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ALZHEIMER'S DISEASE AND TREATMENT

Treating Alzheimer's disease pathology: Present to alternative modalities

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Key words: Alzheimer's disease; Neurodegeneration; Amyloid Beta; Neurofibrillary Tangles.

Abbreviations: AD: Alzheimer's Disease; Aβ: Beta Amyloid; APP: Amyloid Precursor Protein; IL-1β: Interleukin-1 Beta; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor-Alpha; sAPPα: soluble Amyloid Precursor Protein Alpha; CTFα: C-Terminal Fragment Alpha; AICD: Amyloid Precursor Protein Intracellular Domain; sAPPβ: soluble Amyloid Precursor Protein Beta; NMDA: N-methyl-D-aspartate; Ach: Acetylcholine; CSF: Cerebrospinal Fluid; GSH: Glutathione; ROS; Reactive Oxygen Species; LRP1: LDL Receptor-Related Protein 1; CNS: Central Nervous System; BBB: Blood Brain Barrier; SOD: Superoxide Dismutase; NAC: N-Acetyl-Cysteine; CoQ10: Coenzyme Q10; RCC: Respiratory Chain Complexes; MitoQ: Mitoquinone Mesylate; TPP: Triphenylphosphonium; MAO: Monoamine Oxidase; MTDL: Multi-Target-Direct Ligand; HRT: Hormone Replacement Therapy; WHIMS: Women's Health Initiative Memory Study; RA: Trans-Retinoic Acid; SIRT1: Sirtuin-1; BACE1: Beta-Site Amyloid Precursor Protein Cleaving Enzyme1; PS : Presenilin; APH1: Anterior Pharynx Defective 1; PEN2: Presenilin Enhancer 2; GSMs: Gamma Secretase Modulators; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; FC: Fragment Crystallizable; NFTs: Neurofibrillary Tangles; PHF: Paired Helical Filaments; GSK3: Glycogen Synthase Kinase-3; CDK5: Cyclin Dependant Kinase 5; MAPKs: Mitogen Activated Protein Kinase; PP5: Phosphatases 5; AVs: Autophagy vacuole's; fAD: Familial AD; mTOR: Mammalian Target Of Rapamycin; TFEB: Transcription Factor EB; Bcl2: B-cell lymphoma 2

Abstract

The global prevalence of dementia including Alzheimer's disease (AD) is estimated to reach 44 million people. AD is the most common neurodegenerative disorder described by the presence of extracellular amyloid plaques, intracellular hyperphosphorylated tau protein and neuronal loss as main hall marks of the disease. Clinically AD is recognized by the failure of memory and cognitive decline that become severe with the progression of the disease. While most of the cases of AD are sporadic and occurs over the age of 65 years, the disease may also develop in autosomal dominant familial manner and affects the relatively young population. The disease begins due to increased level of oxidative stress which is a hallmark of aging and affects many cellular processes at macromolecular level including alteration of lipids, proteins and DNA by peroxidation, oxidation and methylation. AD may also involve changes in the expression of proteases, contributes to the non-amyloidogenic and amyloidogenic pathways and cleaves the amyloid precursor protein into the soluble non-disease causing and the insoluble disease causing components. FDA approved pharmacological interventions against the disease pathology are acetylcholinesterase inhibitors and NMDA receptor antagonist, both of which are not successful and giving positive results in changing the disease pathology, rather only provide symptomatic treatment. To combat with the disease, now there is a need to focus on factors that can potentially modify the disease pathology or delay the process of disease progression. Several molecular targets such as amyloid peptide, tau protein, neuroinflammation and oxidative stress have also been observed in advanced stage of AD. Therefore, researchers are considering different aspects of disease treatment including amyloid and tau immunotherapy, autophagy, antioxidant and anti-inflammatory therapy, inhibition of tau kinases, secretases modulation and others. Among them we have discussed some of the major therapeutic options that are currently in consideration and for which clinical trials are being going on.

Introduction

Alzheimer's Disease (AD) is the most common neurodegenerative disease defined by the formation of extracellular β-amyloid plaques and intracellular neurofibrillary tangles along with progressive failure of memory and cognition that increases with the increasing concentration of Beta Amyloid (Aβ) and ultimately leads to damaging neuronal processing, tau protein hyper phosphorylation and damage to synaptic processes. The disease usually occurs sporadically but is also inherited in autosomal dominant manner. The onset usually occurs after the age of 50-55 years and the incidence of the disease increases with increasing age, while about half of the early cases are familial. The disease starts with failure of memory and progresses to such a large extent that the patient becomes bedridden and cannot even recognize his environment.

The plaques in the disease are formed by the aggregation of 39-42 amino acid peptides named Aβ produced by the cleavage of Amyloid Precursor Protein (APP). These plaques are surrounded by astrocytes and microglia, the later when activated by the presence of amyloid plaques release inflammatory cytokines like IL-1β, IL-6 and TNF-α. After cleavage of APP the generated Aβ peptide forms oligomers, protofibrils and fibrils by means of self-aggregation. The Aβ oligomers and not the monomers or fibrils play role in disease pathogenesis and cognitive decline.

Aβ is produced from the breakdown of APP by secretase enzymes. APP is cleaved by secretases through two different pathways; the non-amyloidogenic pathway and the amyloidogenic pathway. The non-amyloidogenic pathway yields soluble Amyloid Precursor Protein Alpha (sAPPα) and C-Terminal Fragment Alpha (CTFα) by the action of α-secretase. Later gamma-secretase acts on CTFα and releases P3 and Amyloid Precursor Protein Intracellular Domain (AICD). The amyloidogenic pathway first releases soluble Amyloid Precursor Protein Beta (sAPPβ) by the action of β-secretase and then releases Aβ and AICD by means of γ-secretase.

According to the report of Alzheimer's News Today there is a rapid increase in the number of patients suffering from AD and among them only one in four people with the disease gets diagnosed. Globally 44 million people are estimated to be living with AD or related dementia. In the United States, around 5.5 million people of all ages have AD. Among them, approximately 5.3 million are older than 65 and 200,000 are younger, having early-onset AD [1].

Existing pharmacological management of AD

The Food And Drug Administration (FDA) has approved six drugs to be used in clinical setups for the treatment of AD which include inhibitors of acetylcholinesterase; Donepezil, Rivastigmine, Galantamine and tacrine (discontinued) that point to stabilize the levels of Acetylcholine (Ach) in the synaptic cleft to maintain optimum neurotransmission due to the hypothesis that cholinergic abnormalities during the aging process leads to the development of AD [2-4], memantine; that blocks the N-Methyl-D-Aspartate (NMDA) receptor and the excess of excitatory glutamatergic activity and the combination therapy with memantine and donepezil. Acetylcholine and NMDA receptors are central to the process of memory and learning and in the course of AD and their functions and concentrations are critically compromised.

Since aforementioned treatment options improve only the memory and cognitive functions, without actually slowing down the disease progression and effectiveness of these drugs may vary from patient to patient [5], there are many factors that contribute to the struggle of developing effective treatments for AD. These factors are: failures in clinical studies, gaps in understanding about the precise biological processes and molecular changes in the brain that cause the disease, and long observational time period to investigate the treatment effects.

Alzheimer's disease is still not completely curable and also effectively lacks a logical understanding of the principal event that is triggering the disease. However, a better comprehension of this deadly disorder and the advancement of successful treatments are crucial not only to cure the disease but also to prevent or halt the emerging symptoms of the disease. Keeping these points in mind we have here discussed the possible therapeutic approaches (**Figure 1**) that aimed to modify AD pathology.

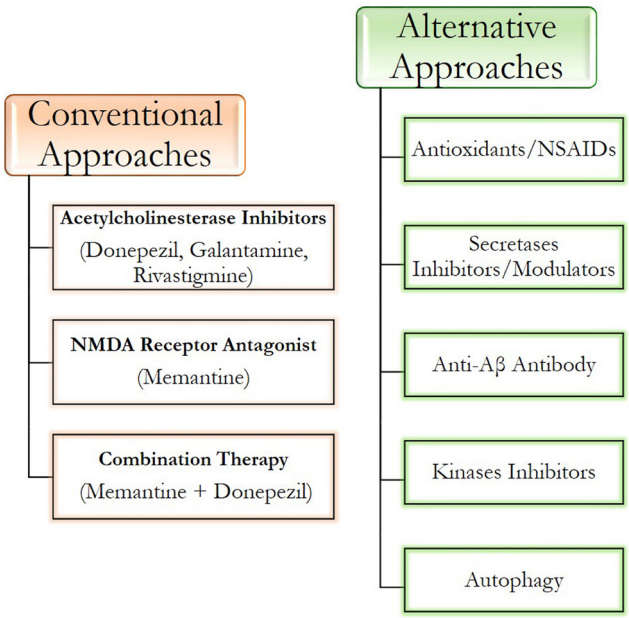

Figure 1: Conventional and alternative approaches againast Alzheimer's diseases.

Linking oxidative stress with AD

With decades of emerging research, it is suggested that neurological tissues of AD patients are subjected to oxidative stress during disease progression. Damages such as protein oxidation, glycoxidation and DNA oxidation are closely associated with the development of AD [6]. Therefore, oxidative stress refers to disproportion among the intracellular production of reactive oxygen species and antioxidant defense processes [7-9]. With the age, there is a decline in capacity of neurons to compensate for reactive oxygen species removal, in such situations a slight cellular stress can results in irreversible injury that provokes the pathogenesis of neurodegenerative disorders [10,11].

The main manifestations of oxidative stress that contributes to damage in AD includes high levels of protein, lipid and advanced DNA oxidation, end products of glycoxidation, deposition of toxic substances for example alcohols, peroxides, cholestones, aldehydes, ketones, free carbonyls and oxidative modulations in nuclear and mitochondrial DNA [12]. The aforementioned elevated oxidative modifications were also measured in Cerebrospinal Fluid (CSF), urine and blood in addition to brain of AD patients [12,13].

A measurable decrease in brain and plasma antioxidants defense mechanisms correlates with age-related memory impairments. Glutathione (GSH) is counted as one of the important aspect of antioxidant defense system which controls the endogenous redox potential in cells [14]. It works by donating electrons to Reactive Oxygen Species (ROS) in order to scavenge them. With increasing age, intracellular concentration of GSH decreases in hippocampal regions of mammalian brain [15,16] that exceeds the ROS production from that of removal thus generates oxidative stress. Therefore, the cause of oxidative stress in AD is due to the imbalance between the radical detoxifying enzymes.

Oxidative stress is thought to occur during early stages of AD, which confirms its role in relation with Aβ presence [17]. Additionally, higher levels of Aβ1-40 and Aβ1-42 have been found in AD cortical and hippocampal regions, which are associated with the elevated levels of oxidative products of lipids, proteins and nucleic acids [18]. In comparison, relatively low levels of oxidative stress markers are reported in the brain regions with low levels of Aβ such as cerebellum [19-21]. Redox proteomics identified the oxidized proteins in early state of the disease and confirmed the oxidation of lipids and proteins in brain niches which have Aβ abundance [22]. It is hypothesized that LDL Receptor-Related Protein 1 (LRP1) protein is oxidized by the Aβ, which results in the accumulation of the neurotoxic Aβ peptide in the brain. LRP1, being the multifunctional protein is responsible for the Aβ efflux from the brain to the blood, beyond the blood brain barrier [23,24]. In AD, Aβ decreases the LRP1 activity by oxidation, [25] thus disrupting its own clearance [26]. Such alteration in the clearance of Aβ leads to an accumulation of Aβ in the brain, which aids in the pathogenesis of AD [27].

Hyperphosphorylated tau is more resistant to proteolytic degradation, which is counted as a major factor in the neurofibrillary degeneration in AD pathology [28,29]. Tau aggregation in fact is an adaptive change taken by neurons to respond to oxidative stress [30-33]. Modifications in Protein tau conformation can also be induced by the 4-HNE (a lipid peroxidation product), which also backs the oxidative stress involvement in disease progression by advancing the formation of neurofibrillary tangles [34].

Antioxidant therapies

One of the promising therapeutic strategies for AD is antioxidants. The disrupted oxidant/antioxidant balance in AD is convinced by evidence and has lead us to the supposition that compounds that have potential to scavenge free radicals or can boost oxidative stress defense mechanisms might be utilized as a therapeutic target for AD. Therefore, several antioxidant molecules have been tested for their improved cognitive actions in clinical studies [35]. Despite successful results of compounds on Aβ pathology or cellular effects in preclinical studies, still the considerable proof of therapeutic potential in humans is missing.

Vitamins and carotene

α-Tocopherol (Vitamin E) is among the key lipid soluble antioxidant, that inhibits the membrane lipid oxidation *in vitro* and the growth of oxidative metabolites induced by Aβ peptide neurotoxicity [36,37]. Array of genes that have direct or indirect link with Aβ clearance are also affected by vitamin E [38]. Vitamin E in therapeutic levels may cross the Blood Brain Barrier (BBB) in the Central Nervous System (CNS) where it has the ability to slow the lipid peroxidation processes [39]. Furthermore, it has been reported through studies done on >4000 individuals, that the levels of memory impairment are directly consistent with the circulating levels of vitamin E, however no such correlations have been found with vitamin A, C, selenium and β-carotene [40]. Several studies support the role of long term supplementation of vitamin E and C with the improvement of cognitive functions and prevent oxidative damage to brain [41].

So far, protection offered by vitamin E in AD patient is still controversial. Despite the available data that vitamin E reduces the plaque burden and lipid peroxidation and potentiates the proteolytic degradation of intracellular and extracellular Aβ [42]. Study conducted in 2009 reported that vitamin E was unable in reducing the plasma levels of oxidative stress in half of the AD patients [43]. Brewer in 2010 proposes the theory behind the ineffectiveness if vitamin E in three main points, in spite of the fact that oxidative stress is triggering the symptoms. Primarily, accumulation of the oxyradical on to the other lipid, instead of oxidized conjugated lipid, due to the unbalanced monotherapy of vitamin E which will be the cause of membrane damage. Therefore, water soluble electron acceptors such as vitamin C, must be included to support vitamin E in systemic removal of ROS. Next, the inappropriate dosage timing of vitamin E might be responsible for the failure of therapy. In the patients with mild cognitive impairment or clinical AD, the synaptic loss and formation of neurofibrillary tangles are irreversible pathological alterations. Treating with vitamin E at this stage would not be beneficial in improving the cognition. Lastly, high-dosage vitamin E is reported to raise mortality rate. Therefore, individualized therapy of vitamin E should be considered, to attain a less oxidized redox amount in plasma, as a non-standard dose may be counterproductive [44].

Vitamin B12 in addition to vitamin E and C may also have some part in the AD treatment [45]. Research studies showed the low levels of vitamin B12 and high serum folate in plasma of cognitive impairment patients [46]. Many evidences proved the elevation of choline acetyltransferase in cholinergic neurons and restoration of cognitive functions in AD individuals after supplementation with vitamin B12 [47]. Hence, it is concluded that vitamin B12 can also serve in multiple antioxidant therapeutic strategies.

Carotenoid is a lipid soluble antioxidant which may hinders lipid peroxidation and improves oxidative status [48]. β-Carotene is one of the most familiar and studied carotenoid which acts as a potent antioxidant that can slake singled oxygen instantly [49].

Antioxidant enzymes

Enzymatic antioxidant systems and cellular molecules are endogenous defense mechanisms that protects against the deleterious effects of free radicals. The three primary enzymes that are responsible for the direct elimination of active oxygen species (superoxide radical and H_2O_2) are Superoxide Dismutase (SOD), catalase and glutathione peroxidase. Additionally, the secondary enzymes like glutathione reductase, systolic GST and glucose-6-phosphate dehydrogenase that functions to reduce peroxide levels and maintain a balanced supply of metabolic intermediates such as GSH and NADPH for optimal working of the primary oxidant enzymes that are stated above [50,51].

The balanced supply of GSH is most significant component that directly scavenges ROS and plays a major role in metabo-

lism of xenobiotics. GSH is exhausted due the increase exposure of xenobiotics in the neutralization process and therefore its efficiency is compromised to serve as an antioxidant. Moreover, GSH is also significant in preserving α-tocopherol and ascorbate in their reduced form so they may work as antioxidants to quench free radicals [52-54].

Mitochondria-targeted antioxidants (MTAs)

Dysfunctioning of mitochondria and compromised energetic metabolism are two key features of AD pathology. Mitochondria can be targeted in two ways i.e., by directly targeting mitochondria through pharmacological approaches or by indirectly hitting the organelle by action on the lifestyle. In this section we will discuss the most popular mitochondrial treatments that are utilized for AD patients these days [55].

Lipoic acid

Lipoic acid is a naturally occurring cofactor present for the mitochondrial enzymes such as α-ketoglutarate dehydrogenase and pyruvate dehydrogenase. Lipoic acid has exhibited several different properties which aids to interfere with the progression and pathogenesis of the AD. For example, lipoic acid strongly chelates with redox-active transition metals, consequently halting the formation of hydroxyl radicals and additionally it also scavenges reactive oxygen species, as a result elevating the levels of reduced glutathione. Moreover, lipoic acid has potential to down-regulate the expression levels of redox-sensitive proinflammatory proteins which includes inducible nitric oxide synthase and TNF. Also, lipoic acid can quench products of lipid peroxidation like acrolein and hydroxynonenal. Research suggests that lipoic acid can be given with natural compounds for example epigallocatechin from green tea, curcumin etc. to additively reduce inflammation, oxidative stress, Aβ levels and plaque load, thereby gives a synergistic benefit in the AD treatment [56,57]. Based on evidences, it can be concluded that Lipoic acid may target the mitochondria which is the most altered organelle involved in AD pathology [58].

N-acetyl-cysteine

N-Acetyl-Cysteine (NAC) has shown to be the precursor of endogenous antioxidant GSH, a primary molecule for the mitochondrial functions maintenance and can also effectively crosses the BBB [59,60]. NAC is the source of cysteine, which is the rate limiting step in the synthesis of glutathione. NAC serves as an antioxidant by boosting GSH levels and interacting directly with the free radicals [61]. Studies also proved ability of NAC to improve neuronal survival [62]. Preclinical studies showed that NAC provide positive effects against Aβ-induced protein, phosphorylated tau levels and lipid peroxidation [63]. It also restores acetylcholine levels and choline acetyltransferase activity [64]. In addition to its GSH modulating and antioxidant properties, NAC also protects against toxicity of Aβ through the stimulation of anti-apoptotic signaling cascades [65].

Since AD is presented with a prominent neuroinflammation element. The main supplier of GSH to neurons and microglia are astrocytes. In the course of chronic inflammation and oxidative stress, astrocytes liberate free radicals and toxic inflammatory mediators speed up microglia activation and neurodegeneration [66]. In this case, NAC halts the inflammatory factor NFκB and inhibits the production of nitric oxide from inflammatory cytokines [67]. Thereby, it is concluded that NAC being a multi targeting compound is capable of modulating Alzheimer's pathophysiology.

Coenzyme Q10

One of the most studied therapeutic strategies in AD is antioxidantsthat directly target mitochondria. With reference to the same, Coenzyme Q10 (CoQ10) an antioxidant has been used that directly targets mitochondria. Endogenously synthesized co-enzyme Q10 (CoQ10, ubiquinone), is a lipophilic antioxidant and has ability to significantly restore proteins, lipids and DNA oxidation specifically mitochondrial DNA. In most of the cells CoQ10 is produced and its function in ATP production is assume as beneficial in improving impaired mitochondrial function and oxidative damage [68,69]. Also, it conserves the mitochondrial membrane potential in the course of oxidative stress and protects the neuronal cells from the toxic effects of amyloid beta.

CoQ10 is quinone structured and is a part of mitochondrial Respiratory Chain Complexes (RCC).In preclinical studies, CoQ10 has significantly prevented the cognitive decline [70], But due to the poor bioavailability in the neurological tissues [71] and electron transfer dependent functioning, [72] it is unsuccessful in human studies. Hence, the Mitoquinone mesylate (MitoQ) was optimized to overcome this issue. MitoQ is an antioxidant that is synthesized from ubiquinone conjugate with Triphenylphosphonium (TPP). To target the molecule of mitochondria, TPP is necessary because it helps in crossing the lipid bilayers which accumulates on the anionic sides of mitochondrial membranes [73].

Mitoquinone mesylate

Mitoquinone mesylate (MitoQ) behaves as ROS scavenger and has been tested in different models of AD. It has successfully showed to protect against oxidative damage, prevent RCC activity, reduced Aβ peptide levels, astrogliosis and synaptic loss to restore cognitive functions [74,75]. To date MitoQ is reported to be tested in small scale clinical trials to investigate its potential effects in cerebrovascular blood flow in AD [76]. Likewise, to MitoQ, some additional antioxidant compounds such as SkQ1, astaxanthin and MitoAPO also positively affect the mitochondrial functions and could be a lead in AD treatment [77-79]. These studies have provided us with the considerable confidence for the therapeutic effectiveness of future therapies that targets mitochondrial disruptions and oxidative stress in AD.

Dietary supplements

Plant compounds such as phenolic acids, flavonoids, alkaloids, carotene, terpenoids etc. are among the most important classes of antioxidants that are exogenous and are a part of human diet. Therefore, it is supposed that antioxidants from natural extracts are an important source in prevention and protection against AD.

One of the studies determined the regular ingestion of antioxidant polyphenols from lemons and apple concentrate and also green tea extract for eight months and presented that they might decrease the homocystein plasmatic concentrations in AD patients, especially in the moderate stage [80].

Several dietary supplements have been displayed to provide treatment for AD like omega-3 polyunsaturated fatty acid, [6] curry spice curcumin and caffeine. Caffeine has strong antioxidant properties and also demonstrated to decrease brain Aβ levels in models of early-onset familial AD [81]. Substantial preclinical data indicates that curry spice curcumin have potent antioxidant, anti-inflammatory and antiamyloid beta pathology activities in AD. Since this compound inhibits enzymes cyclooxy-

genase 2 and lipoxygenase that are in charge of synthesizing proinflammatory leukotrienes, thromboxanes and prostaglandins [82]. Even so, the data regarding the bioavailablity, tolerability and safety of curcumin in elderly population is still missing [83,84]. Additionally, other management options comprise of calorie restriction, environmental enriched exercise and life style modifications have displayed elevated antioxidants in lessening AD neuropathophysiology [85].

Traditional herbal antioxidants

Traditional herbal antioxidants also display potential for the treatment of AD. Scientists reported the three main alkaloids in berberine, Coptidis Rhizoma-groenlandicine and palmatine that successfully restore cognitive functions in AD through both Aβ and choline esterase pathway and also by inhibiting ROS [86]. Moreover, silibinin (silybin), is a flavonoid which was derived from the herb milk thistle (Silybum marianum), have also exhibited antioxidant effects. Silibinin can be potential anti-Alzheimer therapeutic agent as it prevents oxidative damage and memory impairment induced by Aβ [87]. In addition, Ginkgo biloba is a naturally occurring plant that encompasses a variety of compounds like flavonoids and terpenoids which have an ability of quenching free radicals. It is evident from the research studies that Ginkgo biloba can inhibit Aβ aggregation and can reduce amyloid precursor protein *in vitro*. However, there are many conflicts on the effects of Ginkgo biloba that reports it has no potential to alter the amyloid precursor proteins or senile plaques. Several different studies reported the serious side effects that are associated with the use of commercially available ginkgo that counts coma, seizures and bleeding [88]. A systemic review confirmed the moderate neuroprotective effects of Ginkgo biloba, but their clinical application is hard to evaluate [89].

A study reported the antioxidant effects of methanol extracts of cork aok (*Quercus suber*) *in vitro* studies. The most significant antioxidant effects were achieved from the methanol leaf extract of the plant which contains tannins, phenols and flavonoids [90]. The methanolic extract of Atriplex lacianata L. enriched in phenols and flavonoids also possesses significant antioxidant activities [91]. Antioxidant activity of the extracts of *Acanthopanax henryi* leaves which contains caffeoyl quinic acid derivatives and flavonoids were examined by free radical and superoxide anion scavenging [92].

Other antioxidants

Melatonin

Another strong free-radical scavenger and broad spectrum antioxidant is melatonin. In AD, clinical studies with empirical treatment with melatonin have displayed improved neuropsychiatric and cognitive performance [93].

Melatonin is one of the mammalian hormones mainly synthesized in the pineal gland. It scavenges nitrogen and oxygen based reactants that are produced in mitochondria by elevating the expression and activity of glutathione peroxidase, NO synthetase and superoxide dismutase [94]. Ultimately, melatonin contributesin reducing oxidative damage to cells [95]. Studies conducted currently have exhibit that antioxidant melatonin have ability to inhibit Aβ-induced neurotoxicity, [96] diminish tau hyperphosphorylation [97-102] thereby improving learning and memory deficits in *in vitro* and *in vivo* models of AD [102]. Melatonin have also potential to halt the NADPH oxidase phosphorylation through P13K/Akt-dependent signaling cascade in microglia that were exposed to Aβ1-42 in *in vitro* studies [96]. Summarizing all evidences, the above information supports the antioxidant potential of melatonin to be utilized as therapeutic strategy in AD but further clinical data is required to confirm its clinical value for future use.

Monoamine oxidase-B inhibitor

Inhibitors of monoamine oxidase are suggested to be valuable for AD treatment. Monoamine Oxidase (MAO) is an enzyme that catalyzes the oxidative deamination of several xenobiotic and biogenic amines [103]. MAO is present as two separate enzymatic isoforms i.e. MAO-A and MAO-B, which is specific for their substrate and inhibitor aspects. Predominantly catecholaminergic neurons contain MAO-A, on the other hand MAO-B is found in serotonergic glia and neurons [104]. Selective MAO-A inhibitors are employed for the treatment of anxiety and depression [105]. While MAO-B inhibitors have shown to be effective in disorders such as Parkinson's and Alzheimer's disease [106].

AD patients are commonly presented with depressive signs and can be risk factor for disease development [107]. Enhance astrogliosis increases MAO-B levels in the brains of AD patients have also been reported [108]. It is suggested that for the treatment of AD, dual inhibition of both isoforms should be considered rather than MAO-B alone [109]. Selegiline which is familiar as L-deprinyl is a selective inhibitor of MAO-B with potential antioxidant properties and is promising to be used for the treatment of neurodegenerative disorders [110]. It has the ability to protect the vascular endothelium from the noxious effects of Aβ peptide and also enhance the functions of nigral neurons and their survival by halting oxidative deamination [111,112]. In 1997, a study reported that in moderately impaired AD patients, seleginine treatment significantly reduced the neuronal damage and slowed the progression of disease [111]. These evidences suggest that the selegeline may delay the progression of clinically important functional worsening in Alzheimer's patients. It also improves the cognition, mood, behavior and functional ability. But, clinical studies conducted during 2002 declared the lack of therapeutic evidence to be recommended as a treatment for Alzheimer's disease.

Due to the presence of multiple etiologies of AD, single target strategies become difficult to show good therapeutic effect. For that reason, Multi-Target-Direct Ligand (MTDL) displays an effective treatment strategy of AD [113,114]. Combined therapies of AD with anti-AChE and anti-MAO have already been reported. Such as, the derivative N-pyrimidine-4-acetylaniline which possesses AChE and reversible MAO-A inhibitor activity *in vitro* studies has been displayed multi targeted agents against AD [115]. Moving forward, in the development of monoamine oxidase inhibitors, compound JMC49 is also noticeable MAO-B inhibitor and has shown to be a candidate for AD treatment [116,117].

Hormones

Approximately two-thirds in 5.4 million of Americans with Alzheimer's disease are women [118]. Studies done on *in vitro* models supports the neuroprotective actions of estrogen against several cellular insults [119-124] and also gives protection from toxicity of amyloid β [125,126]. However, postmortem investigations uncovered that women with Alzheimer's disease showed reduced levels of brain estrogen [127].

Hormone Replacement Therapy (HRT) has shown to lower

the risk for dementia [128-132]. Nevertheless, the outcomes are controversial after the findings from the Women's Health Initiative Memory Study (WHIMS), which confers that HRT increase the overall risk of dementia [133,134]. Many of these gaps in our knowledge are recently being highlighted by expert panel from a number of Alzheimer's disease organizations. These comprise the prompt need to recognize how estrogen actually influences risk at the molecular level [135,136]. Estrogen has exhibited its antioxidant effects to protect neurons from the Aβ toxicity [112]. Besides its neuroprotective effects, it does not restore the cognition in AD patients [137]. But recommending estrogen as an antioxidant at present to reduce the AD risk has no evidences.

In spite of the current knowledge, there is much doubt regarding the therapeutic success with antioxidant therapy due to certain limitations. Several novel antioxidants exhibited their effects in animal studies but were relatively less effective in clinical trials. The one of the failure in human trials was because of inability to cross BBB. Hence, in future studies, it is necessary to investigate the potential of antioxidant to reduce or slow down the risk of AD progression. Furthermore, combination therapy with antioxidants should be focused rather than the single therapy, in order to assist redox cycling and also expand bioavailability in all cellular compartments.

The physiological and therapeutic role of secretases in AD

The generation of Aβ peptide is the initial step of the amyloid cascade and is generally considered as the main focus of AD pathology. Chemical molecules that can alter the activity of secretases responsible for the production of kind of Aβ peptide are likely to have advantageous effects as they can inhibit Aβ oligomerization and fibrils formation, thereafter improve cognitive deficit associated with the disease pathology. In view of potential anti-AD pharmacological interventions, secretases are divided into two distinct classes based on the cleavage sites present on APP. On one side are the harmful β-, γ-, δ-, and η- secretases that directly or indirectly contribute towards the formation of Aβ and for which inhibitors are needed [138], while on the other side is the α-secretase activation of which is required for beneficial outcomes [139]. Excitingly, activity of α-secretase not only precludes the formation of Aβ peptide but also involves the production of secreted fragment sAPPα which functions as neurotrophic, neuroprotective, memory improving and neurogenesis stimulating factor [140-143].

α-Secretase

The non-amyloidogenic processing of APP occurs between the Lys 16 and Leu 17 amino acid residues of Aβ peptide that lies in the extracellular space [144-146] by zinc metalloproteinases [147]. Experiments conducted on the neuronal and non-neuronal cells revealed that proteinases of the ADAM (A Disintegrin and Metalloproteinase) family possess the activity of α-secretase [148-150]. The most commonly studied α-secretases are ADAM9, ADAM10 and ADAM17 [149-151]. In human brain the expression of ADAM10 is synchronized with the expression of APP, while ADAM17 is less likely to express along with APP [152]. Another member of ADAM family, ADAM19 was proposed as α-secretase, but *in vitro* this protease does not cleave APP derived peptide suggesting that it is indirectly possessing the activity of α-secretases [153]. Concerning the identification of α-secretase studies concluded that ADAM10, and not ADAM9 or ADAM17 is the constitutive α-secretase in primary neurons and in the cells affected in AD [154,155].

Activation of α-secretase in AD

The activity of α-secretase is regulated by many cellular proteins and intracellular signaling pathways which control its activity at different levels. ADAM10 is regulated at the stage of transcription, translation, post translation, protein trafficking and also by regulating cell surface receptors, membrane fluidity and ADAM10-controlling proteins [156]. Various compounds have been presented to activate transcription of ADAM10. Acitretin, which is clinically used to treat psoriasis, is found to induce transcription of ADAM10 gene by activating all the receptors of trans-Retinoic Acid (RA) [157]. In the same manner, compound from red wine, resveratrol has been reported to activate nicotinamide adenine dinucleotide-dependent deacetylase Sirtuin-1 (SIRT1), which intensifies the deacetylation of β receptor of RA, ultimately switching on the transcription of ADAM10 gene [158]. Some natural compounds including a cryptotanshinone from Radix Salvia miltiorrhiza [159] and a marine-derived natural compound bryostatin-1 have also been shown to increase the protein levels of ADAM10 [160]. Furthermore, α-secretase activity is also affected by membrane fluidity. Less cholesterol in the membrane enhances membrane fluidity which disturbs APP internalization [161] thus increasing the processing of APP at the cell surface by α-secretase [162]. Therefore, lipid lowering drugs, for instance HMG-CoA reductase inhibitors were supposed to increase the processing of APP by α-secretase [163]. Similarly, cholesterol-lowering drug lovastatin has been shown to activate activity thereby decreasing the amount of Aβ peptide [161]. Other lipid-lowering agents such as atorvastatin and simvastatin have also been tested to alter α-secretase activity, but they were found ineffective [164,165]. So far, only etazolate, which is an anxiolytic drug and allosterically activate $GABA_A$ receptor [166], has reached to clinical research as a potential candidate for α-secretase activator.

β-Secretase

The function of β-secretase is executed by Beta-Site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) that cleaves APP to produce sAPPβ and CTF99. BACE1 also cleaves APP at another C-terminal site generating CTF89 fragment and subsequently release $Aβ_{11-40}$ after cleavage by γ-secretase. Another protein, BACE2, also possesses β-secretase activity [167], but it is typically limited to glial cells and its expression is less in the brain [168]. It is also not related to the pathogenesis of AD. The amyloid pathology genetic alteration of BACE1 entirely precludes Aβ pathology in AD mouse model expressing Swedish mutation APP670/671 and PSEN1 gene [168,169] or in Tg2576 AD mouse [170]. Mice lacking BACE1 gene do not have β-secretase function and amyloid plaques production in neurons [138,171,172]. BACE1 expression and function are also elevated in AD brains [173,174]. These studies suggest that BACE1 acts as a functionally important β-secretase expressed in the brain that is responsible for the development of AD pathogenesis.

Inhibition of β-secretase in AD

BACE1 protein is critical for the processing of APP and its regulation is important in maintaining the levels of Aβ. Therefore, drugs based on targeting BACE1 gene expression and control are of major concern in the field of drug development against AD. BACE1 gene can be regulated at various levels. To transcriptionally control BACE1 expression, several transcription factors have been recognized to regulate the activity of BACE1 promoter. Post-transcriptionally BACE1 mRNA is modified by alternative splicing, miRNAs at the 3'UTR and uAUGs at the 5'UTR.

Regulation of BACE1 protein also occurs by post-translational modifications that affects protein maturation, stability, trafficking and its enzymatic activity [175].

The discovery and development of BACE1 inhibitors is challenging due to its large substrate binding site, BBB penetration and selectivity [176]. The large size of the BACE1 substrate binding site creates obstruction in the designing of potent inhibitors. The compounds with smaller molecular size cannot fix properly into the binding site to elicit better response while those that fit the binding site mostly have compromised drug-like activity [177]. Furthermore, the catalytic site of BACE1 possesses high similarity with other aspartic proteases [178]. Therefore, the BACE1 inhibitor can possibly inhibit other physiological aspartic proteases such as BACE2 and cathepsins causing issues related to selectivity of the inhibitor [179]. Moreover, it is important for an inhibitor to penetrate the BBB in order to act at the site where Aβ is formed, that is brain [180]. Regardless of major constraints in developing BACE1 inhibitors, BACE1 is generally considered the finest target to reduce Aβ burden and development of potent BACE1 inhibitors holds a key position in academia and industry [179].

Since the pharmaceutical industry lack any FDA approved BACE1 inhibitor, the use of 'fragment-based drug discovery' for the development of BACE1 inhibitors has been shown to be successful which is evident by the clinical research of some of these inhibitors [179]. The first BACE1 inhibitor reached clinical trial is LY2886721 that failed phase II clinical trial due to its significant hepatotoxicity. Likewise, clinical trials RG-7129, BI-1181181, AZD-3839 and LY-2811376 were also ended due to the side effects of these drugs. Some other drug candidates, JNJ-54861911, E2609, AZD-3293 and MK8931 have successfully finished phase I clinical trial and are under further consideration [179].

γ-Secretase

The known γ-secretase is an enzyme that cleaves C-terminal fragment of APP followed by α- or β-secretase cleavage to release the cytoplasmic domain of APP [181]. Unlike the type-I transmembrane monomeric α- and β-secretases, γ-secretase complex is a heterotetramer composed of Presenilin (PS) 1 or 2, nicastrin, Anterior Pharynx Defective 1 (APH1) and Presenilin Enhancer 2 (PEN2) [182]. The catalytic activity of the gamma secretase complex lies in PSs subunit [183].This catalytic site consists of two aspartate residues (at positions 257 and 385 for PS1, 263 and 366 for PS2), embedded in transmembrane domains 6 and 7 in GxGD motifs. Notably, the endoproteolytic cleavage by presenilinase within the E9 domain of the third intracellular loop of PSs to generate NTF and CTF fragments is necessary to achieve an active γ-secretase [184].

Inhibition of γ-secretase in AD

Gamma secretase is recognized as one of the most potential AD therapeutic target because of its role in the last stage of amyloid beta formation and its function in defining the type of C-terminal peptides. Therefore, discovery of gamma secretase inhibitors has gained a lot of expectations [185].

Besides the role of gamma complex in APP cleavage, many other proteins including the cytoskeletal and signaling molecules such as Notch receptors, E cadherin, ephrin B2, CD44, ERBB4, and others are gamma secretase substrates [186]. While inhibition of gamma secretase is evidently favorable for the treatment of AD, simultaneous inhibition of cleavage of other gamma secretase substrates may lead to unfavorable consequences. Moreover, the developed gamma secretase inhibitors which include semagacestat (LY450139) and avagacestat (BMS-708163) were withdrawn from the clinical trials due to their undesirable side effects caused by off-target inhibition. The reason for the failure of semagacestat phase III clinical trials is its incompetence to improve cognitive deficits and its severe adverse effects 188. Failure of semagacestat leads to the development of avagacestat which does not affect Notch [187], however it is rejected from phase II trials because of its serious adverse effects at high doses [188]. As a result, an alternative approach to look for compounds that are capable of altering the activity of gamma secretase rather than inhibiting it entirely has been underlined as a better way to develop AD therapeutics [185].

Modulation of γ-secretase

The first generation of Gamma Secretase Modulators (GSMs) was a subclass of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), documented to lower the amount of larger toxic Aβ42 whereas increasing the shorter non-amyloidogenic Aβ forms, ultimately increasing the Aβ38:Aβ42 ratio [189]. Examples of these modulators include ibuprofen, flurbiprofen, indomethacin, sulindac sulfide, carprofen and tarenflurbil which functions as gamma secretase allosteric inhibitors to bring conformational change in the enzyme so that it specifically inhibits formation of Aβ42 peptide [190]. In the light of these research findings, other NSAID-based GSMs, CHF5074 and EVP-0015962 were also developed less potency, poor drug-like properties and limited BBB penetration these compounds did not reach the goal of drug development against AD [191].

An arylimidazole analog, E2012 has been developed as a non-NSAID GSM prototype [192]. E2012 was the first non-NSAID GSM to be examined clinically with potent inhibitory activity against amyloid beta42 in *in vitro* assays at nanomolar concentrations. But the compound and its derivative E2212 not showed good CNS drug-like effects [185]. Therefore, several other analogs of E2012 have been constructed to improve potency and to overcome the problem of drug unlikeness. Although compounds of this class exhibit inadequate drug-like effect in the CNS, it is important to know that the compounds developed using the pyrimidomorpholine scaffold have better drug-like effects. But development of these compounds with good potent activity stays challenging [185]. Later on, discovery of pyridopyrazine 1,6-dione–based GSMs confer better potency and considerable drug-like effects in the CNS. Notably ether-liked fluorine derivative of pyridopyrazine presented good drug pharmacokinetics as well as more potent activity *in vivo* with EC50 of 6nM [193]. Similarly, alkane-linked compounds of the same class have also been constructed which include benzofuran, tetrahydrofuran and indole analogs [190,194].

Additionally, non-arylimidazole based compounds were also developed among which oxazole based compounds did not exhibit potent and drug-like effects. However, cyanindole compounds displayed good initial safety and potent activity *in vivo* but issues related to the effectiveness as a CNS drug remain a question to be solved [195].

As discussed earlier, natural products provide promising constituents for the drug discovery and development, the same will be applicable to gamma secretase modulators. Triterpene glycosides from *Cimicifuga racemose*, black cohosh were the first to be documented as gamma secretase modulator. However, the first active triterpene glycoside showed activity at nanomolar concentrations but it did not possess good drug-like effects

in the CNS. This issue demanded the discovery of more efficient derivatives [196].

Studies also reported the activity of the aqueous extract of the *Pterocarpus erinaceus* bark in significantly decreasing Aβ42 *in vitro* levels without cytotoxic effects at effective concentration [212]. But identification of bioactive phytochemicals responsible for decreasing amyloid beta load is still needed. In addition to compounds derived from plant sources, some acidic steroids present endogenously have also represented gamma secretase modulating activities without affecting the Notch signaling [197].

It is therefore summarized that development of compounds possessing good drug-like effect in the CNS and excellent potency is highly challenging in the field of drug development [185].

Aβ immunotherapy

There are three possible mechanisms of action of Aβ targeted antibodies (**Figure 2**). First is the direct action of antibody on Aβ protofibrils, fibrils, oligomers or plaques, where antibody disrupts the stability of Aβ species. Second is Fragment, Crystallizable (FC) receptor based phagocytosis by microglial cells and the last is peripheral sink mechanism, where, antibody binds to Aβ present in the plasma, therefore resulting in efflux of Aβ from brain to plasma [198].

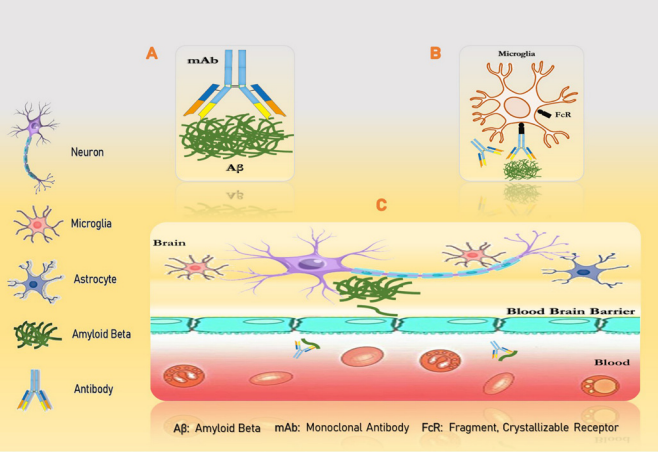

Figure 2: Anti-Aβ antibody targeted immunotherapy, Direct destabilization of Aβ **(A)**. Fc receptor based phagocytosis **(B)**. Peripheral Sink mechanism **(C)**.

The first anti amyloid immune based drug candidate was AN-1792 that aimed to provoke immune response against Aβ and was a pre-aggregated form of Aβ conjugated to QS21 adjuvant. Despite clearing Aβ deposits, this drug was not clinically effective and did not improve cognitive deficit [199]. In addition, AN-1792 induced an immune response in almost 6% of the patients with severe meningoencephalitis-like brain inflammation [199]. Some other safer Aβ antigens and adjuvants were developed later, but they also did not get success to provide clinical benefits. The only drug that is still in the development pipeline is CAD106. Several passive monoclonal antibodies against Aβ have also found to be clinically unsuccessful. Some of the Aβ targeted antibodies (**Table 1**) including crenezumab, gantenerumab, solanezumab and aducanumab which are in phase III clinical research for early AD, asymptomatic patients and prodromal familial AD [198] are discussed here.

Table 1: Anti-Aβ antibodies in phase III clinical research

Drug Candidate	Mechanism of Action	Disease Stage
Crenezumab	mAb	Early AD
Gantenerumab	mAb	Early AD
Aducanumab	mAb	Prodromal
Solanezumab	mAb	Prodromal
BAN2401	mAb	Prodromal

Crenezumab

Crenezumab from Genentech and Hoffman-La Roche is in phase III clinical trial for early stage AD patients. It targets oligomeric and 16-mer fibrillary Aβ types and binds to residues 13 to 16 of oligomeric Aβ forms 44. It also supports Aβ disaggregation by preventing its aggregation [200].

Gantenerumab

Gantenerumab from Hoffman-La Roche and Genentech is developed to treat early stage AD patients by clearing Aβ and is undergoing phase III trials. The affinity of gantenerumab is more against Aβ oligomers (0.6nM) and fibrils (1.2nM) than Aβ monomers (17nM) [201].

Aducanumab

Aducanumab developed and licensed by Neurimmune and Biogen is in the investigational process for treating prodromal AD. Aducanumab binds to Aβ insoluble fibrils and its soluble aggregates and has >10,000-fold increased selectivity for aggregated Aβ forms than the monomers [202].

Solanezumab

Solanezumab is being investigated for patients at high risk of developing AD. Experiments conducted on transgenic mice and human subjects provide data that solanezumab targets Aβ plaques and soluble monomeric form of Aβ [203]. Solanezumab is one of the few Aβ targeted antibodies that have been reported to restore cognitive impairment in some AD transgenic mouse models [204] but not in all [205].

BAN2401

BAN2401 developed by Biogen and Eisai is undergoing experimental testing against prodromal AD. It decreases the burden of Aβ protofibrils in brain and CSF of AD transgenic mice articulating the human Arctic and Swedish APP mutations [206].

Targeting tau kinases in AD pathology

Tau is a protein encoded by gene located on chromosome 17 called mapt gene [207]. This protein referred as IDP or intrinsically dis-ordered protein because of lacking in stable natural structure and show stretchable conformations. These conformations allow the tau protein to interact with other protein as well as itself. Tau protein binds with microtubules and attains a stable structure [208]. It can also gain of toxic function by phosphorylation of tau protein and fragment removal by truncation processes [209].

Tau hypothesis for AD describe the mechanisms through which the Neurofibrillary Tangles (NFTs) of tau protein are formed. NFTs comprises of hyperphosphorylated form of tau protein are the pathological hallmarks of AD [210]. It is ob-

served that hyperphosphorylation of tau protein is directly associated with the disruption of microtubules assembly and already assembled microtubules function [211-214]. The term tau-pathology used when phosphorylated tau protein start hyperphosphorylate and aggregates intracellularly in neuron. Hyperphosphorylated tau redistribute in the neuron from axon to dendritic region in filamentous form [215]. Tau oligomers serve as substrate for the tau-tau binding resulted in tau aggregation [216]. Physiologically the lysosomes and vacuoles are principal agents for elimination of cellular waste. When cellular elimination cycle disrupted the insoluble waste tend to accumulate into the cellular cytoplasm. Truncated tau protein further trigger for the aggregation of tau protein leading the Paired Helical Filaments (PHF) core and then neurofibrillary tangles or NFTs [217]. As the pathology progress these tangles become insoluble with the increase in complexity and abundance of hyperphosphorylated tau and disrupt the function of neuron by disrupting the axonal transport which ultimately leads towards neuronal death [215]. Therefore, tau pathology particularly the hyperphosphorylation of tau protein considered the potential therapeutic target for modulation of the AD.

Lists of strategies are adapted to subsidize the pathogenesis of tau protein in AD model. These strategies include the inhibiting mechanism for hyperphosphorylation of the tau protein, inhibition and clearance of the tau protein aggregation, stabilizing the microtubules and also immunotherapy against tau protein [214].

Inhibitory mechanism of tau protein hyperphosphorylation targets the kinase enzymes which are involved in the tau hyperphosphorylation [218]. Different kinases such as GSK-3β, Fyn kinase, CDK5 and some stimuli associated kinases (JNK and p38) and mitogen activating kinases (ERK1 and ERK2) are involved in hyperphosphorylation of tau protein. Previous studies show higher concentration level of these kinases in the brain of AD patients [219].

Glycogen synthase kinase-3 (GSK3)

GSK3 is a kinase which phosphorylate at serine-threonine position of different proteins and modulates their activity by its phosphorylation [220]. It ubiquitously expressed during the regulation of many signaling pathways particularly in neurodegeneration. Until now two isoforms of the GSK3 are reported, GSK3-α and GSK3-β, while both isoforms are product of two different genes located on chromosomes 19 and 3 share 98% structure similarity [221,222]. Research studies show abundance of GSK3- β expressions in CNS where its level increase with aging [223]. Molecular studies after death on the brain of the AD patients supported the hypothesis that GSK3-β also pathological cause of AD because of presence of hyperactive GSK3- β in the AD patient's brain [224]. It also contributes in the memory consolidation, synaptic plasticity, neurogenesis, long term potentiation and inflammation.

Association of GSK-3β with the neuropathological features of the AD such as Aβ production, tau phosphorylation, memory dysfunction, synaptic failure and neurogenesis is apparent. Therefore, on the basis of this association GSK3-β considered an important therapeutic approach to alleviate pathology of the AD. Phosphorylation and dephosphorylation on different locations are key processes to regulate the function of GSK3-β. Many research studies are conducted for the loss of function of GSK3-β on the AD model and provide sufficient insight information to use it as therapeutic target [225,226].

Instead of high conservation of GSK3- β kinase only few compounds (synthetic and natural) as GSK3-β inhibitors succeeded to clinical trial level after successfully testing in pre-clinical trial. GSK3-β being a part of many fundamental biological processes is main reason for the failure of the clinical trials instead of initially encouraging results [227,228].

The first drug which reached the clinical trial to reduce the tau pathology by targeting inhibition of GSK3-β is known as AZD2558 [229]. It reduced tau phosphorylation successfully in both (*in vivo* and *in vitro*) because of target specificity. However, a lot of severe toxicological side effects associated with this drug prevent to use it for chronic AD patients' treatment. Another drug named AZD1080, which show promising results for reduction of tau hyperphosphorylation *in vitro* as well as in preclinical studies also withdraw from the clinical trials because of severe side effects [229].

Tideglusib being the GSK3-β inhibitor is the only drug to reach the clinical trial phase II for treatment of the AD [230,231]. This drug is used to reduce tau phosphorylation associated pathology in mild to moderate AD patients in clinical trials. These clinical trials provide no promising results regarding significant medical or cognitive improvements in AD patients however they show good drug tolerability [232].

Lithium chloride is CNS drug approved by FDA for bipolar disorders treatment purpose also has inhibitory effect on GSK3-β enzymes. Its activity as GSK3-β inhibitor is weak and unspecific however it shows significant reduction in GSK3-β activity at therapeutic dose with no side effects [233]. Clinical studies show very promising results to use this drug as therapeutic agent against AD. Pilot clinical trials studies with this drug are conducted on AD patients show significantly reduction in tau hyperphosphorylation (tau pathology) and improvement in the impaired cognition in patients with AD [232]. Positive results of the Lithium Chloride against AD suggest that it might be potential therapeutic or disease modifying drug for AD. Further clinical studies with this drug are under the processes.

ANAVEX 2-73 a GSK3- β inhibitor drug is also under clinical trial phase II. Preclinical data of this drug show promising results to reduce tau hyperphosphorylation related pathology in AD patients by GSK3-β inhibitory mechanism [234].

Fyn kinase inhibition

Fyn kinase is one of the important kinase among the other nine kinases of the non-receptor tyrosine kinase (SRK) family [235,236]. This kinase is involved in the production of Aβ plaques and tau phosphorylation of NFTs. Shirazi and wood [237] reported the significant immunoreactivity of Fyn kinase along with abnormal phosphorylated Tau protein in the AD brain compare to the normal brain. On the basis of this many researchers are now focusing to find the possible connections between the Fyn and AD which lead to the down regulation of Aβ on tyrosine kinases. Preclinical data strongly recommend that targeting the Fyn kinase might be potential therapeutic approach for AD. However, Fyn kinase as part of many fundamental physiological processes to maintain the normal function is also a major hindrance because disruption of these physiological processes possess some severe off target consequences. Extreme inhibition of Fyn kinase could have serious side effects on cognition as well as memory functions. These adverse effects could become more severe in AD patients who already have vulnerable state of memory and cognition [238].

Saracantinib is first drug reported to target the Fyn through inhibition of specific SRK in AD. In 2014, a clinical trial with saracantinib on AD patients was launched to study the tolerability, safety and CNS penetration of the drug [238].

Many other tyrosine kinase inhibitors have gone under the pre-clinical and clinical trials to check the therapeutic potential against AD. Masitinib is one of them tyrosine kinase inhibitor with oral availability. Phase II clinical trial with this drug in AD patients show promising results regarding cognition and improvement in daily living [239].

Beta carboline compounds are reported for the reduction of the taupathy in AD. These compounds target the tyrosine kinase called DYRKIA. Over expression of DYRKIA kinase is directly associated with taupathy in AD [240]. Harmine is a beta carboline drug which inhibits the DYRKIA function. Preclinical studies show a significant reduction in tau hyperphosphorylation upon the inhibition of the function of DYRKIA kinase [217].

Cyclin dependant kinase 5

Cyclin Dependant Kinase 5 (CDK5) is unique and important kinase enzyme member of the CDKs family. Research studies provide sufficient data regarding the CDK5 association with the AD pathogenesis. CDK5 is one of the key players in the development of CNS, neuronal movement (migration and differentiation), synaptic and memory processes. Hyperactivity of CDK5 is direct linked with the hyperphosphorylation of Aβ and tau NFTs. This hyperphosphorylation of Aβ and tau protein ultimately leads towards the neurodegenerative disorders such as AD. Presence of CDK5 phosphorylated tau in the AD brain specifically at the hyperphosphorylated sites suggesting it as potential disease kinase. Hence inhibition of CDK5 could be an important target candidate to reduce the tau hyperphosphorylation and potential therapeutic approach against AD [241].

Many drugs which target the GSK3-β kinase inhibitors also target the CDK5 and reduce its level. These drugs are Hymeniialdisine [242], 6-bromoindirubin [243] and Manzamine A analog 95 which inhibit the both kinases [244] and decrease the hyperphosphorylation tau protein with enhanced cognition.

Tamoxifen is a generic drug commonly used for the treatment of breast cancer also show positive results for alleviate the tau hyperphosphorylation associated pathology in AD. This drug inhibits the CDK5 protein by binding the CDK5/p25 subunit and modulates the tau phosphorylation pattern [245].

Mitogen activated protein kinase

Mitogen Activated Protein Kinase (MAPKs) are serine/threonine kinases which have significant role in regulation and maintaining the cellular processes such as cell proliferation, cell differentiation, survival and apoptosis in response the external stimuli [246].

P38 kinase is important MAPK enzyme with association of maintaining the regulation of tau phosphorylation. It is hypothesized that this kinase participates in the hyper-phosphorylation of tau protein under the pathological circumstances [247]. It was reported in 1999 by hanslay and team that p38 MAPK is closely associated with AD. They observed the hyper activity of p38 MAPK in the brain of patients with AD [248]. Research studies confirm that hyper activation of p38 MAPK start at early stage of pathogenesis in AD [249,250]. Specifically, its association with the NFTs pathology in cortex and hippocampus areas in brain of AD patients [251]. Association of p38 MAPK with hyperphosphorylation of NFTs of the disease indicates that inhibition of the p38 MAPK enzyme might b potential therapeutic target to reduce the tau pathology in AD. Therefore, many research groups attempted by direct or indirect p38 MAPK inhibitors to reduce the tau hyperphosphorylation associated pathology of AD.

One commercially available drug SB203580 is conventionally p38 MAPK inhibitor use for reference tools for biological evaluation. Ginsenoside Rg1 is also used as p38 MAPK inhibitor to reduce he tau hyperphosphorylation in Aβ stimulated neurons in Ad model [252]. Trolox, an analog of vitamin E which is water soluble reported for reduction of tau pathology through the inhibitory mechanism of p38 MAPK induced by oxidative stress [253].

Proanthocyanidins, a class of natural flavonoid also reported for decrease the tau phosphorylation in neuronal cells and enhancement of impaired cognition state in AD model. This drug inhibits the cascade activation of p38 induced by ER stress stimuli [254]. The other serine/threonine kinases family of stress induced MAPKs enzymes are a c-Jun N-terminal protein kinases [255].

Inhibition and clearance of tau protein aggregation

Currently, LMTX an important drug which is in clinical trial phase III and providing the promising result in clearance of NFTs and inhibition of their aggregation in AD patients [256]. This drug is derivative of the Methylene blue dye with increase bioavailability and tolerability. It works as blocking the tau phosphorylation and further evading the tau aggregation. Clinical studies showing that it not only prevents the tau protein aggregation also clear the already formed NFTs in AD [257].

Phosphateses also important enzymes which de-phosphorylate the tau protein totally reverse function of the kinases. Phosphatases 5 (PP5) one of the many phosphatases which abundantly present in CNS. Its activity enhanced in the presence of unsaturated fatty acid or long chain fatty Acyl-CoA. Low levels of PP5 indicate the inhibited activity of PP5 on the NFTs along with the low level unsaturated fatty acid leading to imbalance between both processes. This imbalance between phosphorylation and de-phosphorylation ultimately leads towards the tauopathy in AD. On the basis of this PP5 might be potential therapeutic target to alleviate the tauopathy [217].

Autophagy

An extensive amount of data supports that dysregulation of autophagy take place in both AD patients and animal models. Suzuki found that there were many irregular subcellular vesicles and tau proteins accumulated in swollen or dystrophic neuritis in the AD patient brains [258]. In 2005, the Nixon Group found that such vesicles accumulated in dystrophic neuritis in AD brains were immature autophagic vacuoli by the use of immunogold labeling and electron microscopy [259]. Before the synaptic and neuronal loss, in hippocampal neurons of AD mice, unusual increase of immature Autophagy Vacuole's (AVs) in axon was noticed [260,261]. An abnormal accumulation of AVs has also been observed in several other AD animal models including the TgCRND8 mice that overexpresses mutant human APP695 and APPSWE/PS1M146L [262,263]. Through autophagy pathway Tau aggregates are degraded [264,265]. Autophagic gridlock also helps to the development of AD-like tauopathy [266]. The excess of AVs in the brains of AD animal models and AD patients is in clear comparison to the infrequently-observed AVs in

normal brains, which supports that the assembly of pathogenic proteins like as Aβ and tau in AD may be the reason of defective autophagy-lysosome proteolysis pathway [261,262].

Uptill now, APP, PS-1, PS-2 the three main causative genes have been known for early-onset Familial AD (FAD) [267]. Wild type PS1 with no known mutation forms has been found, by controlling the regulation of v-ATPase subunit V0a1 onto lysosome, is vital for lysosome acidification and consequently lead to the regulation of autophagy-lysosome degradation system in a γ-secretase-independent way [268]. Furthermore, study has found that ApoE4 in neuro-2a cells potentiate lysosomal leakages and increases Aβ peptide-induced apoptosis [269]. In brain due to gradual accumulation of AVs and lysosomal shortage is well known to be another hallmark of AD [270] however, autophagy dysfunction is the result or the cause of AD is still a debate [271,272]. Moreover, gender difference may affect the malfunction of auto-lysosome system [228]. In general, data shows that autophagy plays a protective role in early stages of AD, while in more advance stages neuronal degeneration seems to be possible.

Modulation of autophagy as AD therapy

The mammalian Target Of Rapamycin (mTOR) is a well-established main pathway that sense nutrient for cell metabolism by incorporate several signaling cascades into the cells [273-275]. Genetic mTOR signaling reduction in Tg2576 mice brain increased autophagy induction and restored normal signature of hippocampal gene expression, leading to reduced Aβ deposit and alleviated memory deficits (**Figure 3**) [276]. Signaling via mTOR controls tau homeostasis [277]. Pharmacologically dipping mTOR signaling by rapamycin improved tau pathology [278]. Continuing inhibition of mTOR with rapamycin or latrepirdine often inhibits AD like cognitive deficits and reduce Aβ42, amyloid plaques and tau NFTs [279-282]. However, it must be remembered that many other cell function such as gene translation and cell growth has been regulated by mTOR signaling cascade. Long-term inhibition of the mTOR pathway can cause harmful side effects in patients. Rapamycin is therefore not suitable long-term drug candidate. A new definite inducer of autophagy is immediately required for the field. The administration of lentiviral beclin1 vectors in APP transgenic mice, contributes to autophagy induction, and reduces both intracellular and extracellular amyloid pathological condition [283]. The association between beclin1 and its endogenous inhibitor B-cell lymphoma 2 (Bcl2) is reduced by one-point mutation (F121A). Induction of beclin1F121A in mice contributes to the active autophagy in numerous tissues comprising brain, even deprived of any autophagy-induction stimulation. The hyperactive autophagy with beclin1F121A dramatically decreases the deposition of amyloid, and inhibits the cognitive deterioration, and increase the survival rate in AD mouse models [284]. With a gene therapy strategy, cognitive deficiencies in APP/PS1 mice were recovered by rising brain p62 expression. Strategies shown in figure 3 can offer targeted AD therapy more accurately. Autophagy stimulation also decreases neurodegeneration in a mouse model of human tauopathy [285].

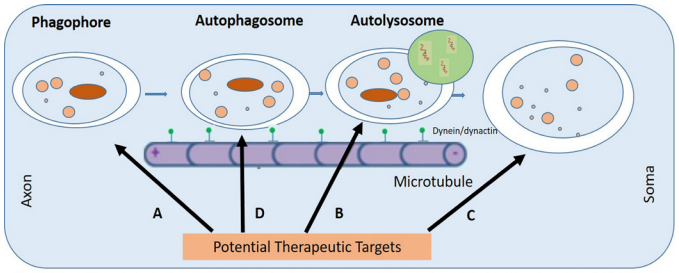

Figure 3: Potential strategies for the treatment of Alzheimer's disease by modulating autophagy. Induction of autophagy (A) in the brain to reduce of Aβ aggregates and NFTs. Stimulation of autophagosome-lysosome fusion (B). Enhancing lysosomal function (C). Stabilizing retrograde transportation of the autophagosomes (D). Green dots are representing molecular motor dynein/dynactin.

On the other hand, contradictory evidence from latest studies in AD models shows the applicability of autophagy induction is uncertain as a common treatment technique for AD. Irregular stimulation of autophagy can result in increase of Aβ production, as aggregated vesicles comprising active γ-secretase machinery [286,287]. More and more research supports the idea that the result of autophagic variation is context-dependent. Research has support autophagosome can be a main pool for Aβ production in AD brain [285]. Stimulation the formation of new autophagosome but not associated with a similar autophagic flux increase that may actually lead to enhance Aβ assembly and catabolic contents leaking from AVs [288]. All of this should be taken into account when taking into consideration autophagic modulation as a treatment, what the autophagy deficiency is, how long and how strong it for modulation? For example, it has been stated that Aβ42-induced cell death can be aggravated by inhibition but not by autophagy [289,290]. So, the advantage of enhanced induction of autophagy thus tends to be contex-dependent, while neuronal survival involves basal autophagy. The results also indicate that the activation of autophagy following the development of mature tangles and plaques had no impact on cognitive impairment or other AD-like pathology, although that autophagy induction decrease solubilized tau, Aβ and amyloid plaques level in 3xTg-AD mice prior to the formation of AD like pathology [282]. Furthermore, the variation in models should partly produce conflicting data on the role of autophagy modulation In addition, systematic research is needed for comprehensive observing the levels of autophagic modulation in different cells (neuron vs. glia) in AD as discussed below.

Furthermore, the aggregation of insoluble Aβ42 can be the direct reason of the of autophagic impairment development over time [291]. In favour of this hypothesis, a recent updated report presented that no transformation into autophagic/lysosomal failure was noticed in TgCRND8 mice when treated with an endogenous inositol stereoisomer i.e. scyllo-inositol, which is known to prevent Aβ42 accumulation and fibril generation before the onset of autophagic/lysosomal failure. In comparison, immature AVs and autophagic/lysosomal substrates were considerably gathered in vehicle-treated TgCRND8 littermates [292].

Recent studies have shown that auto-lysosome dysfunction in AD pathogenesis is triggered by impaired lysosomal proteolytic activity [293]. The genetic ablation of cystatin B, a lysosomal cysteine proteases inhibitor, significantly increase lysosomal activity in TgCRND8 AD mouse model with strong deficiency in proteolytic clearance of autophagic substrates (**Figure 3**), lead-

ing to enhanced clearance of the autophagic substrates, and clear mitigation of memory loss and amyloid pathologies in the animals [263,294]. In therapeutic direction, pharmacological compounds with such special effects would significantly facilitate research [295].

Targeting autophagy through combinational therapy

Theoretically, consecutively use of two pharmacological autophagy-inducers that act via different regulatory pathway will give more beneficial effect. Certainly, by using trehalose or lithium an mTOR-independent autophagy enhancer and the mTOR-dependent autophagy enhancer rapamycin in amalgamation upregulate autophagy more broadly and contributes to a faster clearance of protein aggregates rather than using each alone [296,297]. Furthermore, the use of two drugs in combination may facilitate reduction of the treatment dose rather than in comparison with treatment alone, which could significantly decrease the possibility of aggressive effects. In such a situation, it could be an encouraging mediation approach to moderately upregulate autophagy induction in combination to stimulate the effective completion of autophagic degradation. Though, to target the defective lysosomal proteolysis and the autophagy induction at the same time is still a major challenge.

The Transcription Factor EB (TFEB) seems to fulfill both of these criteria as it coordinately triggers lysosomal biogenesis as well as genes required for autophagosome formation [298]. With its effectiveness has previously been presented under numerous neurological conditions, including lysosomal storage disorders [299], Huntington's Disease (HD) [300] and Parkinson's Disease (PD) [301], it is predictable that in the AD context similar benefits may also be attained. A recent study shows that in addition, TFEB may actually be beneficial for patients with AD treatment [302]. On the other hand, the issues of AV's clearance slowdown and disrupted lysosomal function could be approached by pharmacological treatments which boost the catalytic performance of lysosomal enzymes and instantaneously reducing the burden of auto-lysosomal pathway, as seen in the study of (**Figure** 3) [303].

Other possible approaches

On the other hand, mediations aiming at decreasing the burden to the improper functioning autolysosomal compartments hold some potential target as well. For example, it may all helpful to inhibit Aβ production and oligomerization together with lowering cholesterol. A cholesterol-lowering drug 2-hydroxypropyl-beta-cyclodextrin has been shown to be a potential emerging pharmacological drug for AD model [304]. As for Aβ, a recent study suggests that useful specific inhibitors of Aβ inhibitors may be produce from peptides that interrupt the physical communication among the APP and PS1 [305]. Ultimately, since growing evidence recommends that repairing proper endosomal trafficking (recycling) may have a same effect, an additional possible strategy to solve this issue is to develop selective pharmacological modulators of these procedures. Two current studies providing the first proof of concept test by developing pharmacological stabilizer of the retromer sorting complex for AD treatment [303,306].

It is also very important to investigate biomarkers that can be implemented broadly in clinical settings to determine the therapeutic efficacy of autophagy modulation for a deeper understanding of autophagic malfunction in AD and for the effective creation of therapeutic strategies based on autophagy modulation [307]. Therefore, as proof against autophagy pathway drug ability in the late stage of the disorder, further research may seek to find prevention or therapy trials in the early stage of AD [308].

Conclusion

Besides all the efforts towards the discovery and development of AD therapeutics, the disease is not completely treatable. Therefore, there is a need to discover the undiscovered by understanding the mechanisms of disease pathology and fill the existing therapeutic gaps.

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