Chapter 1

Introduction

1. Introduction

1.1 Neurodegenerative disorders: Alzheimer's Disease

The brain is the most crucial organ in the human body. It controls and coordinates every actions and reaction, permits us to think and feel, and enables us to have feelings and memories, all the things that make us human. Weighing in at 1400 grams, spongy mass of fat and protein is made up of two principal types of cell called neurons and glia each numbering in billions. Additionally, it is made up of special protective layer consist of tightly bound cells that function as a semi-permeable gate, i.e, blood brain barrier (BBB). It maintains the brain atmosphere safe from toxins and harmful substances entering through normal blood circulation [Hofman 2014].

Aging and oxidative stress are key aspects that caused neuronal damage/ degeneration in the brain. Neurodegeneration is a feature of many other debilitating, incurable diseases that are rapidly rising viz. Alzheimer's disease (AD) and Parkinson's disease (PD) [Dugger and Dickson 2017]. The diseases represent an intriguing burden to global population. These age-dependent disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years. Effective therapeutic approaches are dreadfully required, but it would be possible only with intense comprehension of pathological conditions and mechanism of the disease [Gan et al. 2018].

Cognitive deficits occur not only in AD, but also in vascular dementia, frontotemporal dementia (FTD), mixed dementia, and dementia with Lewy bodies (LBD). The pyramidal and extrapyramidal systems are affected in amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and spinocerebellar ataxias (SCAs) [Kumar and Dogra 2008, Magalingam et al. 2018]. Despite their

multiple clinical events, reflecting the loss of neurons and synapses in distinct brain regions, these diseases share common features and pathways.

One such mechanism is the aggregation of nuclear or cytosolic proteins. These include beta-amyloid (Aβ) plaques and inclusions of hyperphosphorylated tau in AD and other tauopathies, aggregates of α -synuclein in PD and other synucleinopathies, inclusions of TAR DNA-binding protein (TDP)-43 in ALS and FTD, and polyglutamine protein aggregates in HD and other CAG-polyglutamine repeat diseases. Abnormalities in protein conformations and their cellular and neuroanatomical distribution establish the major histopathologic features that are essential in making a specific neuropathologic diagnosis [Auld et al. 2002, Hardy 2009].

AD is acknowledged as a progressive multi-factorial neurodegenerative disorder, leads to the progressive loss of functional, mental and behavioral decline. The disease progresses symptomatically from mild to severe and found its place among the eight topmost health complications worldwide. One of the upsetting aspects of the AD is the loss of cognitive abilities that currently accounts for 50 million cases worldwide and the proportion of deaths related to AD is going up. The extensive challenge which AD possesses to healthcare system makes it as crucial for the researchers to develop new therapeutic strategies to fight this disease. Till now, there is no proven way to prevent AD [Bhardwaj et al. 2017].

1.2 Pathophysiology of AD

Since the time of Dr. Alois Alzheimer, worldwide, neuropathologists have recognized Aβ plaques and neurofibrillary tangles (NFTs) in the autopsied brains of AD patients, signifying that these pathologies are root causes for AD. These plaques damage /disrupt the synapse and neuronal circuits. Numerous hypotheses have been established on the

basis of the various contributing factors in order to study this multifactorial disorder (Figure 1.1), such as the cholinergic hypothesis, Aβ hypothesis, tau hypothesis and inflammation hypothesis [Alzheimer's and Dementia 2019, Kumar and Singh 2015].

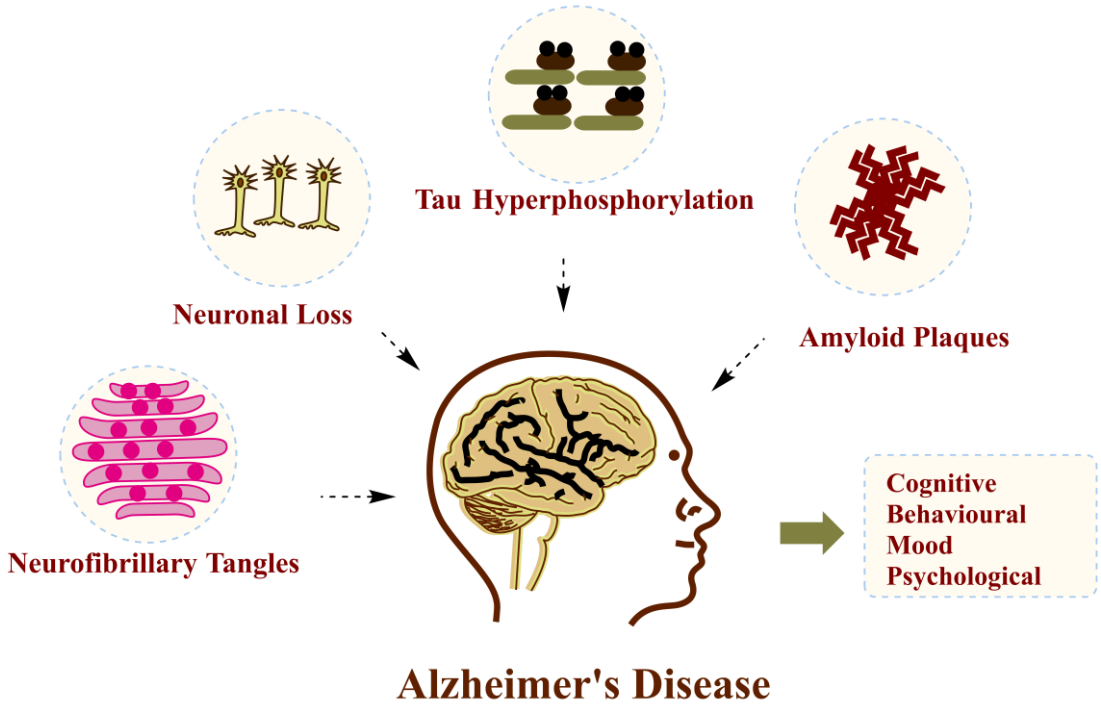


Figure 1.1. Symptoms and risk factors of AD.

Cholinergic neurons are highly essential for function of brain and maintenance of excitation-inhibition balance within neural circuits. Although cholinergic neurons are distributed in various discrete regions, they can project to almost all parts of the brain. These cells release a neurotransmitter called acetylcholine (ACh), which plays crucial role in the regulation of sensory functions, behavioral flexibility, and associative learning. In AD, there is marked deficiency of ACh due to atrophy and degeneration of subcortical cholinergic neurons, mainly those present in basal forebrain, that provides cholinergic innervation to the whole cerebral cortex [Francis et al. 1999, Hampel et al. 2019, Terry et al. 2003].

According to the Aβ hypothesis, the amyloid precursor protein (APP) is typically cleaved by α-secretase and processed by β- and γ-secretases (Figure 1.2) resulting in an imbalance between production and clearance of Aβ peptide. As a result, Aβ peptides voluntarily aggregate into soluble oligomers and unite to form insoluble fibrils and are eventually accumulated in diffuse senile plaques. Some recent studies demonstrated that Aβ (42) oligomers are produced supportive activities of both neurons and its associated astrocytes. It has been observed that Aβ (42) oligomers promote oxidative damage and tau hyperphosphorylation, results in harmful effects on synapses and mitochondria [Adeniji et al. 2017, Hillen 2019, Karran et al. 2011]. Tau is the microtubule associated protein (MAP) of a mature neuron. Tau protein is most copiously expressed in axons of central nervous system neurons but can also be found in the somatodendritic compartment of neurons, oligodendrocytes, and non-neural tissues. The vital functions of tau are to promote assembly and to maintain structure of microtubules [Jouanne et al. 2017].

The most common post-translational modifications of tau proteins are phosphorylation and O-glycosylation. Phosphorylation changes the shape of tau protein and controls its biological activity[Gong et al. 2005, Martin et al. 2011]. Most of the phosphorylation sites are on Ser-Pro and Thr-Pro motives, but a number of other sites have also been reported. Abnormally hyperphosphorylated tau and their incidence in the neocortex is root cause to develop dementia. In AD, brain tau is three to four-fold more hyperphosphorylated than the normal healthy brain tau and in this hyperphosphorylated state it is polymerized into paired helical filaments (PHF) mixed with straight filaments (SF) forming NFTs[Beharry et al. 2014].

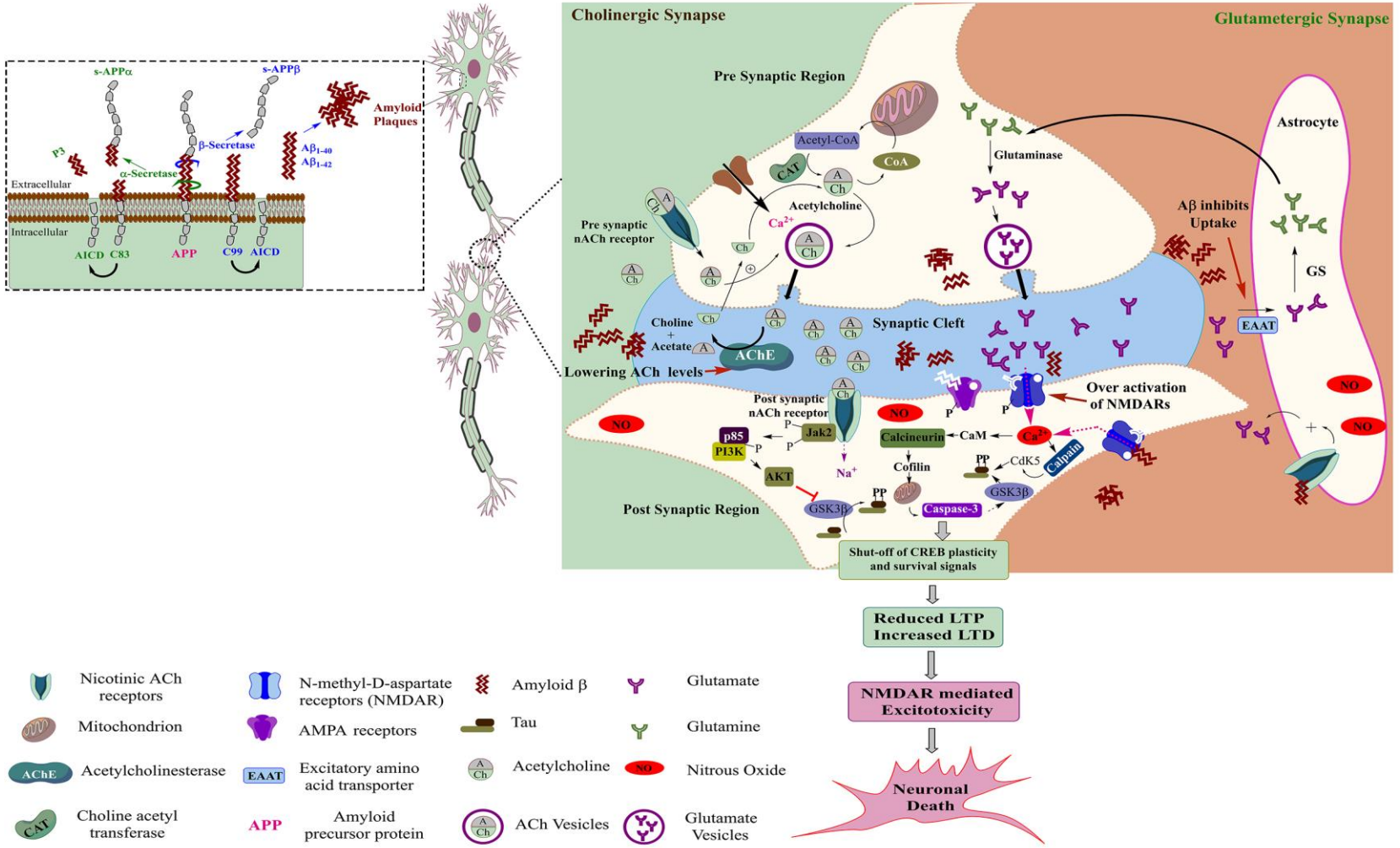


Figure 1.2. Summary of the main pathogenesis hypotheses for AD and treatment strategies.

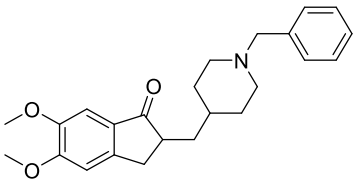
The abnormally hyperphosphorylated tau in AD brain is distinguished from transiently hyperphosphorylated tau by its ability to sequester normal tau, MAP-1 and MAP-2 and disrupt microtubules, and to self-assemble into PHF/SF[Kontaxi et al. 2017]. The majority of tau-based therapeutic approaches against neurodegeneration have focused on modulating tau phosphorylation, given that tau species present within NFTs are hyperphosphorylated[Marcus and Schachter 2011].

1.3 Therapeutic drug targets in AD

The symptoms of AD, cognitive decline and impairment in learning and memory are due to deficit of the neurotransmitter ACh in the brain. The activity of enzymes involved in the synthesis (choline acetyltransferase (CAT) and degradation (acetylcholinesterase (AChE) of ACh were significantly reduced in patients with endstage AD [Davies and Maloney 1976]. Cholinergic abnormalities including variations in reduced choline uptake, ACh release, nicotinic and muscarinic receptor expression, neurotrophin support, loss of cholinergic perikarya and axonal transport may all contribute to development of AD. These studies, composed with the emerging role of ACh in learning and memory, led to the “cholinergic hypothesis of AD” [Francis et al. 1999].

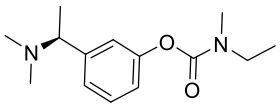
Glutamate is the excitant neurotransmitter in the brain, involved in the excitatory postsynaptic transmission through ionotropic and metabotropic glutamate receptors. There are three classes of glutamate-gated channels and a group of G-protein coupled glutamate receptors (which cause mobilization of Ca^{2+} from internal stores) named according to their activating synthetic agonist: the α-amino 3-hydroxy 5-methyl 4-isoxazole-propionic acid (AMPA) activated receptors, kainate activated receptors, and the N-methyl D-aspartate (NMDA) receptors, have great importance in long-term

adaptive processes. Among these, the ion channels coupled to classical NMDARs are generally the most permeable to Ca^{2+}, that can in turn function as a second messenger in various signaling pathways[Benarroch 2018]. NMDARs plays a essential role in the synaptic transmission and synaptic plasticity thought to underlie learning and memory.



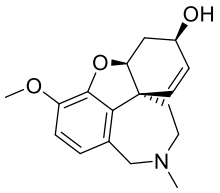
Donepezil (piperidine)

non-competitive and reversible inhibitor



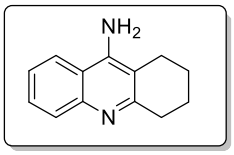
Rivastigmine (carbamate)

non-competitive and reversible inhibitor



Galantamine (tertiary alkaloid)

reversible, competitive inhibitor

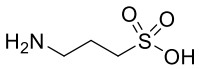


Tacrine (aminoacridine)

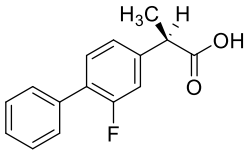
mixed competitive/noncompetitive inhibitor

Withdrawal from market due to toxicity

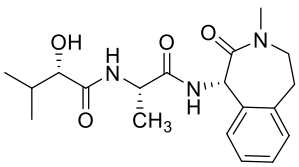
(A) Cholinesterase inhibitors



Tramiprosate



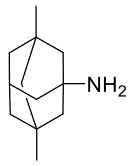
Tarenflurbil



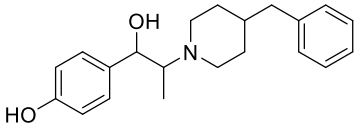
Semagacestat

Discontinued from Phase III clinical trails

(B) Aβ aggregation inhibitors



Memantine



Ifenprodil

(C) NMDA receptor antagonists

Figure 1.3. Chemical compounds used for the treatment of AD.

Impaired synaptic plasticity and dendritic loss in excitatory glutamatergic synapses are primary events in early stage of AD. These synaptic abnormalities are triggered by accumulation of soluble fibrillary β-amyloid (Aβ) oligomers, which bind to several postsynaptic and presynaptic partners. Many of the synaptic effects of Aβ oligomers involve NMDARs and type 1 metabotropic glutamate receptor 5 (mGluR5).

1.4 Current clinical drugs for the treatment of AD

To date, established treatments are only for symptomatic releif, trying to equalize the neurotransmitter disturbance of the disease. Three cholinesterase inhibitors (CIs) are approved for the treatment of mild to moderate AD. A further therapeutic option available for moderate to severe AD is memantine. At the same time antipsychotic and antidepressant treatments are used for the behavioral symptoms of the disease[Areosa et al. 2005, Ballard and Corbett 2010, Birks 2006].

Tacrine, a competitive acetylcholinesterase inhibitor (AChEI) and the first drug to be approved for use in AD by the US-FDA in 1993, was withdrawn from the market in 2013 due to the high incidence of side effects, mostly derived from hepatotoxicity [Qizilbash et al. 1998].

DNZ is a reversible noncompetitive CI approved for treatment of AD and currently the most commonly prescribed drug for the treatment of AD. DNZ is extremely selective for AChE over BuChE activity (405:1). Compared to other approved AChEI, DNZ is similarly effective in improving cognitive and functional decline in AD with comparable safety and tolerability[Birks and Harvey 2018, Jackson et al. 2004]. Rivastigmine, a non-selective pseudoreversible ChE inhibitor, has been reported to have less side effects as well as positive benefit after administration to mild to moderate AD patients[Birks and Evans 2015]. Galantamine, a weak competitive reversible AChEI is

also a potent allosteric modulator of nicotinic acetylcholine receptors in certain areas of the brain, and potentiates the effects of orthoesteric agonists[Akk and Steinbach 2005, Dajas-Bailador et al. 2003].

MMT is a glutamatergic agent, the first and only NMDA receptor antagonist approved by FDA in 2003 for the treatment of moderate-to-severe AD and dementia. Memantine binds to NMDA receptors with a low-micromolar IC_{50} value with strong voltage dependency and rapid unblocking properties[Parsons et al. 2007]. To date, MMT is the only NMDAR antagonist that is clinicallyapproved and preferentially acts as an antagonist of non-synaptic NMDARs. The low affinity antagonist MMT will be the focus of growing interest in targeting extrasynaptic GluN2B containing NMDARs. The GluN2B-containing NMDARs selective inhibition may be beneficial because it prevents neuronal cell death but leaves the protective pathways intact. Therefore, ideal neuroprotective drugs for AD should aim at both the enhancement of synaptic activity and the disruption of extrasynaptic GluN2B-containing NMDAR-dependent excitotoxicity. The non-selective NMDA antagonists cannot be effective as neuroprotective agents[Wang and Reddy 2017, Wang et al. 2013, Wu et al. 2009].

The lack of therapeutic effectiveness of the current available drugs based on the single-target paradigm for the treatment of AD prompted the search of multi targetdirected ligands (MTDLs), designed by various modern medicinal chemistry approaches of different pharmacophoric moieties from well-known bioactive molecules, able to bind to multiple targets associated with AD[Ramsay et al. 2018, Zhang et al. 2017, Zhou et al. 2019].