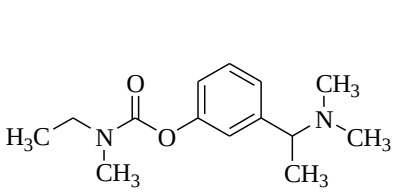
1. Introduction

Alzheimer's disease (AD), one of the major brain diseases, is a complex and progressive neurodegenerative disease which worsens with time. Reports suggest that AD starts 20 years or more prior to appearance of its symptoms, which are generally the small changes in the brain that remain unnoticeable to the affected persons.[1] AD is more prevalent in elderly populations, usually characterized by cognitive impairment with loss of memory, incoherent language and difficulty in learning skills.[2] AD is ranked as the fifth leading cause of death affecting almost 47 million population worldwide, and the number is still rising and is estimated to grow up to 130 million or more by 2050.[3]

Since the discovery of Alzheimer's disease by a German psychiatrist Dr. Alois Alzheimer in the year 1906, researchers have made great efforts to understand and unravel the pathophysiology of AD. However the exact cause of AD still remains uncertain but various causative factors such as misfolding and aggregation of amyloid-β protein, tau protein hyperphosphorylation, oxidative stress, metal ion dyshomeostasis and deficit of acetylcholine levels have been recognized to play important roles in pathophysiology of the disease.



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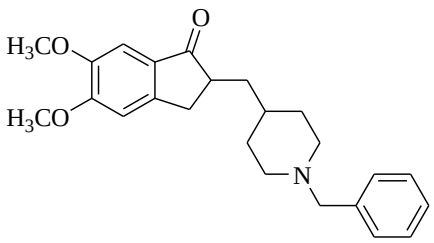

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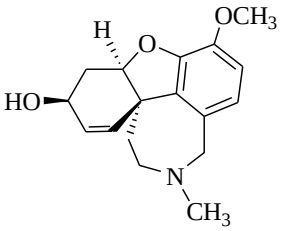

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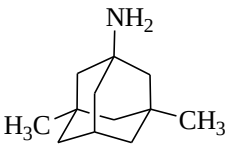

(IV)

Figure 1: Currently marketed drugs for the treatment of AD.

Till date no single drug has been clinically effective to prevent or stop the progress of AD. Currently available drugs in the market for the management of AD (**Figure 1**) include

three acetylcholinesterase inhibitors (AChEIs) viz. rivastigmine (**I**), donepezil (**II**) and galantamine (**III**), and one *N*-methyl-*D*-aspartate receptor (NMDAR) antagonist, memantine (**IV**). These AD treatments are mainly effective to treat mild cognitive impairments (MCI) providing temporary relief from symptoms; however they fail to cure or reverse the progression of AD.[4]

1.1 Risk Factors for AD

In Alzheimer disease attention has been given more to the management of pathological changes and symptoms seen in the brain of AD patients with a little botheration about the risk factors for AD and their management/control. The major non-modifiable risk factors for AD are aging and genetics while the modifiable risk factors include mainly hypertension, diabetes mellitus, hypercholesterolemia, smoking, alcohol, obesity and diet, lack of physical exercise, educational backwardness, and leisure and lack of social activities (**Figure 2**).[5]

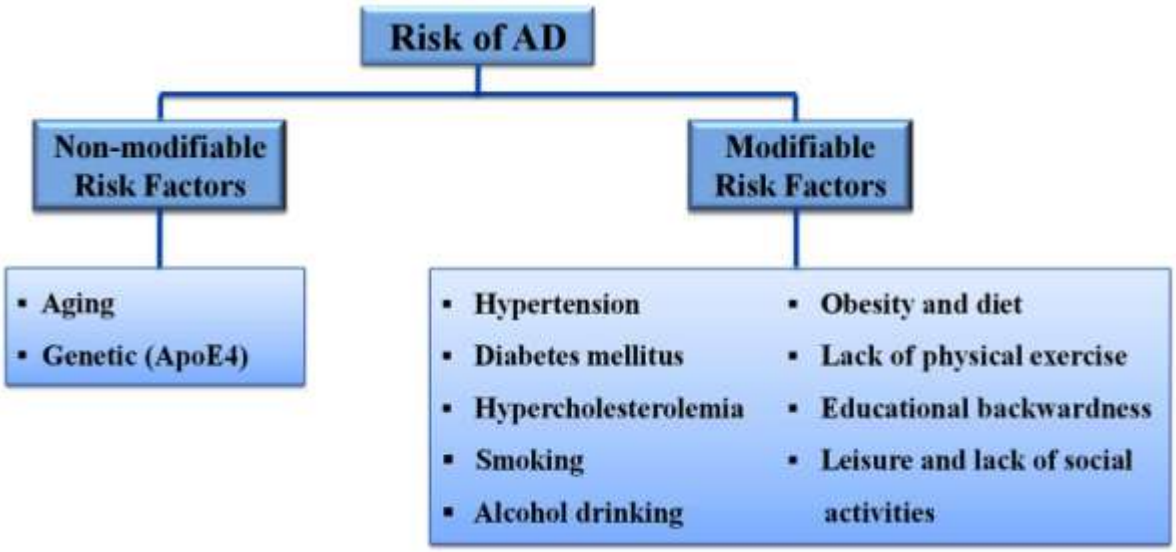

Figure 2: Various non-modifiable and modifiable risk factors for AD.[5]

1.1.1 Non-modifiable risk factors

***Age*:** The risk of AD increases with advanced age. Based on the age of the individuals suffering from AD, AD is of two types, early onset AD (age < 65 years) and late onset AD (age > 65 years). Most of the reported AD cases generally have a late onset whereas the early onset AD cases are very rare. Majority of the early onset cases are familial AD which is caused due to mutations in three genes i.e. Amyloid Precursor Protein (*APP*), Presenilin-1

(*PSEN1*) and Presenilin-2 (*PSEN2*). Besides these, most of the AD cases can be attributed to modifiable risk factors which could be prevented.[5, 6]

***Genetic*:** Other than aging, genetic risk factors are the second most causative factors for AD. Genetic risk factors are generally observed due to genetic polymorphism in the APOE gene encoding the isoforms of cholesterol transporter apolipoprotein E: the ε4 allele (APOE ε4) and due to mutation in the components of the γ-secretase enzyme complex i.e. APP and presenilin. In comparison to the population carrying the most common genotype ε3, presence of one APOE ε4 allele increases the risk of developing AD thrice while the risk of AD is increased by 15 times due to presence of two APOE ε4 alleles, whereas in case of individuals having the APOE ε2, risk of AD is reduced by half and its clinical onset delayed.[7-9] It was reported that APOE ε4 was responsible for both, promotion of Aβ aggregation and reduction in its clearance leading to amyloid plaques and cerebral amyloid angiopathy. It has been considered as one of the major causative genetic factors for AD.[10, 11]

1.1.2 Modifiable risk factors

***Hypertension*:** Hypertension was reported to be concomitant with the increased levels of amyloid plaques and neurofibrillary tangles.[12] Midlife hypertension as well as late-life hypotension exert some detrimental effects in the brain causing cognitive impairments.[5,13] A number of experiments performed on animals revealed the relationship between hypertension and pathophysiology of AD. In an experiment performed on the transgenic AD mice, induced hypertension in the mice by hypertensive drugs, high salt diet, angiotensin II and deoxycorticosterone accelerate accumulation of Aβ as amyloid plaques and cerebral amyloid angiopathy (CAA) causing loss of neuronal cells and subsequent decline in the cognitive functions.[14-16]

***Diabetes mellitus*:** The relationship between diabetes mellitus (DM) and AD still remains unclear as epidemiological studies produced conflicting results wherein some reports revealed such relationship[17-20] whereas other reports failed to reveal it.[21] Although it was observed in clinic-pathological studies that DM diagnosis was not associated with the neuropathological changes in the AD brain but few preclinical studies in AD mouse models reveal the connection between DM and AD pathology.[22-24] Moreover, establishing the mechanism through which DM contributes to Ad pathology and related cognitive impairments, remains an area of active research.[5]

***Hypercholesterolemia*:** Similar to DM, association of hypercholesterolemia with AD is not clearly established. It is even more complex as some experiments showed positive association[25] while others did not detect any significant association between hypercholesterolemia and AD risk.[26] Preclinical studies on diet-induced hypercholesterolemia in transgenic AD mice showed the increased levels of Aβ deposits.[27,28] The use of statins such as atorvastatin and pitavastatin decreased amyloid plaques and microglial inflammation[29] while simvastatin ameliorated cognitive impairments without changing the Aβ levels.[30,31]

***Smoking and Alcohol drinking*:** Smoking is not safe in any manner therefore it is not surprising that smoking increases the risk of developing AD by 2-4 folds. Increased risk of AD and dementia has also been observed in case of passive smokers or second-hand smoking.[5]

In contrast to smoking, a controlled (light or moderate) consumption of alcohol in late life reduces the risk of developing AD and dementia. Surprisingly, moderate alcohol consumers are less prone to AD and dementia compared to non-drinkers. Resveratrol and other polyphenols present in wines promote the APP processing by non-amyloidogenic pathway and also inhibit Aβ aggregation reducing the increased levels of Aβ plaque and oxidative stress. Overconsumption of alcohol (≥ 5 times in 14 days) increases risk of developing AD and dementia.[5]

***Obesity and diet*:** Risk of developing AD and dementia increases with the midlife obesity. Obesity causes mainly early onset AD with increased amyloid plaques and cortical atrophy. A healthy diet consisting of vegetables, olive oil, fruits, fish and whole-grain cereals improves the cognitive deficits and reduces the risk of AD and dementia. In contrast, a diet that contains high saturated fat, low contents of vitamin B_6, B_{12}, E and D causes deposition of Aβ plaques and cognitive impairments increasing the risk of AD and dementia in late life.[5]

***Physical exercise*:** Higher the physical activity, lower will be the risk of developing AD and dementia. An aerobic exercise on regular basis improves the cognitive deficits, promotes the non-amyloidogenic pathway of APP processing and neuronal plasticity.[5, 32, 33]

***Educational backwardness, and leisure and social activities*:** Lack of education, lesser leisure and social activities and even loneliness, single or widower marital status are associated with the risk of AD and dementia. Risk of developing AD and dementia is inversely proportional to the level of education while leisure cognitive and physical activities reduce the risk.[5]

1.2 Pathophysiology of AD

Due to the complexity of AD, etiology of the disease is not yet understood completely. It is mostly caused by genetic, environmental and endogenous factors. Inheritance from the parents has been believed to be one of the major risk factors of the disease. Several hypotheses have also been suggested to explain the causes of the disease which mainly include cholinergic hypothesis, amyloid β-cascade hypothesis, tau hypothesis, oxidative stress and metal ion dyshomeostasis.[34]

1.2.1 Cholinergic hypothesis

According to the cholinergic hypothesis, degeneration of neuronal cells, low levels of neurotransmitter acetylcholine (ACh) and co-related decrease in neurotransmission in the hippocampus and cortex region of the brain mostly cause the cognitive impairments seen in AD patients.[4, 34] Cholinesterases (ChEs), the serine hydrolase enzymes are responsible for the decreased level of ACh in the brain as they rapidly hydrolyze the neurotransmitter into acetate and choline as depicted in figure 3.[35]

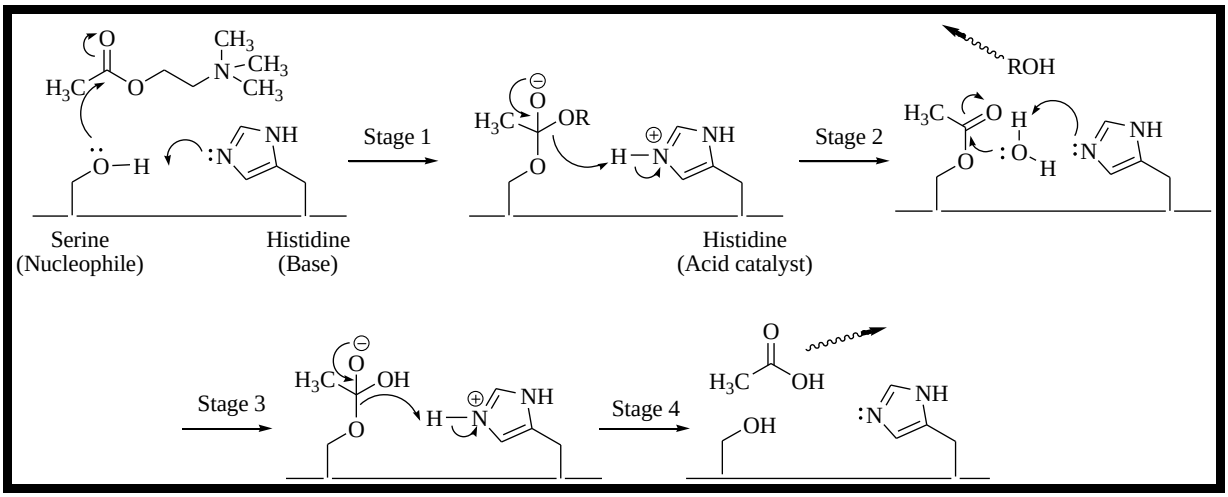

Figure 3: Mechanism of hydrolysis of acetylcholine by AChE.[35]

ChEs are of two types *viz.* acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is present in neuromuscular junctions and cholinergic neurons while BuChE is present in hippocampus and temporal neocortex, and associated with glial cells. Though the location and importance of AChE and BuChE are different but their structures are almost similar to each other. AChE possesses higher affinity for ACh compared to BuChE.[36] ACh is mainly hydrolyzed by AChE rather than BuChE.[37,38]

AChE has two active sites i.e. CAS (catalytic active site) and PAS (peripheral anionic site) as shown in figure 4. CAS consists of two binding locations which actually interact with the substrate ACh. One binding site present at the bottom of a long and narrow pocket has a catalytic triad of Ser200, His440 and Glu327 amino acid residues which catalyses the ester hydrolysis of the ACh, whereas the other binding site, also known as α-anionic site contains an amino acid residue Trp84 interacting with the quaternary ammonium moiety of the ACh.[39] PAS also known as β-anionic site is present at the entrance of the pocket about 14Å apart from the CAS.[40] PAS contains a number of amino acid residues, amongst which Trp279 is the most important that is mostly involved in the interaction with the substrate.[41] PAS plays an important role in the etiology of AD since it interacts with the Aβ peptide leading to its accumulation in the brain in the form of amyloid plaques which further cause the neuronal cell death.[42-44]

BuChE exhibits similar actions like AChE, hydrolysing the ACh into acetate and choline but to a lesser extent compared to AChE.[45, 46] With the progression of AD, it has been observed that the activity of BuChE remains unchanged or gets enhanced in certain regions of brain of AD patients.[47] Moreover, an increased level of BuChE has been observed in late onset AD compensating the decreased activity of AChE due to decreased levels of AChE in the AD brain.[48]

Therefore, both the ChEs have emerged as potential therapeutic targets to develop novel cholinesterase inhibitors (ChEIs) for the management of AD.

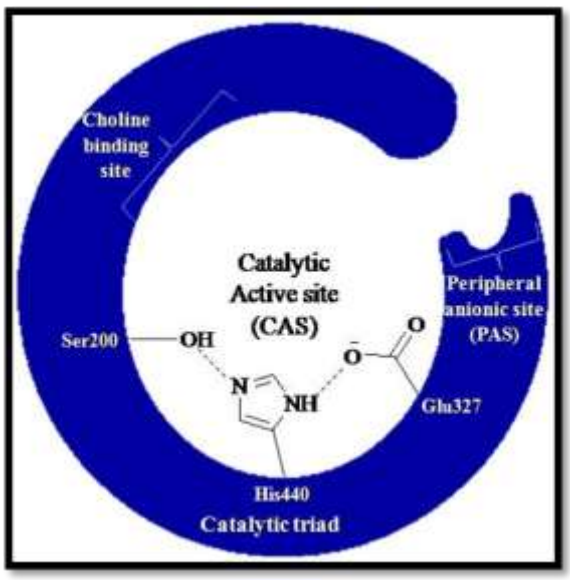

Figure 4: Structural features of the enzyme acetylcholinesterase (AChE).

1.2.2 Amyloid β-cascade and Tau (τ) hypothesis

The major histophathological characteristics of AD are the senile plaques (SPs) and neurofibrillary tangles (NFTs) which are the aggregates of the Aβ-peptides and hyperphosphorylated tau proteins, respectively.[49-51] Amyloid hypothesis suggests that Aβ, the building block of the amyloidogenic pathway, is produced by the abnormal proteolysis of APP which further on aggregation in various parts of the brain form amyloid fibrils causing neuritic injury and cell death (**Figure 5**). There are two major metabolic pathway of APP i.e. non-amyloidogenic and amyloidogenic. In non-amyloidogenic pathway, α-secretase enzyme initially cleaves the APP at α-cleavage site to produce C-terminal fragment α (CTFα) which

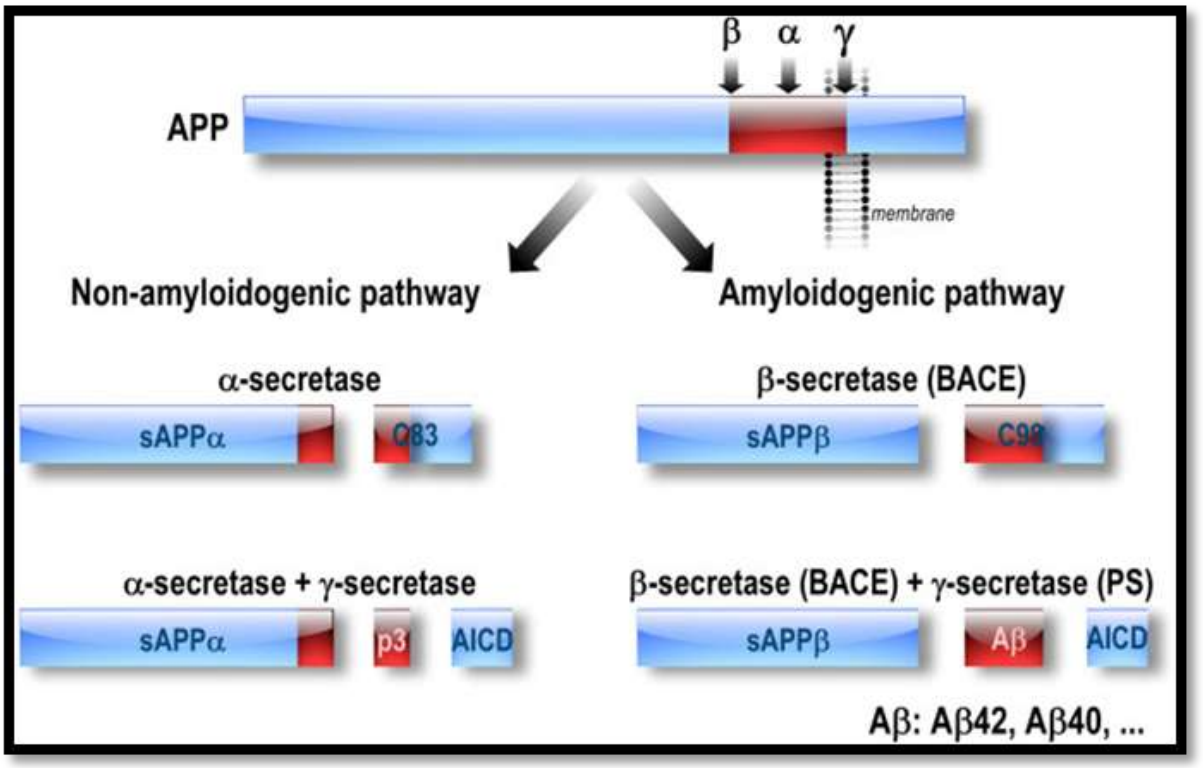

Figure 5: Non-amyloidogenic and amyloidogenic pathway of APP processing.[54]

on further cleavage by γ-secretase form innocent soluble APPα (sAPPα). However, the amyloidogenic pathway of APP processing is initiated with the cleavage of APP at β-cleavage site by β-secretase enzyme, also known as BACE-1 (β-site APP cleaving enzyme 1) producing CTFβ. This CTFβ is then processed by γ-secretase which results into formation of Aβ monomers. These Aβ monomers undergo self-induced, AChE-induced or metal-induced aggregation to form amyloid plaques in the brain which damage the neuronal cells.[52,53]

NFTs are mainly comprised of paired helical filaments (PHFs) of atypical hyperphosphorylated Tau (τ) protein (**Figure 6**). τ-Proteins belonging to the group of microtubules associated proteins (MAPs) are crucial in the normal functioning of neurons as they stabilize microtubules and carry out neuronal trafficking.[55] In the process of formation of NFTs, hyperphosphorylated τ-proteins first develop as amorphous tangles and oligomers, which then form PHFs. These PHFs within the nerve cells combine with other proteins including normal τ-proteins and MAPs to form NFTs.[56,57] Deposition of NFTs in the neuronal cells causes microtubule depolymerization[51,58,59] and interruption in the neuronal transport system leading to the death of neuronal cells.[60-63]

Some of the reports indicate the association of amyloid and tau hypotheses suggesting that Aβ promotes the formation of NFTs, and the oligomers of Aβ and hyperphosphorylated τ collectively cause neurotoxicity.[64-67] Overall, inhibition of Aβ aggregation has been established as the potential therapeutic target to develop clinical agents for AD therapy.

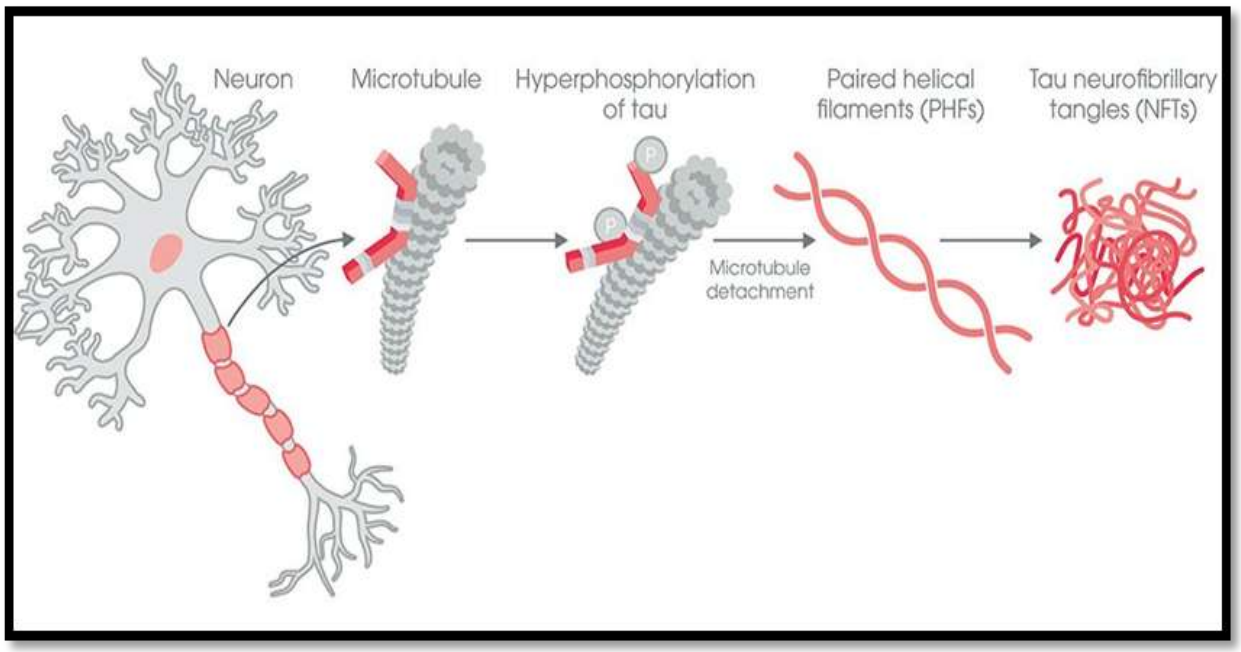

Figure 6: Hyperphosphorylation of tau producing PHFs and NFTs.[63]

1.2.3 Oxidative stress

Reactive oxygen species (ROS) are produced endogenously in various biological processes inevitably at the cellular level.[68] Increasing evidences indicate that the oxidative stress caused by ROS and reactive nitrogen species (RNS) (**Figure 7**) is involved in the neurodegeneration processes occurring in AD.[69] Oxidative stress is responsible to increase

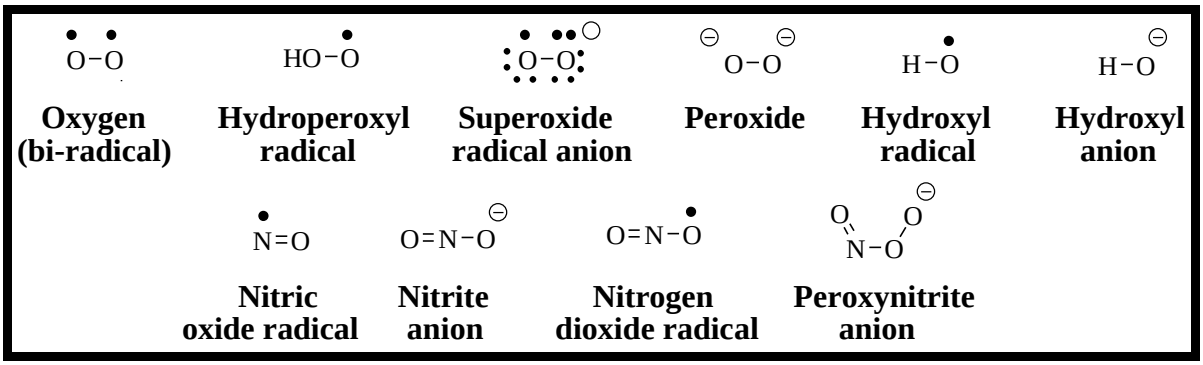

Figure 7: Chemical structures of various reactive oxygen species (ROS, first row) and reactive nitrogen species (RNS, second row).[9]

the activity of β- and γ-secretases and to decrease the activity of α-secretase which subsequently causes increase in production of Aβ.[70,71] Oxidative stress can also lead to mitochondrial dysfunction causing increase in concentration of ROS which react with biomolecules such as lipids, proteins, nucleic acids and carbohydrates (**Figure 8**).[72-74] Thus, efforts have been devoted to develop newer multifunctional antioxidants to manage or target the oxidative stress along with other pathological factors.

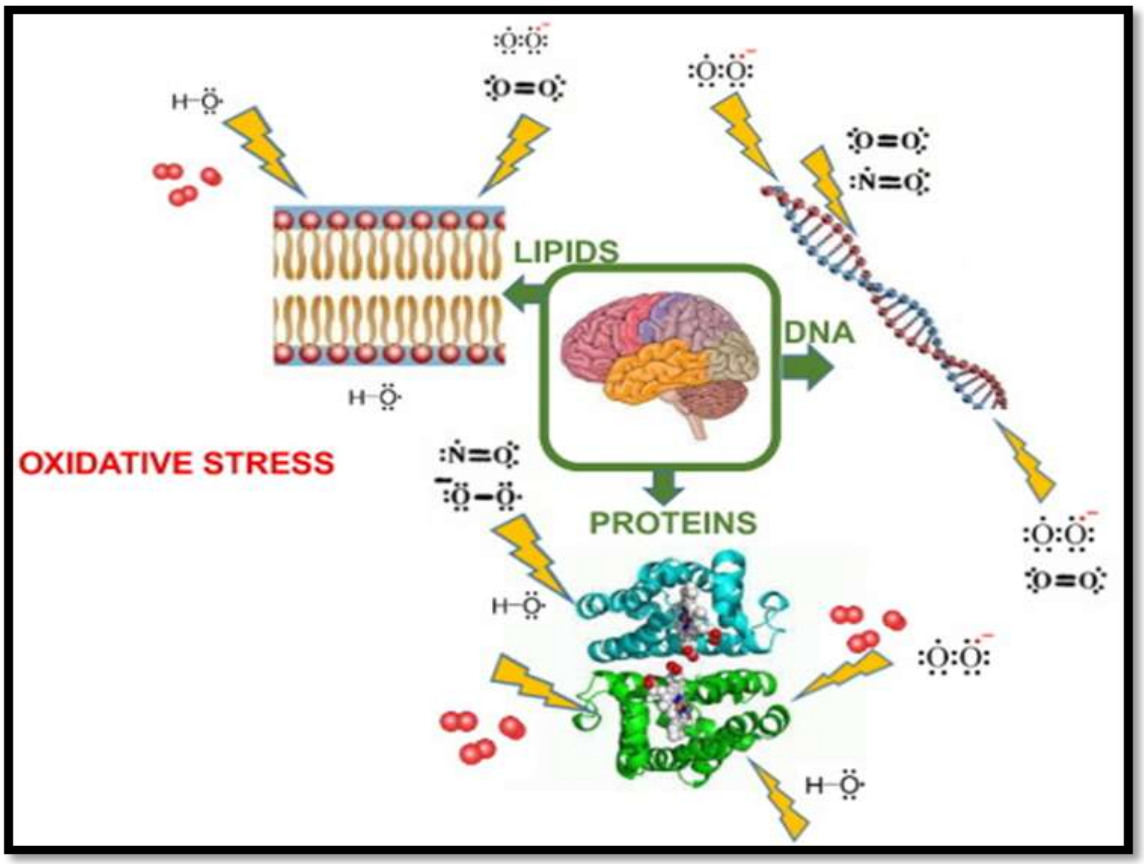

Figure 8: Various ROS and RNS damaging the biomolecules present in the brain.[75]

1.2.4 Metal ion dyshomeostasis

Metal ions play a vital role in various biological processes like metabolism, catalysis and signal transmission to name a few.[9,76] A number of reports have appeared in the literature describing the roles and functions of first-row transition metals such as iron (Fe), copper (Cu) and zinc (Zn). It has been observed that the deregulation of these active metal ions generally lead to increase in the oxidative stress in the brain of AD patients (**Figure 9**).[77,78] Metal ions have also been involved in producing Aβ toxicity and hyperphosphorylation of τ-proteins.[79,80] Interaction of metal ions with Aβ at low physiological concentration causes metal-induced Aβ aggregation.[81] Therefore, clinical candidates having the metal chelating ability would be an additional benefit to combat AD.

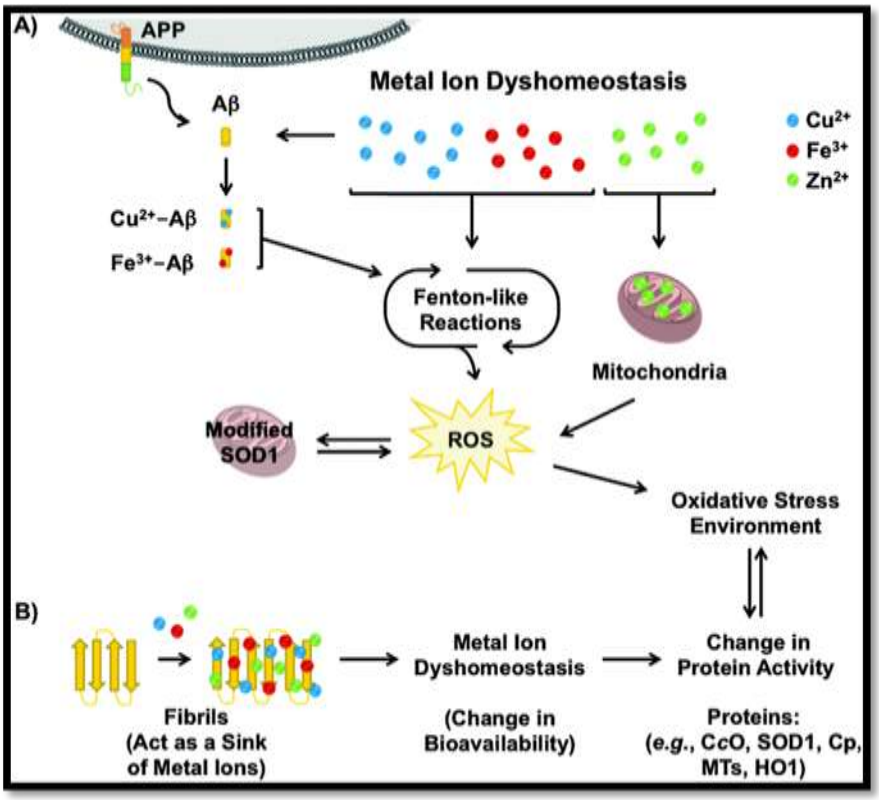

Figure 9: Metal ion dyshomeostasis inducing oxidative stress (**A**) and influencing protein activity (**B**).[82]

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