Section II

Chapter 1: Introduction

1. Introduction

Central nervous system (CNS) is the part of the nervous system that functions to coordinate the activities of all parts of the bodies of multicellular organisms. It consists of the brain and spinal cord. The brain is an assembly of inter-connected neural system that regulates its own activity in a dynamic and complex fashion largely through intercellular chemical neurotransmission.[1] Neurological disorders represent an enormous disease burden, in terms of human suffering and economic cost. A neurological disorder is a disorder of the nervous system of the body that causes structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves and results in a range of symptoms. Examples of symptoms include paralysis, muscle weakness, poor coordination, loss of sensation, seizures, confusion, pain and altered levels of consciousness.

Neurological disorders are developmental, neurodegenerative or psychiatric in nature. Neurodegeneration is the umbrella term for the progressive structural or functional loss of neurons, including death of neurons. The process of neurodegeneration is not well-understood. The greatest risk factor for neurodegenerative diseases is aging. Mitochondrial DNA mutations as well as oxidative stress both contribute to aging.[2] Many of these diseases are of late-onset in nature, meaning there are some factors that change as a person ages.[3] One common factor is that in each disease, neurons gradually lose functions as the disease progresses with age.

Neurological diseases like Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis occur as a result of neurodegenerative processes.[3,4] Among these, Alzheimer's disease is the most common age related neurodegenerative disease. Once this disease begins, it causes progressive destruction of brain cells, which leads to decline in memory and cognitive functions as well as changes in personality and behaviour.[5]

1.1 Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder with a mean duration of around 8.5 years between onset of clinical symptoms and death. Alzheimer's disease leads to nerve cell death and tissue loss throughout the brain. Over a period of time, the brain shrinks dramatically, affecting nearly all its functions. In the brain of Alzheimer's patient the cortex shrivels up, damaging areas involved in thinking, planning

and remembering. This shrinkage is especially severe in the hippocampus, an area of the cortex that plays a key role in formation of new memories. Also ventricles (fluid-filled spaces within the brain) grow larger (Fig. 1.1a).

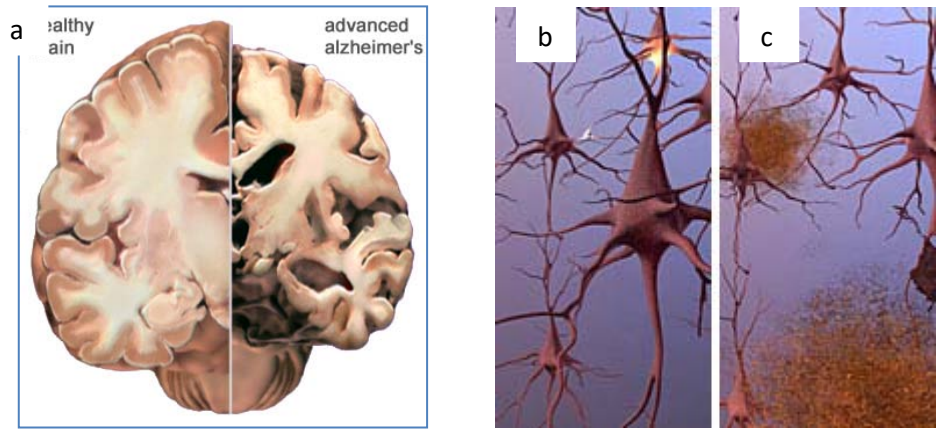

Fig. 1.1 (a) Massive changes in advanced Alzhiemer's brain showing *shriveled cortex*, *shrunk hippocampus* & *larger ventricles.* (b) Normal Neurons and (c) Neuronal tissue showing *Plaques* & *Tangles.*

Microscopy of the brain tissue (affected by AD) shows the presence of dense β-amyloid plaques & neurofibrillary tangles (Fig. 1.1c) compared to the normal brain tissue (Fig. 1.1b). Alzheimer's tissue has fewer nerve cells and synapses than a healthy brain. Plaques and abnormal clusters of protein fragments build up between nerve cells. Dead and dying nerve cells contain *tangles*, which are made up of twisted strands of another protein.[3]

1.2 Treatment strategies in Alzheimer's disease

For the treatment of Alzheimer's disease, a number of strategies are adopted currently and one of them is to control AD through NMDA receptor antagonism (glutamate hypothesis) that we have discussed in Part I. Another stretegy to fight the disease is through the widely accepted hypothesis "chlorinergic hypothesis". In cholinergic hypothesis, majority of the molecules work by inhibiting cholinesterase enzyme but unfortunately the currently available cholinesterase inhibitors are having little control over AD. As a result, research continues to find new and better drug candidates.

While there is no cure for AD, U. S. Food and Drug Administration (USFDA) approved five drugs to treat its symptoms. Among them donepezil, galantamine, rivastigmine and tacrine are "cholinesterase inhibitors". These drugs prevent the breakdown of acetylcholine a chemical messenger present in the brain, vital for learning

and memory. In nut shell we can say that there are only a few drugs available in the market for the management of AD which belong to two different classes of compounds as given below.

1 NMDA receptor antagonist (e.g. Memantine)
2 Cholinesterase inhibitors (e.g. Donepezil, Galantamine, Rivastigmine and Tacrine)

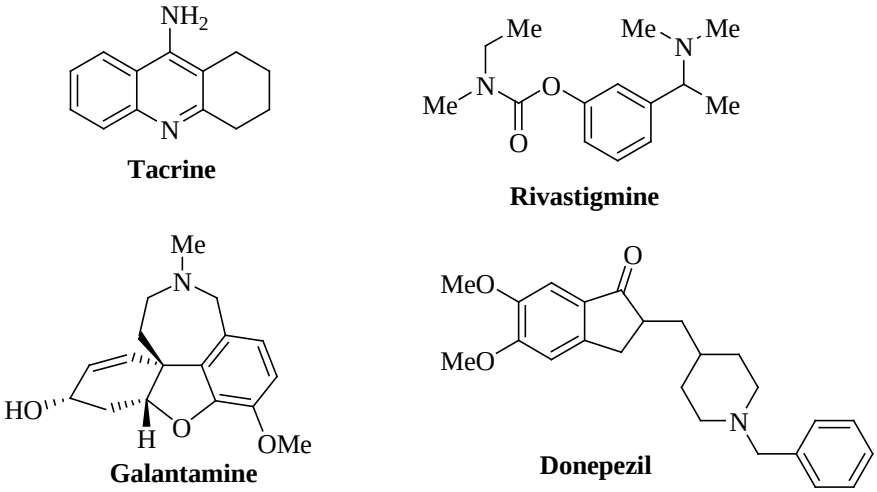

Fig.1.2: FDA approved anticholinesterase drugs for Alzhiemer's disease

NMDA receptor antagonists have already been discussed in the earlier section. In this part cholinesterase inhibitors working through the 'Cholinergic Hypothesis' would be discussed.

1.3 Chlorinergic hypothesis

As per this hypothesis, level of acetylcholine decreases below normal level in Alzheimer's disease. Level of acetylcholine is increased to normal level in certain areas of brain by reducing its hydrolysis by acetylcholinesterase (AChE) enzyme. AChE is an important enzyme that breaks down acetylcholine in the synaptic cleft in neuronal junctions. Inhibition of this enzyme is connected with treatment of several diseases such as Alzheimer's disease, myasthenia gravis, glaucoma and anthelmintic drugs and for the control of insects. Currently available AChE inhibitors in clinical use for the treatment of AD don't have the ability to stop the progress of the disease.

In Alzheimer's disease, many regions of the brain particularly the neocortex and hippocampus, are most badly affected by some characteristic pathology. The pathological

changes in the disease are characterised by extracellular deposition of β-amyloid in senile plaques, intracellular formation of neurofibrillary tangles and the loss of neuronal synapses and pyramidal neurons. These alteration marks in the development of typical symptoms of AD are characterised by impairments of cognitive functions and behavioural disturbances.[6] When systematic biochemical investigations of the brains of AD patients began in the late 1960s and early 1970s, the hope was that a clearly defined neurochemical abnormality would be identified which would provide the basis for the exploration of therapeutic interventions. It was observed in the mid 1970s that there occurred a deficit in the enzyme responsible for the synthesis of acetylcholine (ACh), choline acetyltransferase (ChAT).[7-9] Some reports also claimed that the deficit of presynaptic cholinergic neurotransmitter was due to reduced choline uptake,[10] ACh release[11] and loss of cholinergic perikarya from the nucleus basalis of Meynert.[12] These studies found the basis of "cholinergic hypothesis of Alzheimers disease" in association with the emerging role of ACh in learning and memory.[13] Thus it was proposed that depletion of cholinergic neurons in the basal forebrain along with the loss of cholinergic neurotransmission in the cerebral cortex and other areas significantly affected the cognitive functions in AD patients.[14]

As per cholinergic hypothesis any drug that potentiates central cholinergic activity will control cognition and behavioural problems experienced in AD. As per cholinergic hypothesis, a number of approaches were tried for the treatment of the cholinergic deficit in AD, one of them was replacement of ACh precursors (choline or lecithin) but these agents failed to increase central cholinergic activity. Another widely accepted approach in this hypothesis was the use of cholinesterase (ChE) inhibitors like physostigmine that reduced the hydrolysis of ACh. The newer compounds to target AD include specific M1 muscarinic or nicotinic agonists, M2 muscarinic antagonists, or improved "second generation" ChE inhibitors.[15] Transplantation of ACh rich foetal tissue grafts is another approach, which has been shown to improve the cognitive performance of primates after excitotoxic lesions of cholinergic nuclei.[16] Thus, such approaches may offer additional future possibilities for the treatment for AD; the use of ChE inhibitors is the most well developed and accepted approach to the disease.

1.4 Cholinesterase inhibitors

Certain brain cells release acetylcholine when some massage is to be transferred to the other cells. After receiving the massage by the recipient cells, metabolic enzyme

acetylcholinesterase (AChE) helps to metabolize the secreted acetylcholine in order to maintain the proper functioning of signal transduction. The enzyme AChE also known as acetylhydrolase, is a serine protease that breaks down acetylcholine into choline and acetic acid to terminate neurotransmission at the cholinergic synapses and the choline is further recycled for the next event. In AD there occurs damage and sometimes death of the cells that produce acetylcholine required for the process of signal transduction for transferring massages. Cholinesterase inhibitors also offer some other beneficial effects e.g. galanatamine stimulates ACh release in certain nerve cells and rivastigmine blocks the activity of other enzymes responsible for ACh breakdown.

The beneficial effects of cholinesterase inhibitors are small and vary from person to person, however, cholinesterase inhibitors slow down or delay worsening of symptoms. There is no evidence suggesting benefits of combination therapy in AD but individuals taking extended release dosage forms of cholinesterase inhibitors (ChEIs) experienced better results in moderate to severe disease. ChEIs can improve quality of life for patients with AD and their caregivers by delaying the progression of AD symptoms, for a few months. But the maximum benefit from these agents can only be achieved when medication schedules are fully adhered to by the patient and caregivers.[17] Cholinesterase inhibitors are well tolerated with some commonly occurring side effects i.e. nausea, vomiting, loss of appetite and increased frequency of bowel movements.

Cholinesterase inhibitors are prescribed to treat symptoms related to memory, thinking, language, judgment and other thought processes. Four drugs approved by FDA show their effects in different stages of AD and are commonly prescribed depending on the condition of the patient. Donepezil is approved to treat all stages of AD while rivastigmine and galantamine are approved for mild to moderate stages of AD. Tacrine was the first cholinesterase inhibitor that was approved in 1993 but is rarely prescribed today because of its common side effect i.e. liver damage.

Tacrine, an aminoacridine analog shows various activities such as monoamine oxidase (MAO) inhibition, potassium channel blockade and interaction with muscarinic and nicotinic receptors. However its most prominent action is as a centrally acting reversible cholinesterase inhibitor. The drug is rapidly taken up by the liver during first pass metabolism and cleared rapidly.[18,19] Tacrine has very low bioavailability when taken orally due to its rapid hepatic hydroxylation but its half-life can be increased by higher and

multiple dosing. Relationship of tacrine between administered dosage and its bioavailability is not proportional e.g. doubling the dose may triple or quadruple the bioavailability. Penetration of tacrine into the brain is very rapid where its concentration is tenfold higher compared to that in plasma.[19,20] Tacrine's pharmacokinetics shows dose nonlinearity, extensive distribution, and rapid elimination through hepatic transformation, mainly into the hydroxyl metabolite velnacrine. A large number of clinical studies of AD patients reported for the efficacy of tacrine on the progression of AD suggested that when tacrin was given and tolerated at a high dose of >80 mg/day, patients were less likely to be hospitalised compared to those on lower doses or those who discontinued the drug.[21-23] Among the patients who received tacrine in clinical trials, nearly half of them had serum alanine aminotransferase (ALT) levels greater than the upper limit. After discontinuation of tacrine the elevated ALT level got decreased. Hence, careful monitoring of ALT levels was necessary for all patients on tacrine.[24]

Donepezil is a specifically designed piperidine derivative with reversible acetylcholinesterase inhibitory activity. Donepezil shows higher specificity for acetylcholinesterase enzyme compared to tacrine[25] and lacks peripheral anticholinesterase activity in tissues such as cardiac and smooth muscles of the gut.[26] The drug shows linear pharmacokinetics which is an advantage over tacrine. Also donepezil displays longer half-life of over 70 hr,[27] get metabolised by hepatic cytochrome P450 system and exhibits slow excretion through kidneys.[28] When the drug was first launched, the major problem was its cost rather than its efficacy. Later on various clinical studies were performed with 1, 2, 5 and 10 mg/day doses that showed dose dependent improvements in the patients in which 10 mg dose was well tolerated with better efficacy.[29] The drug showed no additional adverse events even after 98 weeks clinical trial duration, except for some common side effects like gastrointestinal disturbances which were common for donepezil.

One of the benzazepine derivatives, galantamine was introduced into the market for the treatment of mild to moderate vascular dementia and AD.[30] Galantamine is a potent allosteric potentiating ligand of human nicotinic acetylcholine receptors (nAChRs) in certain areas of the brain, as well as a weak competitive and reversible cholinesterase inhibitor in all areas of the body.[31] Absorption of galantamine is rapid and complete with a half-life of seven hours. Through nAChRs, galantamine increases acetylcholine release along with its cholinesterase inhibitory action and thereby increases action of acetylcholine. 75 % of the drug is metabolised in the liver through hepatic CYP2D6 and

CYP3A4 enzymes. Side effects of galantamine are very similar to that of other cholinesterase inhibitors, i.e GI disturbances. Probably, other cholinesterase inhibitors are better tolerated practically but, careful and gradual administration of galantamine over more than three months may lead to equivalent long-term tolerability.[32]

Rivastigmine, a newer brain selective carbamate is an AChE inhibitor known as a 'pseudo-irreversible' inhibitor because it mimics ACh by binding with the enzyme AChE forming a carbamylated complex. Rivastigmine prevents enzyme-catalysed hydrolysis of ACh for several hours after the drug has been eliminated from the plasma. Hence, with a half-life of 1h only, rivastigmine has a duration of action of about 10 h.[33,34] Rivastigmine exhibits marked CNS selectivity like donepezil, showing specific effect in the cortex and hippocampus.[35] The plasma protein binding of the drug is less and it is inactivated by cleavage during the enzyme inhibition so, the drug is rapidly excreted through the kidneys. Clinical trials of the drug in AD patients confirm better results with low (4 mg/day) to higher (6-12 mg/day) doses. Rivastigmine showed marked adverse effects, increased risk for mortality convulsions and changes in ECG. Nausea and vomiting are common for rivastigmine along with other side effects like fatigue, asthenia, dizziness and somnolence.[36]

1.5 Quinazolinones in the treatment of AD

Pharmacologic treatments employ medication to slow or stop an illness or treat its symptoms. All approved drugs, temporarily improve symptoms of Alzheimer's disease by increasing the amount of neurotransmitters in the brain. The effectiveness of these drugs varies from person to person. However, none of the treatments available today for Alzheimer's disease slows or stops the damage to neurons that causes Alzheimer's symptoms and eventually makes the disease fatal. Many factors contribute to the difficulty in developing effective treatments for AD. These factors include the high cost of drug development, the relatively long time needed to observe disease progression in Alzheimer's, and the structure of the brain, which is protected by the blood-brain barrier through which a few drugs can cross. Hence there is an urgent need for the development of new molecules for the treatment of AD, which could offer better control and cost effectiveness.

Heterocycles are among the earliest organic compounds, recognised as discrete substances which gained much interest among the chemists worldwide for their diversified

biological activities. 4(3*H*)-Quinazolinones and their derivatives constitute an important class of heterocyclic compounds. They occupy an important position in medicinal and pesticide chemistry, presenting a wide range of bioactivities. As medicines, many of them display antifungal,[37] antimicrobial,[38] anti-HIV,[39] antitubercular,[40] anticancer,[41] antiinflammatory,[42] anticonvulsant,[43] antidepressant,[44] hypolipidemic,[45] antiulcer,[46] analgesic[47] or immunotropic activities[48] and are also known to act as thymidylate synthase,[49] poly(ADP-ribose) polymerase (PARP),[50] and protein tyrosine kinase inhibitors.[51] As pesticides, they are used as insecticides,[52] fungicides[53] and antiviral agents[54] such as TMV and CMV inhibitors. In light of the growing number of applications in recent years, there has been an enormous increase in the interest among biologists and chemists about the bioactivity of quinazoline derivatives.