

Recent Progress in Alzheimer's Disease: Pathophysiology, Newer Natural & Synthetic Inhibitors, and Therapeutic Targets

Sweta Chandrol¹, Sandeep Gupta², Prashant Tiwari³

¹Faculty of Pharmaceutical Sciences, Shri Shankaracharya Technical Campus, Bhilai-490020, Dist: Durg, Chhattisgarh, India; ²LCIT College of Pharmacy, Bilaspur-495223, Chhattisgarh, India; ³Department of Pharmacology, College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru-560078, Karnataka, India.

ABSTRACT

The inability to remember recent events, process new information, or complete even the most basic of tasks is a hallmark of Alzheimer's disease, an irreversible neurodegenerative brain illness. Among those 65 and older, it is the leading cause of dementia. When a person's cognitive and behavioural skills deteriorate to the point that they become a burden in everyday life, we say that they have dementia. There is currently no known way to halt the progression of AD or stop its symptoms from occurring. Huge literature survey was conducted from various popular medical databases such as Google Scholar, PubMed, ScienceDirect, etc. through the internet and the available data were classified according to their targets. The article focuses on basics of AD, History, Symptoms, Stages, Beta-amyloid peptide, Modulators of amyloid aggregation, Amyloid beta-oligomer, Targets for inhibiting the β -amyloid production (Monoclonal antibodies, Hormones, Inhibition of $A\beta$ -peptide formation, Inhibition of $A\beta$ -peptide formation [molecules like Posiphen, JNJ-54861911, LY3202626, E2609, AZD-3293, CNP520, MK-8931, LY2886721, and LY2811376], and Clearance of $A\beta$), and List of beta-amyloid inhibitors in clinical trials. This literature evidences may be a convenient reference (particularly, the low molecular-weight inhibitors) to the modern-day researchers (pharmacognosists, pharmacologists, clinicians, medicinal chemists, medical practitioners, scientists, etc.) working dedicatedly in AD research. This study will certainly open novel avenues for ligand development and revolutionary applications in pharmacotherapeutics.

Keywords: Alzheimer's disease, Beta-Amyloid, Tau protein, Cholinesterase, Inhibitors, Clinical trials

INTRODUCTION

Alzheimer's disease (AD) is a progressive age-related neurodegenerative disorder and is the most common form of dementia among the elderly. It is generally diagnosed in individuals over the age of 65 years. The main pathological hallmarks of AD are the accumulation of amyloid plaques, or senile plaques, containing extracellular deposits of the amyloid- β peptide ($A\beta$) and the presence of intraneuronal neurofibrillary tangles (NFTs), which result from hyperphosphorylated tau-protein. The oxidation of lipids, proteins, and nucleic acids in neurons is also a hallmark pathological feature of AD. The etiology of AD is still unknown, but several factors have been suggested that appear to reduce the incidence of the disease.^{1,2}

Three main approaches have been taken:

1. The first involves the reestablishment of neurotransmitters levels, with the inhibition of cholinesterases such as acetyl cholinesterase (AChE), butyryl cholinesterase (BChE), and monoamine oxidase (MAO) enzymes.^{3,4}
2. The second one concerns neuroprotection where oxidative stress is considered to be an early event in the pathological cascade for the disease, suggesting the potential use of antioxidants to limit the effects of free radicals on nerve cells.^{5,6}
3. The third approach deals with specific aspects related to AD, including the decrease in the production or aggregation of $A\beta$ peptide, and inhibition of γ and β -secretase enzymes which play a critical role in the amyloidogenic and tau protein pathways, among

Corresponding Author:

Ms. Sweta Chandrol, Research Scholar, Faculty of Pharmaceutical Sciences, Shri Shankaracharya Technical Campus, Bhilai 490020, Dist. Durg, Chhattisgarh, India; E-mail: sweta.chandrol@gmail.com

ISSN: 2231-2188 (Print)

ISSN: 2231-685X (Online)

Received: 24.11.2023

Revised: 20.12.2023

Accepted: 03.01.2024

Published: 10.01.2024

others. Intracellular NFT is made up of paired microtubule-associated protein tau helical filaments, which are abnormally hyperphosphorylated.^{7,8}

The emphasis on studies today has switched from protein deposits to studies on the function of activating effectors, soluble oligomeric A β and P-tau. A lot of research is also dedicated to studying how A β and P-tau contribute to AD-related toxic events, how they induce improvements in the expression of other essential brain proteins, and eventually how they cause neurodegeneration. However, in order to obtain a deeper understanding of the mechanism of neurotoxicity and to ensure successful treatment, it is also important to discern how both A β and P-tau communicate. AChE is a central enzyme in the cholinergic nervous system, as reviewed thoroughly in this special issue. Many various types of neurons deteriorate through the development of AD, and there is a deep depletion of forebrain cholinergic neurons, which is followed by a gradual decrease in acetylcholine.^{9,10}

AChE is regulated by both the acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT) and the acetylcholine-hydrolyzing enzyme AChE. To a large degree, therapies intended to reverse the cholinergic deficit are focused on the relevance of cholinergic activity in cognition. Despite the general reduction in AChE activity in the AD brain, current AD treatment is primarily based on AChE-I antagonists, which improve cholinergic transmission but have limited and intermittent therapeutic effects. For almost 50 years, it has been well known that the distribution of AChE molecular types in the AD brain is especially disturbed, but the physiopathological importance and consequent effects of these fascinating changes in AChE organisms remain unclear. A typical characteristic of AD neuropathology is an improvement in AChE levels around amyloid plaques and NFT, but the importance of this rise remains to be determined.^{11,12}

In other words, AChE activity upregulation following long-term AChE-I therapy has been documented in a variety of studies over the past decade. The limited efficacy and persistence of AChE-II can be linked to all these anomalies in AChE expression patterns, as well as AChE upregulation in response to persistent inhibition.^{13,14}

ADNI's main successes were as follows:

1. Production in a multicenter environment of systematic techniques for clinical studies, positron emission tomography (PET), magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) biomarkers;^{15,16}
2. Elucidation of trends, imaging transition frequencies, and biomarker measures of CSF in control subjects, patients with MCI, and patients with AD. CSF biomarkers are compatible with β -amyloid cascade and

tau-mediated neurodegeneration theories for AD expected disease trajectories, while brain atrophy and hypometabolism levels show predicted trends, but show varying rates of change depending on area and seriousness of disease;^{17,18}

3. The evaluation of alternate medical categorization approaches. The best classifiers currently integrate optimal features from a number of modalities, including MRI, fluorodeoxyglucose-PET, CSF biomarkers and clinical trials;^{19,20}
4. In mildly symptomatic or even non-symptomatic subjects, β -amyloid, CSF biomarkers, and tau, as well as amyloid PET, can represent the earliest steps in AD pathology and are leading candidates for the detection of AD in its preclinical stages;^{21,22}
5. Improving the quality of clinical trials by recognizing the samples who are more likely to suffer imminent potential clinical deterioration by using more responsive outcome tests to minimize sample sizes. Baseline cognitive and/or MRI tests typically expected a better potential regression than other modalities, whereas the most successful outcome measures were found to be MRI measures of improvement;^{23,24}
6. Confirmation of the CR1, CLU, and PICALM AD risk loci and recognition of new nominee risk loci;^{25,26}
7. Worldwide influence in Europe, Asia, and Australia through the development of ADNI-like programmes;^{27,28}
8. Understanding the biology and pathobiology of normal ageing, MCI, and AD by combining ADNI biomarker data with ADNI clinical data to promote studies to address disputes regarding contradictory theories about AD etiopathogenesis, thus advancing efforts to identify disease-modifying ADNI drugs;^{29,30}
9. The construction of networks to allow all raw and processed data to be exchanged without embargo by interested science investigators worldwide. The ADNI research was expanded by a two-year 2009 Grand Opportunities grant and a renewal of the ADNI (ADNI-2) from October 2010 to 2016, with an additional 550 participants participating in the study.^{31,32}

HISTORY

In 1906, AD was first described by Dr. Alois Alzheimer in his patient known only as Auguste D who experienced memory loss and physiological change that he noted in the autopsy where the brain was shrinking in and around nerve cells in brain. The abnormal clumps called amyloid plaque and tangled bundles of fibers called neurofibrillary were found in the brain.³³

SYMPTOMS

- **Cognitive:** mental decline, difficulty thinking and understanding, confusion in the evening hours, delusion, forgetfulness, mental confusion, difficulty concentrating, and inability to create new memories.
- **Behavioral:** aggression, agitation, difficulty with self-care, irritability, meaningless repetition of own words, and restlessness.
- **Mood:** anger, loneliness, and mood swings.
- **Psychological:** depression and hallucination.
- **Common:** inability to combine muscle movements, jumbled speech, and loss of appetite.^{34,35}

STAGES OF ALZHEIMER'S DISEASE

- **Stage one:** No impairment
- **Stage two:** very mild cognitive decline
- **Stage three:** mild cognitive decline
- **Stage four:** moderate cognitive decline
- **Mild stage:** dementia³⁶

CAUSES OF ALZHEIMER DISEASE

There are various factors which leads the healthy brain to AD brain:

Neurofibrillary tangles

Tau protein phosphorylates and aggregates within neuronal cytoplasm which ultimately forms neurofibrillary tangles. When tau phosphorylates, it detaches from the microtubule, thus it dissociates, and the cytoskeleton structure gets dispersed. A small number of neurofibrillary tangles are universal consequences of aging. An increase in the population of tangles is the prime and possibly the main mechanism of neuronal death in AD. Neurofibrillary tangles mostly occurs in the areas of the hippocampus which are involved in the storage of permanent memories, and hence memory is impaired in the early stages of AD.^{37,38}

Senile Plaques

Senile plaques are formed from the β -amyloid peptide. It is a peptide of 39-43 amino acid residues produced by proteolytic cleavage of a large precursor known as the amyloid precursor protein (APP), which is encoded by a gene located at chromosome 21 in humans. Enzyme secretase (secretase and γ -secretase) is responsible for proteolysis of APP at position 597 and 637-639 and the release of β -amyloid fragments. A β is a major protein constituent of the senile plaques found in the brain of AD patients.^{39,40}

ROLE OF GENE

Genes are involved in the development of AD as some mutations increase the production of short forms β -amyloid while the others favor the formation of long-form β -amyloid which aggregates more readily. Mutation in other genes coding for the novel proteins presenilin-1 and presenilin-2 are reported to account for the majority of early-onset, familial dominant inherited AD.^{40,41}

ROLE OF ENVIRONMENTAL FACTORS

Metals such as aluminum and lead are linked with a number of neurodegenerative disease including AD and causes toxicity to a number of organs in the human body. Copper and arsenic disrupt the homeostasis of amyloid- β -protein. Chronic exposure to pesticides like organophosphates leads to cognitive and psychomotor impairment. Metals like aluminum copper, iron, lead, cobalt, cadmium, manganese, mercury, arsenic, selenium, and zinc directly or indirectly affect the healthy brain and may increase the chances of neurodegeneration. Insecticides or pesticides such as organochlorides and organophosphates may increase the risk of dementia in AD. Industrial and commercial pollutants such as brominated flames retardants show impaired learning and memory and simultaneously decrease hippocampus cholinergic receptors. Air pollutants involve nickel like toxic particulate matter. In the nickel nanoparticles model of air pollution, there is an increased report in the levels of A β -40 and A β -42 levels in mice brain.⁴²

PATHOPHYSIOLOGY

Alzheimer's disease occurs due to the formation of senile plaque and neurofibrillary tangle in the brain (mostly hippocampus and cortex region), where the senile plaque is formed at the surface of the neuron and NFT is created inside the neuron. A senile plaque is formed by β -amyloid protein and NFT is formed by tau protein due to hyperphosphorylation. Amyloid precursor proteins are present on the surface of the neuron and are normally cut by the enzymes such as β -secretase and γ -secretase enzyme and this cut part is referred to as β -amyloid. This is collected in forming a fiber-like structure called senile plaque. NFT is formed and the signal is transferred from soma to the synapse, further, the signal is transferred from a neuron which is made up of microtubules. These microtubules are stabilized by tau protein. In Alzheimer's disease, this tau protein becomes defective and removes from microtubules. The defective tau protein is assembling in neurons and formed filament. Without the skeleton, the neurons do not generate and the connection between neurons is lost. An abnormal accumulation of tau filament in neuron form

NFT. Senile plaque and NFT are deposited at the synaptic junction due to a decrease the released and concentration of acetylcholine.⁴³

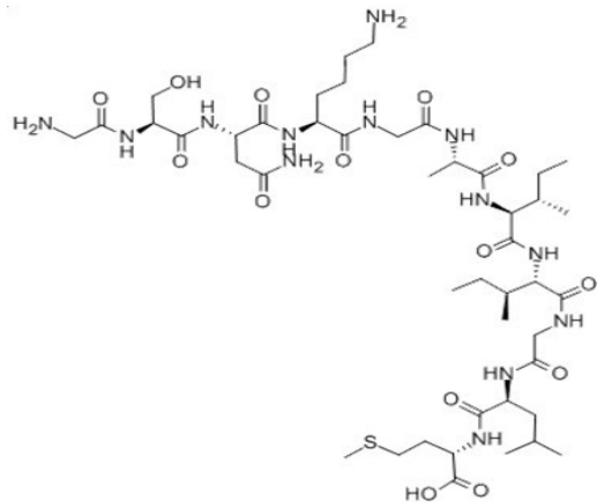
STRUCTURE OF PAIRED HELICAL FILAMENTS (PHF)

Most of the neurological disease involves the formation of dense fibrous aggregates. These aggregates are morphologically different from the normal components of the neuronal cytoskeleton. There are two types of filaments; PHF (paired helical filament) and SF (straight filament) where the former constitutes the principal component that is neurofibrillary tangles in AD. PHFs are the double-helical stock of morphological units, each with a C-shaped cross-section displaying three domains. When seen under an electron microscope, PHFs have a fuzzy-coat that can be stripped off by pronase to leave a pronase-resistant core. The earlier immunological studies showed that tau is associated with PHF as well as with some anti-tau antibodies which decorate fuzzy PHF's but not stripped PHF's. The second minor class filaments found in the AD brain are straight filaments, having 15 nm wide dimension when seen in an electron microscope. Isolated filaments from AD brain and antibody labeling of sectioned material shows SF's share epitope with PHF's.⁴⁴

BETA-AMYLOID PEPTIDE

The β -amyloid peptide ($A\beta$) plays a central role in the neuropathology of Alzheimer's disease (AD).¹³ $A\beta$ is a peptide of 39-43 amino acid residues produced by proteolytic cleavage of a large precursor known as the amyloid precursor protein (APP), encoded by a gene located at chromosome 21 in humans. APP is an integral membrane glycoprotein, with a short cytoplasmic C-terminal tail and a large extracellular N-terminal domain. The class of enzyme known as secretases is responsible for the proteolysis of APP and release of $A\beta$. $A\beta$ is released following cleavage of APP at positions 597 and 637-639 by β -secretases and γ -secretases, respectively.

γ -secretases may cleave APP at the C-terminal end of A β at four different positions, giving rise to A β peptide. The exact position of C-terminal cleavage appears critical to the development of AD, since the generation of the more amyloidogenic peptides is strongly correlated with the development of AD. A β is the major protein constituent of the senile plaques found in the brains of AD patients. A β forms characteristic non-covalent fibrillar aggregates both *in vitro* and *in vivo*, and its aggregation and ensuing amyloid deposition in the brain have been related to AD neurotoxicity.⁴⁵



MODULATORS OF AMYLOID AGGREGATION

- Plasma protein
- Glicosaminoglycans
- Apolipoprotein
- Complement C1q
- Acetylcholinesterase
- Laminin
- Entactin
- Phospholipids
- Ganglioside
- Glycerol
- Metals⁴⁶

TAU PROTEIN

Tau protein belongs to the family of microtubule association protein. They are mainly present in neurons, where they assemble tubulin monomers into the microtubule networks that keep the cell shape and it acts as axonal transport. The human adult brain consists of six different isoforms which are regulated by an alternative splicing mechanism. Tau proteins are major components of intraneuronal and glial fibrillar lesions. Molecular analysis depicted that abnormal phosphorylation might be an important event in the pathogenesis where tau is the remarkable marker of the neurodegenerative process.⁴⁷

GENE ORGANIZATION

The human tau gene is one of the unique and spotted over a hundred kb on the long arm of chromosome-17, denoting the position at 17q21 which contains 16 exons.⁴⁸

SPLICING

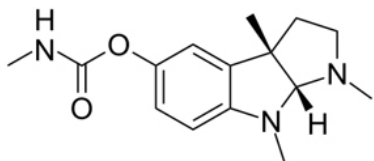
Primary tau transcript contains 16 exons but three of them; exons 4A, 6, 8 are not present in any mRNA in the human brain. Exon-1 is a part of the promoter and is transcribed but not translated. Exon-14 is usually identified in mRNA. Some of the consecutive exons are 1, 4, 5, 7, 9, 11, 12, 13 [36, 39, 40, 41], and 2,3,10 are brain-specific of adult. Alternative splicing of these three exons allows for six combination viz. (2-3-10-), (2+3-10-), (2+3+10-), (2-3-10+), (2+3-10+), (2+3+10+).^{39,40,41} Primary tau transcript gives rise to six mRNA.⁴⁹

STRUCTURE AND ROLE OF TAU PROTEIN

Tau isoforms are produced from a single gene through alternative mRNA splicing. In the adult brain, tau protein consists of a family of six isoforms which range from 352 to 442 amino acid with a molecular weight range from 45 KDa to 65 KDa. There are two terminals present in tau; one is the carboxy-terminal (C-terminal) and the other is amino-terminal. Tau isoforms differ from each other by the presence of either 3 or 4 repeat regions in the C-terminal and the absence or presence of one or two inserts in the amino-terminal part (N-terminal). Tau isoforms are differentially determined during growth so that they have specific physiological roles independently. Tau isoforms may be dispersed in the neuronal subpopulation. N-terminal called as projection domain because it projects from the microtubule surface where it interacts with other cytoskeleton elements as well as the plasma membrane whereas C-terminal is called the microtubule-binding domain because it binds on microtubules and stabilizes it.⁵⁰

ESTABLISHED SYNTHETIC CHOLINESTERASE INHIBITORS

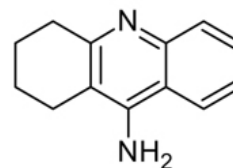
Physostigmine



Physostigmine is a carbamate ester and an indole alkaloid that was the first ChE inhibitor investigated for the treatment of AD. It was isolated from the seeds of *Physostigma venenosum*, a para-sympathomimetic plant alkaloid. The blood-brain barrier (BBB) can move through, has a short half-life, and a small therapeutic index. It also has a number of side effects such as nausea, vomiting, diarrhoea, dizziness, and

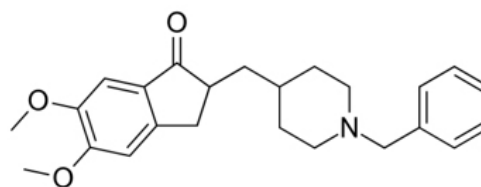
headaches. It was previously used for glaucoma treatment and delayed gastric emptying. However because of the drawbacks listed above the drug was not licenced and was discarded for AD use. With a lower side effect profile, the newer medications proved to be more successful.⁵¹

Tacrine



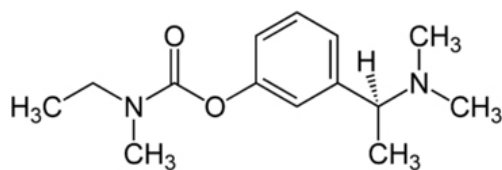
Tacrine is a 1,2,3-triazole derivative that acts both as AchE and BuChE. It was first approved for the treatment of AD in 1993 for its efficacy on the ADAS-Cog and on the global measure compared to placebo in phase-II and phase-III clinical trials. For AD subjects, it was found to be quite limited owing to poor tolerance and precipitation of a number of side effects including vomiting, nausea, diarrhea, dizziness, syncope, and seizures. Also, the route of administration and patient compliance were challenging due to 4-times a day dosing regimen as a result of a short half-life. In addition, patients under treatment with this drug are required to have periodic blood monitoring due to hepatotoxicity. Finally, owing to the aforementioned liver toxicity, which was believed to be caused by the preference for BuChE and the availability of less toxic, better tolerated medications with a simplified dosing schedule, tacrine was discontinued.⁵²

Donepezil



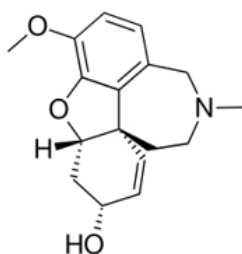
Donepezil is a piperidine derivative that acts as a selective and reversible inhibitor of AchE. Donepezil was approved in 1996 for the treatment of mild-to-moderate AD. A high dose (23mg) formulation was approved for use for moderate to severe AD. It is well absorbed with a relative oral bioavailability of 100% and reaches peak plasma concentrations in 3-4 hrs. The elimination half-life is ~70 hrs and is approximately 96% bound to human plasma proteins. It is primarily metabolized by CYP₄₅₀ isoenzymes 2D6 and 3A4 and undergoes glucuronidation. It is often assumed that there is an extra mechanism of action rather than just a ChEI. Donepezil is known to function not just at the level of the neurotransmitter, but even at the molecular and cellular level in virtually any stage involved in AD pathogenesis.⁵³

Rivastigmine



It is a carbamate derivative of reversible cholinesterase inhibitor that is selective for the central nervous system and is used for the treatment of AD. It is a small molecule and has easy BBB permeability which enables fantastic BuChE and AChE inhibitory properties. Rivastigmine was approved for the treatment of mild-to-moderate AD in the year 2000 and has since gained approval for PAD after a trial with 699 patients with mild-to-moderately severe AD. In capsular form, the drug has been frequently associated with side effects like nausea, vomiting, anorexia, and diarrhea. In 2007, Rivastigmine, due to small molecule characteristics, was reformulated for delivery through a transdermal patch which resulted in significantly lowered GI side effects compared to the oral capsule. It is minimally metabolized by the CYP450 cytochrome system with weak binding to plasma proteins (~40%). The duration of ChE activity in cerebrospinal fluid is ~10 hrs after a 6-mg oral dose.⁵⁴

Galantamine



It is a benzazepine derivative obtained from norebelladine. It is found in *Galanthus* and Other Amaryllidaceae. It has been noted that the medicinal activity of Galantamine is primarily due to its sensitising action on nAChRs rather than general cholinergic stimulation due to cholinesterase inhibition. For the treatment of mild-to-moderate AD, it was accepted. A substantial increase in the ADAS-cog score of 3.3 points for the 16 mg/day group and 3.6 points for the 24 mg/day group was correlated with the administration of this medication, as well as a noticeable improvement in the physiological, emotional and functional symptoms of AD compared with placebo.⁵⁵

CONCLUSION

The emerging details of AD pathophysiology provide possible therapeutic targets. Although, no effective treatment

is yet available to stop AD or to prevent its development, this review may inspire motivated researchers for future product developments. As reviewed above, different approaches addressing distinct aspects of the disease are being pursued in an attempt to develop effective therapies. The stimulation of the physiological mechanism of clearance of the peptide and inhibition of amyloid aggregation may eventually constitute an effective approach to the prevention of AD. The drug development process progresses from phase-I to phase-III created rays of hope for future arrival. This literature evidences may be a convenient reference (particularly, the low molecular-weight inhibitors) to the modern-day researchers (pharmacognosists, pharmacologists, clinicians, medicinal chemists, medical practitioners, scientists, etc.) working dedicatedly in AD research. This study will certainly open novel avenues for ligand development and revolutionary applications in pharmacotherapeutics.

ACKNOWLEDGEMENT

The authors would like to acknowledge Chairman, Vice-Chairman, Secretary, Principal, Faculties, and Non-teaching staffs for their kind support.

CONFLICT OF INTEREST

None declared.

SOURCE OF FUNDING

No agency provided any funds.

AUTHORS' CONTRIBUTION:

Sweta Chandrol: Writing manuscript, Literature review, Analysis

Sandeep Gupta: Concept, Suggestions, Revisions

REFERENCES

1. Kowall NW, Budson AE. The handbook of Alzheimer's disease and other dementias. John Wiley & Sons; 2011.
2. Taylor R. Alzheimer's from the inside out. Health Professions Press; 2007.
3. Morrison AS, Lyketsos C. The pathophysiology of Alzheimer's disease and directions in treatment. Adv Stud Nurs. 2005;3(8):256-70.
4. Alzheimer A. Über eine eigenartige Erkrankung der Hirnrinde. ZentralblNerven Psych. 1907;18:177-9.
5. Bondi MW, Edmonds EC, Salmon DP. Alzheimer's disease: past, present, and future. J Int Neuropsychol Soc. 2017;23(9-10):818-31.
6. Munoz DG, Feldman H. Causes of Alzheimer's disease. Can Med Assoc J. 2000;162(1):65-72.
7. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-59.
8. Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, et al. Neuronal loss correlates with but exceeds

- neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997;41(1):17-24.
9. Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurol*. 1998;50(1):136-45.
 10. Rogers SL, Friedhoff LT. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US multicentre, randomized, double-blind, placebo-controlled trial. *Dement Geriatr Cogn Disord*. 1996;7(6):293-303.
 11. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun*. 1984;120(3):885-90.
 12. Defelice FG, Ferreira ST. Physiopathological modulators of amyloid aggregation and novel pharmacological approaches in Alzheimer's disease. *An Acad Bras Ciênc*. 2002;74(2):265-84.
 13. Verbeek MM, Ruiter DJ, De Waal RM. The Role of Amyloid in the Pathogenesis of Alzheimer's Disease. *Biol Chem*. 1997;378:937-50.
 14. Sandbrink R, Hartmann T, Masters CL, Beyreuther K. Genes contributing to Alzheimer's disease. *Mol Psychiatry*. 1996;1(1):27-40.
 15. Campbell A. The potential role of aluminium in Alzheimer's disease. *Nephrol Dial Transplant*. 2002;17(2):17-20.
 16. Basha MR, Wei W, Bakheet SA, Benitez N, Siddiqi HK, Ge YW, et al. The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and β -amyloid in the aging brain. *J Neuro Sci*. 2005;25(4):823-9.
 17. Shih RA, Hu H, Weisskopf MG, Schwartz BS. Cumulative lead dose and cognitive function in adults: a review of studies that measured both blood lead and bone lead. *Environ Health Perspect*. 2007;115(3):483-92.
 18. Baum L, Chan IH, Cheung SK, Goggins WB, Mok V, Lam L, et al. Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability. *Biometals*. 2010;23(1):173-9.
 19. Singh NK, Chhillar N, Banerjee BD, Bala K, Basu M, Mustafa M. Organochlorine pesticide levels and risk of Alzheimer's disease in north Indian population. *Human Exptoxicol*. 2013;32(1):24-30.
 20. Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. *Environ Health Perspect*. 2004;112(9):950-8.
 21. Baldi I, Lebailly P, Mohammed-Brahim B, Letenneur L, Dartigues JF, Brochard P. Neurodegenerative diseases and exposure to pesticides in the elderly. *Am J Epidemiol*. 2003;157(5):409-14.
 22. Yegambaram M, Manivannan B, G Beach T, U Halden R. Role of environmental contaminants in the etiology of Alzheimer's disease: A review. *Curr Alzheimer Res*. 2015;12(2):116-46.
 23. Viberg H, Fredriksson A, Eriksson P. Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol Appl Pharmacol*. 2003;192(2):95-106.
 24. Kim SH, Knight EM, Saunders EL, Cuevas AK, Popovech M, Chen LC, et al. Rapid doubling of Alzheimer's amyloid- β 40 and 42 levels in brains of mice exposed to a nickel nanoparticle model of air pollution. *F1000Res*. 2012;1:70.
 25. Goedert M. Tau protein and the neurofibrillary pathology of Alzheimer's disease. In: *Apolipoprotein E and Alzheimer's Disease 1996* (pp. 103-125). Springer, Berlin, Heidelberg.
 26. Crowther RA. Straight and paired helical filaments in Alzheimer disease have a common structural unit. *Proc Natl Acad Sci*. 1991;88(6):2288-92.
 27. Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. *Nature*. 1963;197(4863):192-3.
 28. Wischik CM, Crowther RA, Stewart M, Roth M. Subunit structure of paired helical filaments in Alzheimer's disease. *J Cell Biol*. 1985;100(6):1905-12.
 29. Crowther RA, Wischik CM. Image reconstruction of the Alzheimer paired helical filament. *EMBO J*. 1985;4(13B):3661-5.
 30. Wischik CM, Novak M, Thøgersen HC, Edwards PC, Runswick MJ, Jakes R, et al. Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci*. 1988;85(12):4506-10.
 31. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, Crowther RA. Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci*. 1988;85(13):4884-8.
 32. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci*. 1975;72(5):1858-62.
 33. Buée L, Bussi re T, Bu e-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Rev*. 2000;33(1):95-130.
 34. Neve RL, Harris P, Kosik KS, Kurnit DM, Donlon TA. Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2. *Mol Brain Res*. 1986;1(3):271-80.
 35. Andreadis A, Broderick JA, Kosik KS. Relative exon affinities and suboptimal splice site signals lead to non-equivalence of two cassette exons. *Nucleic Acids Res*. 1995;23(17):3585-93.
 36. Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. *Biochem*. 1992;31(43):10626-33.
 37. Himmler A, Drechsel D, Kirschner MW, Martin DW. Tau consists of a set of proteins with repeated C-terminal microtubule-binding domains and variable N-terminal domains. *Mol Cell Biol*. 1989;9(4):1381-8.
 38. Nelson PT, Stefansson K, Gulcher J, Saper CB. Molecular evolution of τ protein: implications for Alzheimer's disease. *J Neurochem*. 1996;67(4):1622-32.
 39. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron*. 1989;3(4):519-26.
 40. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *EMBO J*. 1989;8(2):393-9.
 41. Sawa A, Oyama F, Matsushita M, Ihara Y. Molecular diversity at the carboxyl terminus of human and rat tau. *Mol Brain Res*. 1994;27(1):111-7.
 42. Kosik KS, Orecchio LD, Bakalis S, Neve RL. Developmentally regulated expression of specific tau sequences. *Neuron*. 1989;2(4):1389-97.
 43. Coyle JT, Price DL, Delong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. *Sci*. 1983;219(4589):1184-90.
 44. Kosik KS, Kowall NW, McKee A. Along the way to a neurofibrillary tangle: a look at the structure of tau. *Ann Med*. 1989;21(2):109-12.
 45. Goedert M, Jakes R. Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization. *EMBO J*. 1990;9(13):4225-30.

46. Brandt R, Léger J, Lee G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *J Cell Biol.* 1995;131(5):1327-40.
47. Hirokawa N, Shiomura Y, Okabe S. Tau proteins: the molecular structure and mode of binding on microtubules. *J Cell Biol.* 1988;107(4):1449-59.
48. Danao K, Kodape Y, Mahapatra D, Borikar S, Karande N. Highlights on Synthetic, Natural, and Hybrid Cholinesterase Inhibitors for Effective Treatment of Alzheimer's Disease: A Review. *Int J Cur Res Rev.* 2021;13(11):27-34.
49. Cummings J, Lee G, Mortsdorf T, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2017. *Alzheimers Dement.* 2017;3(3):367-84.
50. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. *Alzheimers Dement.* 2018;4:195-214.
51. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Res.* 2018;7:1161.
52. Cummings J, Blennow K, Johnson K, Keeley M, Bateman RJ, Molinuevo JL et al. Anti-tau trials for Alzheimer's disease: a report from the EU/US/CTAD Task Force. *J Prev Alzheimers Dis.* 2019;6(3):157-63.
53. Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2019. *Alzheimers Dement.* 2019;5:272-93.
54. Bauzon J, Lee G, Cummings J. Repurposed agents in the Alzheimer's disease drug development pipeline. *Alzheimers Res Ther.* 2020;12(1):1-6.
55. Khoury R, Patel K, Gold J, Hinds S, Grossberg GT. Recent progress in the pharmacotherapy of Alzheimer's disease. *Drugs Aging.* 2017;34(11):811-20.