Predisposition and the role of Nanotechnology in AD treatment

Two genetic / hereditary factors found to predispose a person to Alzheimer's (particularly early onset Alzheimer's disease) and these are either suffering from Down's Syndrome (a genetic defect) or a family history of dementia (a genetic / hereditary condition) as it seems there is a slightly higher risk of developing the condition if a first degree blood relative (parent / brother / sister) has developed it previously. Researchers have linked at least ten percent of late onset Alzheimer's to the inheritance of a gene mutation (on chromosome 14) that directs production of apolipoprotein (ApoE) – a cholesterol carrying protein. There have also been other genetic mutations identified that may account for a predisposition to Alzheimer's and these occur on chromosomes 1, 12, 19 and 21.

One of the treatment strategies of AD remission caused by genetic predisposition is the use of nanotechnology in which encapsulated nanoparticles in the form of small interfering RNAs (siRNAs) could silence genes encoding APP and tau in cell culture models [33]. The siRNAs could also have the potential to inhibit β and γ secretases that generate $A\beta$ proteins. The siRNAs are very delicate and can get easily degraded in the physiological environment, so to maintain efficacy in vivo, the siRNAs must be delivered into the appropriate neurons. The delivery of siRNAs to neurons in the brain can be optimally achieved by encapsulating them in nanoparticles that can cross the blood brain barrier (BBB) or by expressing them in viral vectors, have demonstrated that silencing β -secretase activity using siRNAs delivered through lentiviral vectors reduced amyloid production in transgenic mice that express APP. Translational blocking of APP using antisense oligodeoxynucleotides reduced cerebral amyloid and acetylcholinesterase activity in Tg2576 transgenic mice.

2.1.4 Mitigation via inhibition of tau hyperphosphorylation and prevention of tau aggregation.

As hyperphosphorylated tau is associated with NFTs and tau-related neurodegenerative diseases, inhibition of the responsible kinases has been a top strategy for targeting tau for the prevention or treatment of AD [34]. Inhibitors of GSK3 β and CDK5 have been of particular interest. Treatment with the GSK3 inhibitor LiCl induces a reduction of tau phosphorylation has been shown to decrease tau aggregation and reduce axonal degeneration in tau transgenic mice [35].

LiCl is not selective for GSK3 β versus GSK3 α and can have many other off-target effects, making assessment of its ability to inhibit tau phosphorylation via GSK3 β problematic. However, an apparently selective GSK3 inhibitor AR-A014418 was also found to reduce tau phosphorylation, filamentous tau, and axonal degeneration after oral administration for one month in tau transgenic mice [35]. An analog of AR-A014418 entered into clinical trials for AD in 2007, but its development has since been discontinued. Another selective GSK3 inhibitor, Nypta (Tideglusib), is noncompetitive for ATP [36] and appears to be well tolerated and to improve cognitive performance in a 4–6-week clinical trial [37].

2.1.5 Tau clearance

Stimulating the natural ability of cells to clear away misfolded or aggregated proteins has been another strategy for targeting aberrant tau. One specific approach is by enhancing degradation of tau by the ubiquitin-proteasome system. Misfolded proteins are tagged with chains of ubiquitin that enable interaction with and breakdown by the proteasome. This process can apparently be stimulated with inhibitors of the ATPase activity of heat shock protein (HSP) 90, a molecular chaperone involved in protein refolding. Inhibition of this HSP90 activity results in increased expression of HSP70, which together with CHIP (the carboxyterminus of HSP70-interacting protein) is involved in the ubiquitination of misfolded proteins [38]. Knockout of CHIP in mice results in elevated phosphorylated tau, suggesting that HSP70/CHIP is involved in the degradation of this particular form of tau [39]. Consistent with this notion, HSP90 inhibitors increase proteasomal degradation of phosphorylated tau through HSP70/CHIP in cell culture [39,40] and decrease hyperphosphorylated tau in tau transgenic mice [41].

Toward the identification of small-molecule stimulators of the ubiquitin-proteasome system to enhance the clearance of deleterious proteins, the discovery that a proteasome-associated deubiquitinating enzyme, USP14, inhibits proteasomal degradation of proteins by trimming off ubiquitin chains inspired a high-throughput screen to find inhibitors of USP14 activity [42]. The hit compound that emerged from this screen, dubbed IU1, reversibly inhibited USP14 and did so selectively over other deubiquitinating enzymes. IU1 could also stimulate the degradation of tau in a concentration-dependent manner in cell culture, and this tau degradation required USP14 and active proteasome complexes. The degradation of another protein implicated in neurodegeneration, TDP-43, was likewise enhanced by IU1, while ubiquitin-independent substrate of the proteasome was not affected.

Monomeric, misfolded tau can be degraded by the proteasome, but aggregated forms of tau cannot, as such aggregates cannot pass through the proteasome pore. However, protein aggregates as well as whole intracellular organelles can be cleared through a process called autophagy (literally, self-eating, [43]. In one type of autophagy (macroautophagy) protein aggregates or organelles are enveloped into vesicles that then fuse with lysosomes for degradation. Some evidence suggests that reduction of this process can lead to neurodegeneration [44] and that enhancement of autophagy can help clear out aggregated proteins associated with

neurodegeneration [45]. The immunosuppressant drug rapamycin can enhance macroautophagy and prevent plaques, NFTs, and cognitive deficits in an AD mouse model [46]. However, the safety of this agent for long-term treatment of AD in humans is a serious concern. Recent results suggest that trehalose also activates macroautophagy, reduces insoluble tau, and is neuroprotective in a tau transgenic mouse model [47], although trehalose has multiple biological effects and the safety of this agent at chronic pharmacological doses is likewise unclear. Other means of pharmacologically increasing autophagy that are more specific may be required, but even then, the consequences of chronic stimulation of autophagy are unclear.

One possibility process in which antibodies prevent tau pathology is that they enter neurons through endocytosis, although there is no evidence that this occurs, and even if it did, the antibodies would have to somehow escape the endocytic vesicles to gain access to tau in the cytoplasm. Another possibility is that tau is secreted from neurons to some degree and that this allows the spread of tau pathology from neuron to neuron possible. In this scenario, anti-tau antibodies would interact with tau at synapses and intercept misfolded or aggregated tau before it is taken up by the neighboring neuron. Consistent with this idea is the fact that tau is found in the CSF and is considered a promising biomarker for AD [48].

2.2 PHARMACOLOGICAL REGIMENS IN CLINICAL TRIALS

2.1.1 BACE1 inhibitors

1. Small molecule BACE1 inhibitor: LY2811376

The pharmaceutical company Eli Lilly was among the first to develop and test orally bioavailable non-peptidic BACE1 inhibitors in humans. The small molecule BACE1 inhibitor LY2811376 showed satisfactory pharmacokinetic and pharmacodynamic characteristics in preclinical animal models that translated to a Phase 1 clinical trial in humans [49]. However, chronic toxicology studies in rat showing non-clinical non-target associated pathology in retina and brain precluded the clinical development of this molecule. Although it was discontinued, LY2811376 demonstrated the feasibility of developing a potent brain-penetrant orally bioavailable small molecule BACE1 inhibitor and represented the first reported translation of reduced CSF biomarkers of BACE1 cleavage from preclinical animal models to humans.

Lilly advanced a next-generation compound, LY2886721, into Phase 1 and 2 clinical trials to determine its safety and tolerability, pharmacokinetics, and pharmacodynamics. Similar to LY2811376, LY2886721 was a potent orally bioavailable small molecule BACE1 inhibitor that robustly decreased levels of A β in the brains of preclinical animal models. However, unlike LY2811376, treatment with LY2886721 did not appear to be toxic to the retina or brain. Forty-seven healthy volunteers were given daily oral doses of either LY2886721 or placebo for 14 days in Phase 1 [50]. Either a multiple ascending dose (5, 15, and 35 mg) or a single dose (70 mg) followed by a multiple ascending dose was performed in two Phase 1 study designs (NCT01227252, NCT01534273). Over the course of the 14-day study, LY2886721 was reported to be safe and well tolerated. Plasma half-life of LY2886721 was ~12 hours, compatible with once

per day dosing. Dose-dependent decreases of both plasma and CSF A β 40 levels resulted from LY2886721 administration. A β 40 levels in the CSF were decreased up to 74% with the highest dose of LY2886721. Levels of A β 42 and sAPP β in the CSF were both reduced to a similar extent as CSF A β 40 by LY2886721. Interestingly, levels of sAPP α , the α -secretase cleavage product, were increased in CSF [51], an observation that is consistent with BACE1 inhibition, since β - and α -secretase compete for processing of APP. A Phase 1 study of LY2886721 in patients with AD was also conducted (NCT01807026).

The positive Phase 1 trials led to a six-month Phase 2 trial of 35 or 70 mg of LY2886721 dosed orally once per day in 130 patients with prodromal AD, also known as amnesic mild cognitive impairment (MCI), or mild AD (NCT01561430) [52]. Recently, Lilly voluntarily terminated the Phase 2 trial because a small number of subjects that were given LY2886721 developed abnormal liver biochemistries. The company reported that the LY2886721-related liver abnormalities did not appear to be associated with the BACE1 mechanism of action, a conclusion supported by a normal liver phenotype of BACE1 $-/-$ mice. It is not uncommon that some small molecules in clinical development are discontinued because of abnormal liver function as a non-target related side effect. Thus, the termination of LY2886721 does not necessarily suggest that BACE1 is not a viable drug target.

II. Small molecule BACE1 inhibitor: Mk-8931

Mk-8931, a small molecule BACE1 inhibitor developed by the pharmaceutical company Merck was tested in 88 healthy volunteers (18–45 years old) as a two-part randomized, double-blind, placebo-controlled Phase 1 clinical trial [53]. Single and multiple (daily for 14 days) oral doses of MK-8931 were analyzed for safety, tolerability, pharmacokinetics, and pharmacodynamics. In healthy volunteers, MK-8931 was well tolerated and no serious adverse events were reported. Determining whether MK-8931 was able to enter the brain and engage its target, the β -secretase enzyme, were primary goals of the study. To do so, biomarkers of BACE1 activity were measured in the CSF, including A β 40, A β 42, and sAPP β , the latter being the BACE1-cleaved ectodomain of APP. MK-8931 markedly reduced levels of A β in the CSF in a sustained and dose-dependent manner. A single oral dose of 100 or 550 mg of MK-8931 decreased CSF A β 40 levels by 75% or 92%, respectively, at 36 hours after dosing. Levels of A β 42 and sAPP β in the CSF were also reduced to similar extents. Multiple oral dosing of MK-8931 lowered A β levels in the CSF by over 90%. MK-8931 has a plasma half-life of ~20 hours, suggesting that a single daily oral dose may maintain stable drug levels in vivo.

A randomized, double-blind, placebo-controlled Phase 1b trial of MK-8931 in 32 mild to moderate AD patients (mean age and Mini-Mental State Examination (MMSE), 73 yrs. and 22, respectively) was also conducted for safety, tolerability, pharmacokinetics, and pharmacodynamics (NCT01496170) [54]. One of three doses (12, 40, or 60 mg) of MK-8931 or placebo was given once each day orally for 7 days and levels of A β 40, A β 42, and sAPP β in the CSF were measured. Similar to the healthy volunteers, MK-8931 strongly decreased levels of A β in the CSF in a sustained and dose-dependent fashion. Daily dosing of 12, 40, or 60 mg reduced CSF A β 40 by 57,

79, or 84%, respectively, and resulted in similar reductions for CSF A β 42 and sAPP β . MK-8931 did not appear to cause serious adverse events in the AD patients during the course of the study. Importantly, the MK-8931 Phase 1b results suggest that the pharmacokinetic and pharmacodynamic properties of BACE1 inhibitor drugs are not significantly altered by the presence of high amyloid loads in the brains of AD patients.

Encouraged by the positive results of the MK-8931 Phase 1 and 1b studies, a Phase 2/3 combined clinical trial (the EPOCH study, NCT01739348) was started in late 2012. EPOCH is a 78-week, randomized, placebo-controlled, parallel-group, double-blind clinical trial to evaluate the safety and efficacy of 12 or 40 mg/day oral dosing of MK-8931 versus placebo in mild to moderate AD patients. In Phase 2 the trial will evaluate 200 AD patients and will enroll up to 1,700 patients for Phase 3. Primary efficacy outcomes are the changes from baseline in the Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog) and the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) scores.

A recent interim safety analysis in 200 AD patients treated with MK-8931 for at least 3 months suggested that the drug was well tolerated and that the EPOCH study proceed without changes to the protocol. Enrolment in the trial has continued with up to 1960 patients expected for Phase 3. An additional clinical trial (the APECS study, NCT01953601) has also commenced, consisting of a 104 week randomized, placebo-controlled, parallel-group, double-blind Phase 3 study to evaluate the safety and efficacy of 12 mg or 40 mg per day oral dosing of MK-8931 versus placebo in 1500 patients with MCI. The primary efficacy outcome in APECS is the change from baseline in the Clinical Dementia Rating Scale-Sum of Boxes (CDR-SB) score. Secondary outcome substudies are included in both EPOCH and APECS to measure AD biomarkers, consisting of cortical amyloid load, CSF A β and tau, and hippocampal volume. The Phase 3 efficacy studies for EPOCH and APECS are expected to conclude in 2017 and 2018, respectively.

III. Small molecule BACE1 inhibitor: AZD3293

The AstraZenica BACE1 inhibitor, AZD3293, was recently tested for safety, tolerability, pharmacokinetics, and effects on plasma and CSF A β levels in healthy young (18–55 yr) and elderly (55–80 yr) subjects (Alexander). Phase 1 randomized, double-blind; placebo-controlled SAD and MAD studies were conducted. In the SAD study (NCT01739647), 1 to 750 mg doses of AZD3293 were administered to 7 young cohorts, while an elderly cohort received 15 mg (8 subjects/cohort). In the MAD study (NCT01795339), multiple once-daily doses of AZD3293 ranging from 15 to 70 mg were administered to 5 cohorts (two elderly) for 2 weeks. AZD3293 was well tolerated with no serious adverse events reported up to the highest dose given (750 mg) in the SAD study. The half-life of AZD3293 was 11–20 hours and thus compatible with once daily dosing. Pharmacokinetic parameters of AZD3293 between elderly and young subjects were indistinguishable. In the MAD study, the 15 or 50 mg doses reduced CSF A β 40 and A β 42 concentrations by a constant 50 or 75%, respectively. Additionally, AZD3293 administration produced dose-dependent decreases and increases of sAPP β and sAPP α concentrations in the CSF, respectively, that had similar timelines as the reductions in CSF A β 40 and A β 42 [55]. The Phase 1

studies of AZD3293 in health subjects (NCT01739647) and AD patients (NCT01795339) have been completed, and combined Phase 2/3 trials in 1,551 MCI and mild AD patients are planned (20 mg or 50 mg doses, 104 week duration, AMARANTH trial (NCT02245737)). Recently, AstraZenica and Lilly entered into a partnership to jointly develop AZD3293 for AD.

IV. Small molecule BACE1 inhibitor: E2609

The drug company Eisai has developed an orally bioavailable small molecule BACE1 inhibitor, E2609, that has shown robust lowering of cerebral A β in preclinical and clinical studies. E2609 was first clinically studied in healthy volunteers in randomized, double-blind, placebo-controlled Phase 1 trials [56,57]. A single oral ascending dose (SAD) study (73 subjects) and a 14-day multiple oral ascending dose (MAD) study (50 subjects) tested E2609 in two separate Phase 1 clinical trials (NCT01294540 and NCT01511783, respectively). The SAD study analyzed plasma A β levels following E2609 administration ranging from 5 to 800 mg (9 cohorts), while the MAD study measured both plasma and CSF A β levels in response to E2609 doses ranging from 25 to 400 mg (5 cohorts). The E2609 plasma half-life of 12–16 hours is compatible with once per day dosing. Each of the two Phase 1 studies showed robust dose-dependent decreases of levels of A β in the CSF and/or plasma. CSF A β levels were reduced up to 85% at the highest dose of E2609 (400 mg) in the MAD study. Similar decreases in levels of sAPP β in the CSF were observed, while CSF levels of sAPP α were increased. E2609 appeared to be safe and well tolerated, as no serious adverse events were reported in either Phase 1 study. Eisai has recently completed a Phase 1 trial of E2609 in subjects with MCI or mild AD (NCT01600859), and a Phase 2 clinical trial of E2609 is planned. Recently, Eisai and Biogen Idec entered into a partnership to jointly develop E2609 for AD.

Although small molecules that directly inhibit BACE1 enzyme activity are leading therapeutic approaches, potential alternative strategies to reduce BACE1 processing of APP are being explored. As noted, BACE1 levels are significantly elevated in AD brain and might accelerate the production of A β . Therefore, approaches to lower and normalize BACE1 levels in the brain might slow AD progression and avoid possible untoward side effects caused by direct BACE1 enzyme inhibition. Consequently, efforts are underway to elucidate the mechanisms of BACE1 elevation in AD in order to identify drug targets that could block the BACE1 increase when inhibited. BACE1 undergoes complex regulation at the transcriptional, translational, and post-translational levels, all of which appear to have a role in elevating BACE1 levels and activity in AD [58, 59]. Much evidence suggests that BACE1 is a stress response protease that is increased by oxidative stress, inflammation, hypoxia, and trauma, among other insults that occur in AD [60, 61, 62]. Even A β itself increases BACE1 levels in neurons [63, 64], suggesting a vicious pathogenic cycle whereby A β could accelerate its own production through BACE1 elevation. Which, if any, of these complex multi-layered regulatory mechanisms might yield therapeutic strategies for lowering BACE1 levels in AD is unclear, but continuing research in this important area may reveal promising new AD drug targets in the future.

Another class of alternative therapeutic strategy for BACE1 inhibition involves immunotherapy approaches to reduce BACE1 processing of APP. The first of these strategies employs antibodies directed against the β -secretase cleavage site of APP that sterically block access of the BACE1 active site to APP [65]. These anti- β -site APP antibodies decrease A β production cultures cells and when injected i.v. reduces amyloid plaque pathology in the brains of APP transgenic mice [66]. Other immunotherapy approaches include anti-BACE1 antibodies that are not directed against the active site but instead target an exosite on the surface of the BACE1 catalytic domain that can allosterically regulate enzyme activity [67]. This exosite is located on structurally adjacent regions of the C, D, and F loops of the enzyme [68]. Exosite antibody binding to BACE1 alters structural features and dynamic characteristics near the substrate binding cleft of the enzyme. Additionally, transport of BACE1 antibodies across the BBB has been facilitated by engineering one arm of the antibody to recognize transferrin receptor (TfR), which shuttles transferrin across the BBB for the delivery of iron into the brain [69,70]. These bispecific BACE1-TfR antibodies accumulate in the brain and reduce endogenous A β levels in mice to a much greater extent than monospecific BACE1 antibodies. Moreover, TfR bispecific antibodies could be useful for treating other neurologic diseases amenable to immunotherapy. These antibody approaches are currently in preclinical phases.

2.1.5 Tau aggregation or assembly inhibitors (TAAI)

The assembly of tau into neurotoxic oligomers or fibrils makes blocking tau-tau interactions a reasonable strategy for therapeutic intervention. A number of screens in search of compounds that inhibit tau self-assembly have been carried out, and a variety of compound types have been identified, including phenothiazines, porphyrins, polyphenols, cyanine dyes, aminothienopyrazines, anthraquinones, phenothiazolyl hydrazides, and rhodanines [71].

The most advanced tau assembly inhibitor is the phenothiazine dye methylene blue (Rember). In 2008, this compound was reported to slow cognitive decline in a small Phase II clinical trial over almost one year, although this result was complicated by the fact that the highest dose led to lower than expected drug exposures, and no evidence for target engagement was reported [72].

A study in a tau transgenic mouse model, however, showed that very high doses that lowered soluble tau levels also resulted in cognitive improvement [73]. While a Phase III trial would help clarify the efficacy of methylene blue in humans, no such trial has yet been initiated. However, a second-generation methylene blue analog, LMTX, is allegedly in line for a Phase III trial.

2.3 FUTURE PHARMACOLOGICAL THERAPEUTIC TARGET

2.3.1 Genetic deletion of GPR3: A new concept to halt the progression of Alzheimer's disease

Study by Huang *et al* [74] revealed a protein that appears to be a key to the development of Alzheimer's disease and this according to the researchers might provide a new drug target for dementia. Huang *et al* [74] found that the deletion of the G protein-coupled receptor 3 (GPR3) gene, a protein expressed in the brain, alleviated the cognitive deficits and reduced amyloid

pathology is a potential AD therapeutic target and provides the validation needed for future development of GPR3 modulators.

2.3.2 Modulation of β - and γ -secretases: A potentials therapeutic Target for AD intervention

Alzheimer's disease (AD) is characterized by extraneuronal deposition of the amyloid β protein in the form of plaques and the intraneuronal deposition of the microtubule-associated protein in the form of filaments. Amyloid β ($A\beta$) peptide is a major components of senile plaques formed as a result of proteolytic cleavage of amyloid precursor protein (APP) by the secretases. According to the β -amyloid theory, it has been proposed that $A\beta$ peptide deposits or even the partially aggregated soluble form are responsible for triggering a neurotoxic cascade of events which ultimately results in neurodegeneration [75]. The extracellular accumulation of amyloid plaques composed of the β -amyloid ($A\beta$) peptide represents one of the two defining lesions in Alzheimer's disease (AD) brain, the other being intracellular aggregation of hyperphosphorylated tau into neurofibrillary tangles. Results indicate that amyloid deposition begins ~10-20 years before the onset of dementia, suggesting that cerebral accumulation of $A\beta$ has as a critical early role in AD pathogenesis [76].

If so, then inhibition of $A\beta$ accumulation in the brain may benefit AD, if given early enough during the course of the disease. Neurons are the major producers of $A\beta$ in the brain, although glia, in particular astrocytes, may also contribute to $A\beta$ generation, particularly during physiological stress that causes glial activation as happens in AD. The formation of $A\beta$ is a sequential proteolytic process beginning with the cleavage of amyloid precursor protein (APP) by the β -secretase enzyme, which generates the amino (N) terminus of $A\beta$ and yields the membrane bound C-terminal fragment C99 [77]. γ -secretase cuts C99 to release $A\beta$, which is secreted from the cell [78,79]. Interestingly, the γ -secretase cut is imprecise and creates $A\beta$ isoforms of different lengths at the carboxy (C) terminus, of which the longer isoforms are highly associated with AD. Processing of APP by both β - and γ -secretases is necessary for the generation of $A\beta$, suggesting that inhibition or modulation of either or both of these proteases in the brain should decrease $A\beta$ levels and be beneficial for AD.

CONCLUSION

In the meantime, however, one or more of the current approaches may eventually bear fruits and bring light at the end of the tunnel in the form of an approved drug for AD especially the very latest discovery involving genetic deletion of GPR3 protein. The protein thought to be largely responsible for the buildup of beta amyloid plaques in the brain as its removal result in robust cognitive improvement and reduced signs of dementia in the brain so these efforts should continue. Unstinting, unalloyed, and unflinching support towards the ongoing search for an effective means of targeting tau should be sustained and possibly invigorated as the world awaits a cure for this insidious, intractable but surmountable monster called AD.

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