Chapter 1
Introduction

1. Introduction

1.1 Neurodegenerative disorders

Neurodegenerative disorders are incurable and debilitating conditions, that result in progressive loss of nerve cells and problem with movement and mental functioning. These are a major threat to human health and are more prevalent in elderly population. Neurodegenerative diseases include, Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Huntington's disease, Fronto-temporal dementia and spinocerebellar ataxis(1). Mentioned diseases are very much diverse in their pathophysiology, with some affecting the cognitive functions while others affecting the motor functions (2).

1.1.1 Alzheimer's disease

It is one of the most common neurodegenerative diseases and accounts for more than 80% of dementia cases worldwide in older adults. It leads to the progressive loss of mental, behavioral, functional decline and ability to learn. AD is primarily characterized by the progressive loss of memory associated with other cognitive deficits. The global burden of the population suffering from AD was assessed at 44 million in 2015. This number is expected to double by 2030 and triple by 2050, if no effective treatment is in place (4). The two strong hypotheses being advocated in the pathogenesis of AD are based on protein 'plaques' and 'tangles,' that hamper the usual cognitive activity of the brain. Several efforts have been made to design therapies which interfere with these structures (plaques' and 'tangles'). AD is one of the major amyloidosis, with two types of amyloids deposited in the brain. These are (i) amyloid β (Aβ) forming aggregates

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which constitute plaques and cerebrovascular amyloid angiopathy and (ii) tau protein which forms neurofibrillary tangles (NFTs), dystrophic neurites, and neuropil threads The senile or neuritic plaques and NFTs are the characteristic lesions found in different areas of the brain *i.e.* temporal lobe and cortical area (5).

1.1.2 Parkinson's disease (PD)

PD is second most common progressive neurodegenerative disorder affecting older population(3). There is loss of dopaminergic neurons from substantia nigra and development of neuronal lewy bodies which lead to motor impairment(4). Tremors and bradykinesia are the characteristic features of Parkinson's disease(5).

1.1.3 Amylotrophic lateral sclerosis (ALS)

ALS is a rare late-onset fatal neurodegenerative disorder, characterized by progressive muscular paralysis resulting from degeneration of motor neurons from primary motor cortex, corticospinal tracts, spinal cord and brain stem. Due to the loss of motor neurons, brain's ability to control and initiate muscle movement is completely lost (6).

1.1.4 Hutington's disease

Hutington's disease is an inherited disease caused by progressive breakdown of nerve cells(7). It affects a person's physical and mental abilities during his young age. It is caused due to dominantly inherited trinucleotide repeat (i.e. CAG) expansion in hutingtin gene located on chromosome 4. At the cellular level, faulty hutingtin gene results in neuronal dysfunction and cell death through various mechanisms *i.e.* proteosis, transcription, direct neurotoxicity and mitochondrial dysfunction(8). Currently there is no disease modifying treatment for this, but clinical developments in recent years have focused on supportive and symptomatic management of the disease (9).

1.1.5 Frontotemporal dementia (FTD)

FTD is a collective term used to describe neurocognitive syndrome, that encompasses progressive dysfunction in behavior, language, and executive functioning(10). It is second most common cause of dementia after Alzheimer's disease. Currently, there is no approved drug for disease modifying purpose however, asymptomatic treatments such as selective serotonin reuptake inhibitors and atypical anti-psychotic agents are typically used(11).

1.2 Pathophysiology of Alzheimer's disease

The atrophy of cerebral cortex and loss of cortical and subcortical neurons are significant characteristics of the AD(12). Amyloid plaques, insoluble aggregates of hydrophobic β-amyloid peptide and neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein, are pathological markers of AD (13). In ordinary individuals also, small number of β-amyloid plaques and neurofibrillary tangles can be observed, but they are abundant in patients with AD(14). In advanced cases, β-amyloid plaques and NFT are numerous and most abundant in hippocampus and associative regions of the cortex(15).

In AD, there is marked deficiency of acetylcholine due to atrophy and degeneration of subcortical cholinergic neurons, mainly those present in basal forebrain, that provides cholinergic innervation to the whole cerebral cortex(16). The cortical and hippocampal targets, that receive cholinergic input are also destructed(17). The deficit is not only related to acetylcholine but involves other neurotransmitters, including serotonin, glutamate, and neuropeptides too(18).

1.2.1 Role of amyloid precursor protein (APP)

APP is a 770-amino acid membrane protein and is expressed by many cells, including CNS neurons. Its cleavage involves three proteases (secretases)- α, β, and γ. The

cleavage by α-secretase produces soluble APP (sAPP), which is believed to have neurotopic and neuroprotective properties(19). Aβ formation involves cleavage at two different points by β- and γ-secretases. The enzyme γ-secretase (large intramembrane complex of several proteins) lacks precision and cuts APP at different points generating Aβ fragments of different lengths, including $A\beta_{40}$ and $A\beta_{42}$(20). Mutations in this region of APP gene affect the preferred cleavage point and leads to overproduction of $A\beta_{42}$. Both proteins aggregate to form amyloid plaques, but $A\beta_{42}$ shows a stronger tendency to do so as compared to $A\beta_{40}$ and is mainly responsible for amyloid formation(21). Mutations in presenilin genes result in increased γ-secretase activity, as presenilin proteins form part of γ-secretase complex. These mutations in AD increase the ratio of $A\beta_{42}$:$A\beta_{40}$ that can be detected in plasma and serves as a marker for the familial AD. Mutations in ApoE4 gene (for lipid transport protein), that facilitates the clearance of Aβ oligomers, also predispose to AD, because the mutant form of ApoE4 proteins is less efficient in this function(22).

1.2.2 Role of beta-secretase (BACE)

Amyloid plaques, primarily composed of Aβ, progressively develop in brains of AD patients, and mutations in three genes (APP, PS1, and PS2) cause early on-set familial AD (FAD) by increasing synthesis of toxic Aβ42 peptide(23). Given the strong association between Aβ and AD, therapeutic strategies to lower the concentration of Aβ in brain should prove beneficial for the treatment of AD. Aβ is a proteolytic product of large Type I membrane protein, amyloid precursor protein (APP)(24). Two proteases, called β- and γ-secretases, cleave APP to generate the Aβ peptide. The molecular identities of these proteases were unknown for over a decade. The γ-secretase has been tentatively identified as the presenilin proteins, PS1 and PS2, and β-secretase has been shown to be the novel transmembrane aspartic protease, β-site APP Cleaving Enzyme 1

(BACE1; also called Asp2 and memapsin2)(25). BACE 2, a novel protease homologous to BACE1, was also identified, and the two BACE enzymes define a new family of transmembrane aspartic proteases. BACE1 exhibits all the properties of β-secretase, and as the key enzyme that initiates the formation of Aβ and thus is an attractive drug target for AD(26).

1.2.3 Role of amyloid beta (Aβ)

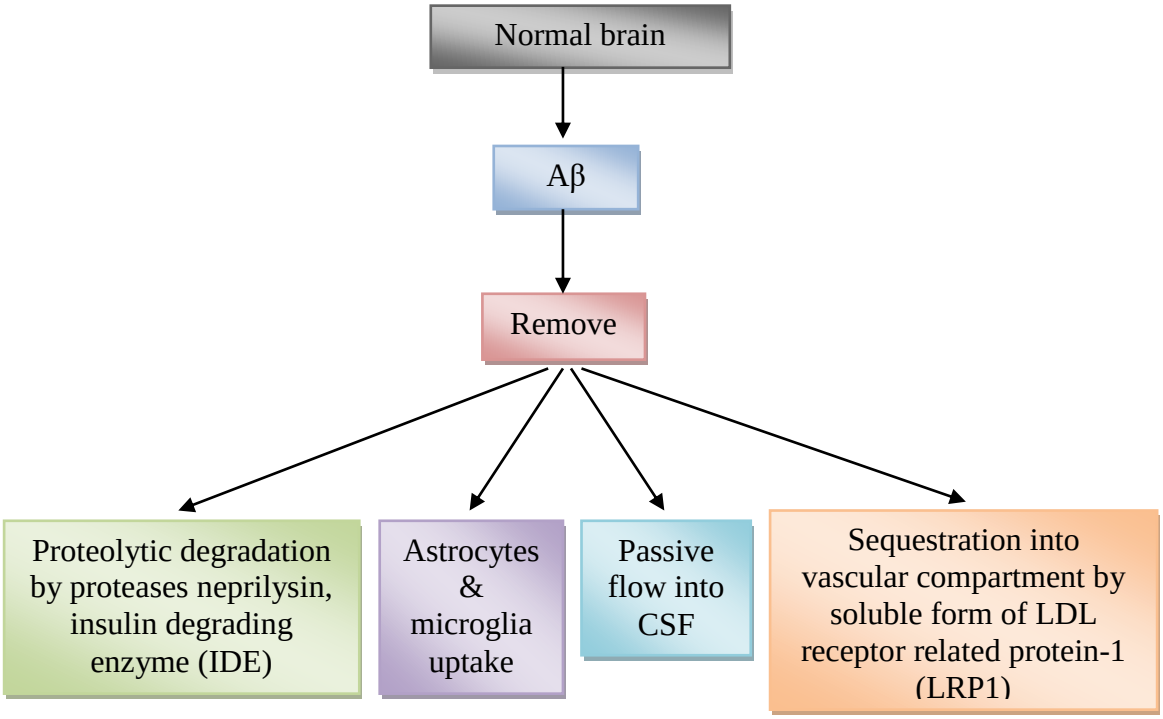
Aggregation and accumulation of Aβ in brain may result, from increased production of Aβ, decreased degradation, or reduced clearance across the BBB. Aβ oligomers impair synaptic functions and related signaling pathways, changing neuronal activities, and trigger the release of neurotoxic mediators from glial cells(27). Fibrillar amyloid plaques displace and distort neuronal processes. The lipid transport protein apoE4 impairs Aβ clearance and promotes Aβ deposition. When expressed within stressed neurons, apoE4 is cleaved into neurotoxic fragments that disrupt the cytoskeleton and impair mitochondrial functions. Tau, which is usually most abundant in axons, becomes mislocalized to the neuronal soma and dendrites and forms inclusions called neurofibrillary tangles (NFTs). The α-synuclein may also self-assemble into pathogenic oligomers and form larger aggregates (Lewy bodies). Both tau and α-synuclein can also be released into the extracellular space, where they may spread to other cells. Vascular abnormalities impair the supply of nutrients and removal of metabolic byproducts, cause microinfarcts, and promote the activation of glial cells (**Figure 1.1)**(19).

1.2.4 Role of glutamate

The ability for thinking and remembering is derived from various input and output pathways between hippocampus and neocortex. These pathways rely on signaling mediated by neurotransmitter glutamate(28). In AD patients, there is a disruption in glutamatergic neurotransmission cycle at the glial cell reuptake of free glutamate from

synapse. Neuropathologic studies have shown decreased levels of glutamate reuptake in the frontal and temporal cortices of AD patients, possibly because of oxidative modification of the glutamate transporter 1 molecule(29).

Figure 1.1 Clearance pathway of Aβ in normal brain



Also, diminished uptake by vesicular glutamate transporter has been reported in AD patients. These neuropathologic studies, suggest the involvement of glutamatergic system in AD. As per this glutamatergic hypothesis, inefficient removal of free glutamate from synapse results in presence of abnormally high levels of synaptic glutamate under resting conditions. Additionally, as the decrease in vesicular glutamate uptake causes less glutamate to be stored in each vesicle, neurons are left with fewer neurotransmitter molecules to release into the synaptic cleft at the time of neuronal activity(30).

1.2.5 Acetylcholine

In attentional processing, acetylcholine (ACh) plays a major role. The area throughout whole neocortex receive cholinergic inputs from basal forebrain, and thus ACh function

extends beyond attentional processing, and cholinergic signaling has an impact on cognition also(31). The cholinergic hypothesis of AD states that cortical deficiencies in cholinergic neurotransmission contribute to the specific cognitive deficits seen in patients; however, the extent to which it is directly related to cholinergic signaling is not clear(32).

In AD, the activity of choline acetyltransferase, the enzyme involved in ACh synthesis is reduced to 35% to 50% of normal levels. Synaptic reuptake of choline is reduced to 60% (approximately) of normal levels in AD(33). Direct measurement reveals that synthesis level of Ach is decreased by half in affected patients, that can be correlated with increasing severity of dementia in AD patients(34).

1.2.6 Role of NMDA receptor

AD is characterized by loss of synapses, deposition of Aβ plaques, NFTs, and hyperphosphorylated tau(35). These changes are associated with NMDARs activation and oxidative stress which ultimately result in AD pathology. Further, Aβ also triggers NMDA-mediated Ca^{2+} influx, excitotoxicity, and stress-related signaling pathways in neurons which may worsen aging-related increase in oxidative stress, impaired energy metabolism, and defective Ca^{2+} homeostasis(36). NMDARs are cationic channels gated by the neurotransmitter glutamate, which play an essential role in excitatory transmission, synaptic integration, learning and memory in the central nervous system CNS. NMDAR activation, excessive Ca^{2+} fluxes, and free radical generation are associated with synaptic dysfunction and tau phosphorylation(36). Excessive amount of glutamate is associated with intense transient influx of Ca^{2+}, leading to mitochondrial functional impairment characterized by activation of permeability transition pores in the inner mitochondrial membrane, cytochrome c release and depletion of ATP, and simultaneous formation of ROS. Therefore, it is evident that proper NMDAR and

synapse function are necessary for learning and memory, and abnormalities in function of NMDAR and synapse may participate in pathogenesis of AD at synaptic level(30).

1.2.7 Role of Neuropeptides

Several studies of neuropeptide abnormalities have also been reported in AD. The neuropeptides that are considered to be altered in AD include somatostatin, neuropeptide Y, corticotrophin-releasing factor, vasopressin, oxytocin, neurotensin, substance P, vasoactive-intestinal peptide, β-endorphin, α-melanocyte stimulating hormone, cholecystokinin, and galanin (**Table 1.1**)(37, 38).

Table 1.1 Various pathological factors involved in AD

Neuropeptide	Normal Brain	Alzheimer's Disease Brain
Somatostatin	High	Markedly reduced in all areas of cortex
Neuropeptide Y	High	Normal or slightly reduced in cerebral cortex
Corticotrophin-releasing factor	Low	Markedly reduced in all areas of cortex
Vasopressin	Low	Moderately reduced in hippocampus
Oxytocin	Low	Slightly increased in hippocampus
Neurotensin	Low	Normal in hippocampus, low in amygdala and septum
Substance P	Medium	Normal or moderately reduced in cerebral cortex
Vasoactive-intestinal peptide	High	Normal or slightly reduced in cerebral cortex
β-Endorphin	Low	Slightly reduced in cerebral cortex
α-Melanocyte-stimulating hormone	Medium	Reduced in all area of cerebral cortex
Cholecystokinin	High	Normal or slightly reduced in temporal lobe
Galanin	High	Normal or increased in cerebral cortex

1.2.8 Neuroprotective role of Estrogen in Alzheimer's disease

The occurrence of AD is significantly correlated with loss of estrogen in women after menopause. In a study using more than 5,000 brain samples, a significant increase of NFT formation was observed in women compared to men(39). This global change of AD pathology in women appears to be closely associated with greater cognitive loss relative to that in men(40). Recent results indicate that the decline of estrogen levels in

the brain may make neurons more susceptible to age-related neurodegenerative processes, also suggesting the protective roles of estrogen against AD(41). However, the clinical relevance of estrogen-based hormone replacement therapy for AD and cognitive aging remains undetermined. Pathophysiological pathway of AD are illustrated in **Figure 1.2**.

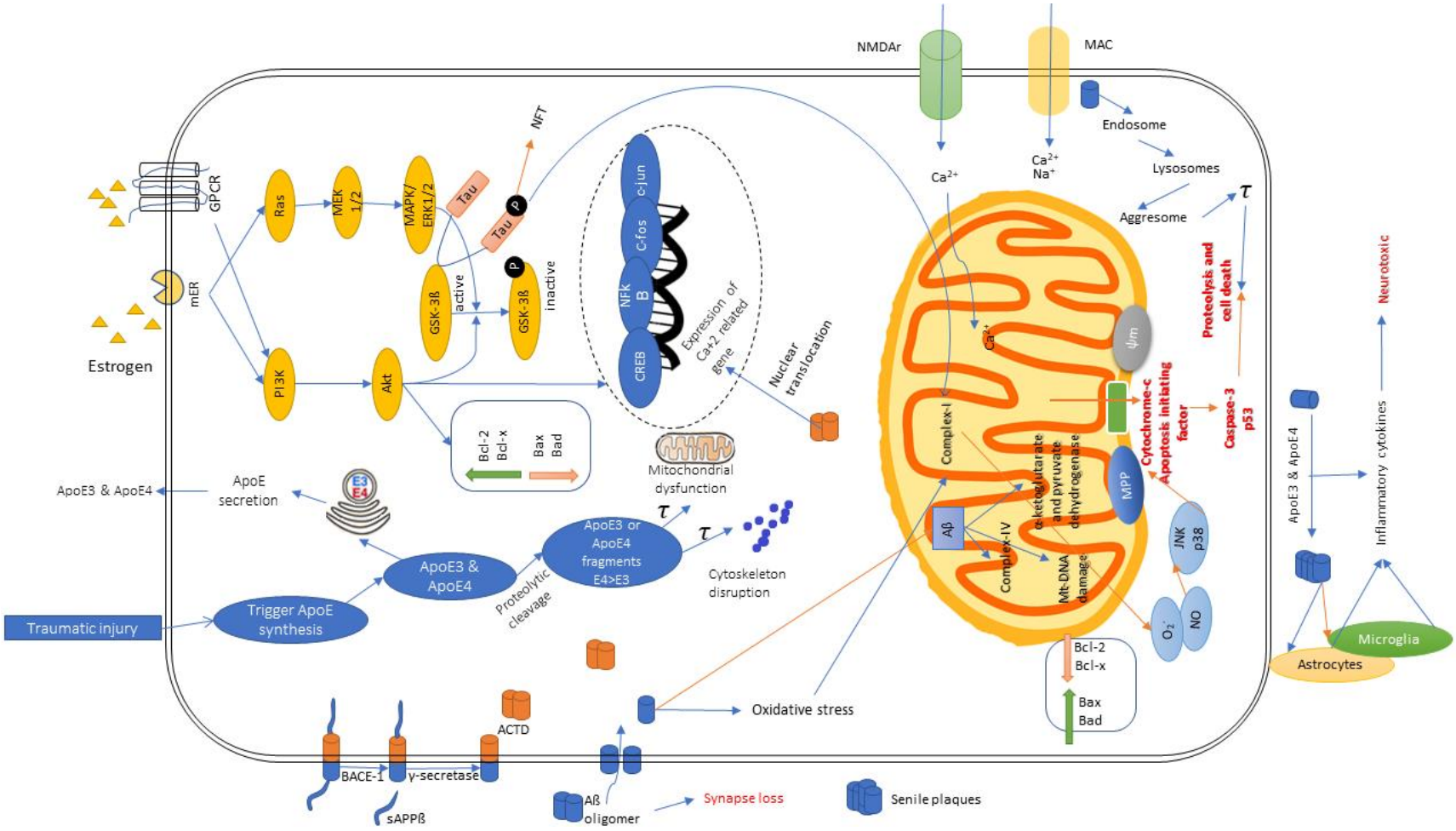
1.3 Stages of Alzheimer's disease

1.3.1 Early stage

The initial stage of AD is often ignored. Relatives, friends, and sometimes professionals as well see it as "old age," just a regular part of aging process. As the inception of disease is gradual, it is difficult to ascertain exactly when it begins(42). The person may:

- have problems talking correctly (language problems)
- have significant loss of memory mainly for things that have just happened
- not beaware the time of day or the day of the week
- become lost in familiar places
- have difficulty in decision making
- become inactive and unmotivated
- show mood changes, depression or anxiety
- react unusually or aggressively on occasion and loss of interest in activities

Figure 1.2 Various pathophysiological pathways of Alzheimer's disease



1.3.2 Middle stage

As the disease progresses, limitations become more explicit and restricting(43). The person with dementia has difficulty with everyday living and:

- may become very forgetful, especially of recent events and people's names
- can no longer manage to live unaccompanied without problems
- is unable to cook, clean or shop
- may become hugely dependent on family members and caregivers
- need help with personal hygiene, *i.e.*, washing and dressing
- has increased difficulty with talking
- shows problems with wandering and other behavior such as repeated questioning and calling out, clinging and disturbed sleeping
- becomes lost at home as well as outside
- may have hallucinations

1.3.3 Late stage

The late stage is nearly total dependence and idleness. Memory disturbances become severe, and the physical side of the disease are more apparent. The person may:

- have difficulty in eating
- be incapable of communicating
- not recognize relatives, friends and familiar objects
- have trouble in understanding what is going on around them
- be unable to find his or her way around in the home
- have trouble in walking
- have difficulty in swallowing
- have bladder and bowel incontinence
- display inappropriate behavior in public

- be confined to a wheelchair or bed

1.4 Treatment of Alzheimer's disease

1.4.1 Pharmacological treatment

A significant treatment approach has involved attempts to increase the cholinergic function of the brain.

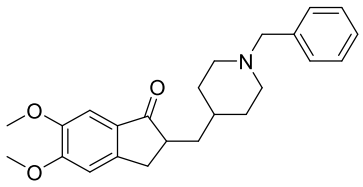
1.4.1.1 Precursors of acetylcholine synthesis

This was an early approach and involved use of choline chloride and phosphatidylcholine (lecithin), The precursors for synthesis of ACh, but they didn't show any clinically significant efficacy.

1.4.1.2 Acetylcholinesterase (AChE) inhibitors

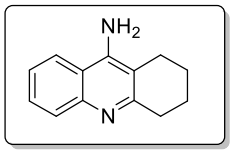
AChE enzyme is responsible for the catabolism of Ach, physostigmine is a reversible AChE inhibitor, but its use is limited due to short half-life and tendency to produce symptoms of systemic cholinergic excess at therapeutic doses(44). Four AChE inhibitors, currently approved by the FDA, for treatment of AD are tacrine (Now withdrawn), donepezil, rivastigmine, and galantamine. Tacrine is a potent centrally-acting inhibitor of AChE(45). Its side effects are significant and dose-limiting and include abdominal cramping, anorexia, nausea, vomiting, diarrhea, and elevations of serum transaminases. Thus, it is not used often clinically. Donepezil is a selective inhibitor of AChE in the CNS and little effect on AChE in peripheral tissues(45). Rivastigmine and galantamine are dosed twice daily and produce similar degree of cognitive improvement. Adverse effects associated with donepezil, rivastigmine, and galantamine are like tacrine, but less frequent and severe and include nausea, diarrhea, vomiting, and insomnia(46). Further, unlike tacrine, they are not associated with hepatotoxicity (**Figure 1.3**).

Figure 1.3 FDA approved AChE inhibitors for Alzheimer's disease.



Donepezil (piperidine)

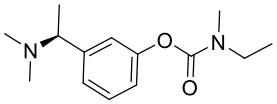
non-competitive and reversible inhibitor



Tacrine (aminoacridine)

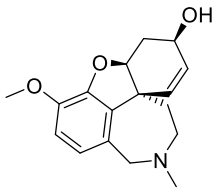
mixed competitive/noncompetitive inhibitor

Withdrawal from market due to toxicity



Rivastigmine (carbamate)

non-competitive and reversible inhibitor



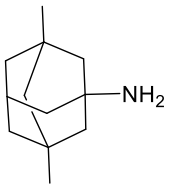
Galanthamine (tertiary alkaloid)

reversible, competitive inhibitor

1.4.1.3 NMDA receptor antagonist

It is an alternative strategy for treatment of AD and includes the use of memantine(47). It produces a dose-dependent blockade of NMDA receptors. Adverse effects of memantine are usually mild, reversible and may include headache or dizziness (**Figure 1.4**).

Figure 1.4 Memantine as NMDA receptor antagonist used in the treatment of Alzheimer's disease.



Memantine

1.4.1.4 Matrix Metalloproteinases (MMP 2 & 9) inhibitors

In normal human brain, the matrix metalloproteinase (MMPs) levels are quite low. However, it is reported to be upregulated during neurological disorders including AD and after injuries. The altered MMPs expression in AD had been reported previously(48). Brkie *et al.,* measured the expression of MMP gene in choroid plexus epithelial (CP) and cerebrospinal fluid (CSF) to establish a correlation between MMPs and Aβ induced blood-CSF barrier (BCSFB) leakage. An increase in MMPs activity was observed simultaneously in CSF with $A\beta_{1-42}$ oligomer injection. Intracerebroventricular co-treatment of Aβ oligomers with GM6001, a non-selective MMP inhibitor, could reduce the Aβ oligomers mediated BCSFB disruption(49). It was found that MMP-2 and 9 were engaged in different pathological cascade of AD(50). The role of MMP-9 in Aβ promoted neurotoxicity in AD has not been understood completely(51).

1.4.2 Non-pharmacological therapy for AD

These therapies does not involve medication and are often used with a goal of maintaining or improving cognitive function, the ability to perform activities of daily living or overall quality of life. They may also be used with the aim of decreasing behavioral symptoms like depression, apathy, wandering, sleep disturbances, agitation and aggression. The examples include computerized memory training, listening to favorite music as a way to stir recall, and incorporating special lighting to lessen sleep disorders. The non-pharmacologic therapies have not been shown to alter the course of AD, as with current pharmacologic therapies. The reviews and meta-analysis of non-pharmacologic therapies, tested in randomized controlled trials, have found that some are beneficial to people with Alzheimer's dementia. Among these are exercise and cognitive stimulation. A meta-analysis reported that aerobic exercise and a combination

of aerobic and non-aerobic exercise can improve cognitive function. Further, systematic review found that exercise has a positive effect on overall cognitive function, some aspects of well-being and is associated with slower rate of cognitive decline in people with Alzheimer's dementia(52).

1.4.2.1 Vagus nerve stimulation

The basis behind effectiveness of VNS, on cognition, is nucleus of solitary tract in brain that is the main rely station for afferent vagal nerve fibers(53). This nucleus has widespread projections to many area in forebrain and brain stem including amygdala and hippocampus that are involved in learning and memory function(54). VNS cause changes in electrophysiology and metabolic profile of these structures.

1.4.2.2 Deep brain stimulation

It is one of the neuromodulation approaches considered for AD. It modulates the activity of neural elements by implanted electrodes in a key brain region with an internal pulse generator(55). The use of DBS in AD is based on rationale that apart from neurodegenerative disorder, AD can also be considered as neural circuit disorder because it affects several integrated cortical and subcortical pathways involved in memory and cognition(56).