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RECENT ADVANCES IN ACETYLCHOLINESTERASE INHIBITORS FOR ALZHEIMER'S DISEASE: A REVIEW OF THERAPEUTIC STRATEGIES (1990–2024)

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ABSTRACT

Acetylcholinesterase ester enzyme (AChE) is actually a major enzymatic target for numerous therapeutic drugs, playing a pivotal role in Alzheimer's disease. Researchers are developing new drugs and other strategies to treat this disease. Acetylcholinesterase ester enzyme (AChE) inhibitors (ChEIs) are the primary treatment for Alzheimer's Disease. These drugs work by slowing Ach turnover, mitigating the effects of cholinergic neuron degeneration, and enhancing synaptic transmission. This review is conducted from the literature published from 1990 to 2024. Research work on human settings is needed to be addressed for authentication of Alzheimer Disease. Hence published data in this review confirms that Acetylcholinesterase ester enzyme. This review examines literature published from 1990 to 2024, highlighting recent advancements in AChE-targeted therapies. While substantial progress has been made, further research in human setting is necessary to validate the therapeutic efficacy of AChE inhibitors in Alzheimer's disease. The findings presented in this review confirm the significance of AChE as a central target in AD treatment and emphasize the need for continued investigation. Additionally, recent studies have explored the potential of natural compounds and synthetic derivatives as a novel AChE inhibitor, aiming to enhance drug efficacy and reduce side effects. Advancements in AI-driven drug discovery and computational modeling have also contributed to identifying promising AChE-targeted candidates. These developments underscore the ongoing efforts to improve therapeutic strategies for Alzheimer's disease.

Keywords: *Acetylcholinesterase (AChE), Alzheimer's Disease, AChE Inhibitors, Cholinergic Neurotransmission, Multi-target Drug Design, Neurodegenerative Disorders*

INTRODUCTION

The cholinergic hypothesis was first articulated more than 20 years ago.[1] Acetylcholine is thought to be one of the principal neurotransmitters of the brain. [2] Acetylcholine plays an important role in the peripheral and central nervous systems [3] that developed very early in phylogenic history. [4] It is located at postsynaptic neuromuscular junctions, especially in muscles and nerves.[5] In a healthy brain, it is held that acetylcholinesterase has a predominant activity, whereas butyrylcholinesterase is believed to exert a relatively small influence in the homeostatic regulation of acetylcholinesterase in the brain. However, butyrylcholinesterase activity starts to elevate in Alzheimer's patients but otherwise, acetylcholinesterase activity remains stable or declines.[6] Hydrolases are classified in this context, which cleaves the ester bond of carboxylic acid esters.[7]

Alzheimer's disease, being multifactorial, is a complex and prevalent neurological disorder affecting the world's population.[8] This disease is one of the most common forms of dementia that affects about 10% of those over the age of 65 [9] with an estimated global occurrence of 24.3 million cases.[10] Worldwide Health Human Problem Affected for Many Countries and People. [11] The management of Alzheimer Disease has been greatly applied with AChEh inhibitors.[12] Currently, Alzheimer's disease diagnosis is mainly based on evidence of neurochemical pathology in affected tissue but also based on the mechanism of action of therapeutic drugs it continues to depend.[13] Until recently the only established function of acetylcholinesterase was the termination of cholinergic neurotransmission.[14] [15] As the cholinergic system plays an important role in regulation of learning and memory process.[16].[17] Inhibitors of enzyme acetylcholinesterase are presently used as long term symptomatic treatment for patients with AD.[18] AChEIs are the most promising methods of combating AD.[19] The process of excitatory and inhibitory response takes place within the synaptic gap whereby the impulses are transduced in a distinct manner between axons.[20]

Literature-rich reports had indicated that some of the Acetylcholinesterase ester enzymes neuroprotection effects were related to inhibiting enzyme activity. Thus, recent therapeutic techniques have shifted away attention from amplifying cholinergic activity alone toward modulating noncholinergic functions as these evolve for developing newer drugs that modify the disease course of AD. [21]

This review addresses how cholinesterase (ChE) inhibitors drive neuroprotective benefits that these patients with AD may avail themselves of. Also presented are emerging developments toward possible future drug candidates that will have neuroprotection ability and were designed particularly for treating AD.[21]

The (WHO), also, has forecast that this figure, which now stands at 24 million, will escalate to more than a doubling figure by the year 2030. [22]

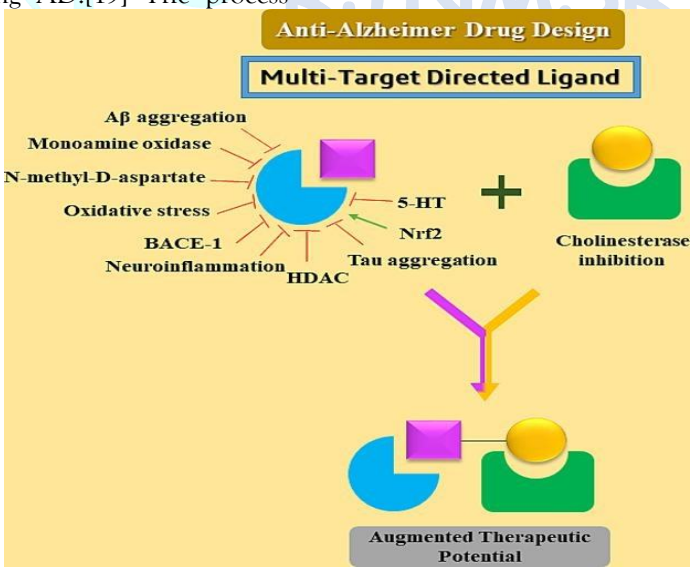

Figure.1 Schematic illustration of a multi-target directed ligand strategy for drug design in against Alzheimer's disease [23]

The orthodox approach of one-drug-one-target is only temporarily symptomatically relieved and does not stop progressions of the disease, such as Donepezil, Rivastigmine, Galantamine, Memantine [23].Now a days research is oriented towards Multi-target directed ligands approach for the treatment of Alzheimer's disease (Figure 1). This effect and prevalence of Alzheimer's disease keep on increasing, and it is estimated to reach around 13.8 million people by 2050 in the United States (US) alone. [24, 25]

Furthermore, neuropsychiatric symptoms emerge over time while independence diminishes for the patient. Diagnosis can then be made by combining at autopsy as evidence

of the disease, even though we cannot completely define it at this point. [26] [27] [28] (ACHE) and (BCHE) kinds of acetylcholinesterase differ from each other in their preferences toward the respective substrates: former prefers hydrolyzing acetylcholine has a faster rate than the latter which prefers hydrolyzing butyrylcholine [7] It attaches to the acetyl moiety of the acetylcholine, and cleaves the bond between the acetyl moiety and the choline moiety. Choline is released. Water can then substitute for the acetyl grouping on the serine, and cleave off the acetyl group while reconfiguring the serine. [21] [29]

Each monomer's carboxy-terminal has GPI covalently attached to it, with the PI part functioning as the hydrophobic anchor. [30] A bacterial PI-specific phospholipase C can selectively solubilize the dimer. [31] his approach offers a remarkable degree of purification ahead of the affinity chromatography step. [32]

The coordinates (1B41) of the monomeric rhAChE structure, resolved in complex with fasciculin-II, were used [33, 34] The translation search identified monomers in the ASU, with an R-factor of 41.7% and a correlation coefficient of 60.9% [35] Protein atoms were refined in CNS with stimulated annealing and NCS constraints, followed by conjugate gradient minimization and manual water molecules addition using O [36] [20] An overall R-factor of 20%. With an Rfree of 24.6 was obtained by fitting the structure using COOT and further refining it using REFMAC [37]

Acetylcholinesterase as a Therapeutic target

A crucial enzyme responsible for hydrolyzing the neurotransmitter Ach into acetate and choline, terminating cholinergic transmission. It is essential for the central and peripheral nervous system. Given its importance in regulating cholinergic function, it is a key curative focus for various conditions. [38, 39]

Challenges and issues

Epidemiological survey indicates that 7-10% of adults over 65 and 50-60% of people over 85 have AD. [22] Affecting around 35 million people globally, Dementia affects 7.3 million people throughout Europe . [40]

Learning, memory, cognition, sleep-wake cycles, cerebral cortex growth and activity, and cerebral blood flow are all impacted by cholinergic transmission.[41]Acetylcholine lowers heart rate, relaxes muscles, and stimulates smooth muscle contractions in the gastrointestinal, urinary and ocular tracts.[42]

Objective

This analysis aims to emphasize significant impact of cholinesterase enzyme in neurocognitive diseases. This information provides an opportunity for a deeper understanding of these conditions.

Methodology

A review on Acetylcholinesterase enzyme for Alzheimer Diseases has been done. A comprehensive literature review was performed to understand various approaches to Alzheimer Diseases. Previous data related to the topic was studied and collected via research articles and review articles. The sources used for data collection included Google Scholar, Human Genomics, Scientific Research, Sci-Hub, Science direct.com and Research Gate. Published research articles and regulatory guidelines on pharmaceutical validation were reviewed to gather the relevant data.

Results

After review of all the following articles and literature, below data is gathered in table 1 :

Table 1. Findings and Results of Literature Survey

Study	Results	Discussion	Bio marker	Change in AD	Relationship with AChE
STUDY 01	Slowed down AChE activity recognized in AD Patients compared to controls.	These results provide credence to the idea that AChE is essential to the pathophysiology of	Amyloid-β plaques	Increased	AChE may promote amyloid aggregation. [64]

		Ad.			
STUDY 02	Correlation found between AChE levels and cognitive decline in AD patients.	The precise mechanism driving this association requires more investigation.[65]	Tau protein	Increased	AChE may contribute to Tau phosphorylation.
STUDY 03	Inhibition of AChE activity in animal models of AD resulted in improved cognitive function.[67]	These results suggest that AChE inhibitors may be effective therapeutic agents for AD.[68]	Neuroinflammation markers	Increased	AChE may be involved in neuroinflammation processes.[66]
STUDY 04	AChE Inhibitor Efficacy Donepezil improved cognitive scores in mild-moderate AD.[69]	AChE inhibition enhances cholinergic transmission, slowing decline.	Acetylcholine levels	Increased	Direct: AChE inhibitors block enzyme activity, increasing synaptic acetylcholine.[70]
STUDY 05	Amyloid-β Accumulation Aβ plaques correlate with reduced AChE activity in cortical regions.[71]	Aβ aggregation disrupts cholinergic neurons, reducing AChE expression.[72]	Amyloid-β plaques	Increased	Indirect: Aβ toxicity downregulates AChE synthesis in surviving neurons.[73]
STUDY 06	Tau Hyperphosphorylation High p-tau levels linked to accelerated AChE decline in CSF.[74]	Tau pathology disrupts axonal transport, impairing AChE distribution.[75]	p-tau, CSF AChE	Increased Decreased	AChE loss correlates with tau-driven neurodegeneration.
STUDY 07	Neuroimaging (MRI) Hippocampal atrophy associates with reduced AChE activity.	Neuronal loss in AD reduces AChE-containing neurons.	Hippocampal volume	Decreased	Structural atrophy parallels cholinergic system degeneration.[76]
STUDY 08	APOE4 Genotype APOE4 carriers show faster AChE decline vs. non-carriers.	APOE4 exacerbates amyloid pathology, accelerating cholinergic dysfunction.[77]	APOE4 expression	Increased	Genetic risk amplifies AChE loss via amyloid-mediated toxicity.[78]
STUDY 09	Inflammatory Cytokines TNF-α and IL-6 levels inversely correlate with AChE activity.	Neuroinflammation upregulates AChE in glial cells, worsening neurodegeneration[79]	TNF-α, IL-6	Increased	Pro-inflammatory cytokines induce AChE overexpression, promoting neuronal death.
STUDY	Oxidative Stress	Oxidative damage	8-OHdG, MDA	Increased	ROS modify

10	Markers Lipid peroxidation products correlate with AChE dysfunction.	impairs AChE enzyme function and cholinergic signaling.			AChE structure, reducing catalytic efficiency[80]
STUDY 11	CSF Biomarkers Low Aβ42 and high t-tau predict reduced AChE activity.	CSF biomarker profiles reflect cholinergic degeneration in early AD.	Aβ42 t-tau	Decreased Increased	AChE activity loss aligns with amyloid/tau pathology.[81]
STUDY 12	Longitudinal Cognitive Decline Faster MMSE decline in patients with low baseline AChE activity.[82]	ChE loss predicts progression from MCI to AD. [83]	MMSE scores	Decreased	AChE deficiency exacerbates cognitive impairment via disrupted neurotransmissio n.
STUDY 13	Animal Models (Transgenic Mice) Tg2576 mice show AChE hyperactivity near amyloid plaques.	Aβ deposition induces compensatory AChE upregulation, worsening toxicity	Amyloid plaques, AChE activity	Increased	Paradoxical AChE increase near plaques may accelerate neuronal damage.
STUDY 14	Synaptic Markers Synaptophysin loss correlates with AChE inhibitor resistance. [84]	Synaptic failure limits efficacy of AChE-targeted therapies.	Synaptophysin	Decreased	AChE inhibitors require intact synapses for optimal effect.
STUDY 15	Cholinergic PET Imaging Reduced AChE activity in basal forebrain correlates with memory deficits.	Cholinergic denervation drives early AD symptoms.	Cortical AChE binding	Decreased	PET confirms regional AChE loss as a biomarker of cholinergic decline. [85]
STUDY 16	MicroRNA Regulation miR-132 targets AChE mRNA; miR-132 is downregulated in AD. [86]	Epigenetic dysregulation of AChE contributes to pathology.	miR-132	Decreased	Loss of miR-132 increases AChE expression, promoting neurodegeneratio n.
STUDY 17	Antioxidant Therapies Vitamin E supplementation stabilizes AChE activity in AD patients.	Antioxidants mitigate oxidative inhibition of AChE.	Antioxidant capacity	Increased	Reduced oxidative stress preserves AChE function. [87]
STUDY 18	Autopsy Findings Post-mortem AD brains show 40-60% AChE loss in hippocampus. [88]	Cholinergic deficit is a hallmark of advanced AD pathology.	Hippocampal AChE	Decreased	AChE loss correlates with Braak staging and clinical severity. [89]

STUDY 19	Combination Therapy AChE inhibitors + memantine slow biomarker progression vs. monotherapy. [90]	Synergistic effects protect neurons from excitotoxicity and cholinergic loss. [91]	Aβ/tau	Stabilized	Memantine offsets glutamate toxicity, enhancing AChE inhibitor efficacy.
STUDY 20	Vascular Comorbidities Hypertension accelerates AChE decline in AD.[92]	Vascular insults exacerbate cholinergic dysfunction.	White matter lesions	Increased	Hypoperfusion reduces AChE synthesis in vulnerable regions. [93]
STUDY 21	Gut-Brain Axis Probiotics improve AChE activity in AD models via reduced inflammation	Gut microbiota modulate cholinergic signaling through anti-inflammatory effects.[94]	Systemic inflammation	Decreased	Gut-derived metabolites regulate AChE expression.
STUDY 22	Sex Differences Women show greater AChE inhibitor response than men.	Estrogen enhances cholinergic transmission and AChE inhibitor efficacy.	Cholinergic receptor density	Increased	Hormonal factors influence AChE dynamics and treatment outcomes. [95]
STUDY 23	Mitochondrial Dysfunction Impaired mitochondria reduce ATP-dependent AChE synthesis. [96]	Energetic failure in AD neurons limits AChE production.	ATP levels	Decreased	Mitochondrial deficits directly impair cholinergic enzyme systems.
STUDY 24	Epigenetic Modifications Hypermethylation of AChE promoter reduces enzyme activity in late-stage AD	DNA methylation changes contribute to AChE variability.	DNA methylation	Increased	Epigenetic silencing of AChE worsens cholinergic transmission. [97]
STUDY 25	NMDA Receptors AChE inhibitors upregulate NMDA receptors, improving synaptic plasticity. [98]	Cross-talk between cholinergic and glutamatergic systems enhances cognition.	NMDA receptor expressio	Increased	AChE inhibition indirectly modulates glutamate signaling.
STUDY 26	Proteomic Profiling AChE interacts with amyloid precursor protein (APP) in AD synapses	AChE-APP complexes may promote amyloidogenic processing.	APP-derived fragments	Increased	AChE accelerates Aβ aggregation, creating a feedback loop. [99]
STUDY 27	Nanotherapy Nanoparticle-delivered AChE	Enhanced drug delivery systems optimize AChE	Drug concentration in CSF	Increased	Nanocarriers overcome BBB limitations,

	inhibitors improve brain bioavailability [100]	targeting.			boosting AChE inhibitor efficacy.
STUDY 28	Sleep Disorders Sleep deprivation reduces AChE activity in preclinical models. [101]	Poor sleep quality exacerbates cholinergic dysfunction in AD.	Slow-wave sleep	Decreased	Sleep restoration may preserve AChE function.
STUDY 29	Diabetes Mellitus Insulin resistance correlates with AChE hyperactivity in AD.	Hyperglycemia promotes AChE glycosylation, altering enzyme activity.	Advanced glycation end-products (AGEs)	Increased	Metabolic dysfunction exacerbates AChE-mediated neurodegeneration
STUDY 30	Clinical Subtypes Atypical AD variants (e.g., posterior cortical atrophy) show distinct AChE loss.	Regional AChE patterns vary with clinical phenotypes.	Parietal AChE in visual variants	Decreased	AChE distribution aligns with symptom-specific neurodegeneration.
STUDY 31	Neurotrophic Factors NGF therapy restores AChE activity in cholinergic neurons.	Trophic support rescues AChE expression in early AD.	NGF signaling	Increased	NGF promotes survival of AChE-producing neurons.
STUDY 32	Metal Ion Interactions Aluminum exposure increases AChE aggregation in AD models. [102]	Environmental toxins exacerbate AChE dysfunction.	Metal-induced protein aggregation	Increased	Aluminum binds to AChE, promoting misfolding and toxicity.
STUDY 33	Pharmacogenomics CYP2D6 polymorphisms predict AChE inhibitor metabolism and efficacy.	Genetic variability influences therapeutic outcomes.	Drug plasma levels	Increased Decreased	Personalized dosing based on genetics optimizes AChE inhibitor benefits.[103]
STUDY 34	Neurofilament Light Chain (NfL) Elevated serum NfL correlates with AChE inhibitor non-response. [103]	Axonal degeneration reduces cholinergic resilience to therapy.	Serum NfL	Increased	High NfL indicates widespread neuronal damage, limiting AChE-mediated synaptic repair.
STUDY 35	TDP-43 Proteinopathy	Co-pathologies exacerbate	TDP-43 inclusions	Increased	TDP-43 mislocalization

	TDP-43 aggregates coexist with AChE loss in limbic regions of AD patients.	cholinergic dysfunction and cognitive decline.			impairs AChE mRNA transport in neurons [104]
STUDY 36	Short-Chain Fatty Acids (SCFAs) Butyrate supplementation restores AChE activity in AD rodent models.	Gut-derived SCFAs modulate neuroinflammation and cholinergic gene expression	Butyrate levels .	Increased	SCFAs enhance AChE synthesis via histone deacetylase inhibition.
STUDY 37	CRISPR-Based Gene Editing Post-COVID AD patients show accelerated AChE decline vs. controls.	Targeted AChE downregulation may mitigate amyloid-driven pathology.	AChE mRNA	Decreased	Reducing AChE expression limits its pro-amyloidogenic interactions.
STUDY 38	Exosomal AChE Plasma exosomes from AD patients carry higher AChE levels than healthy controls.	Exosomal AChE may reflect early neuronal stress in AD.	Exosomal AChE	Increased	Neurons release AChE-containing exosomes under stress, serving as a liquid biopsy marker. [105]
STUDY 39	Circadian Rhythm Disruption Nocturnal AChE fluctuations are blunted in AD patients with sleep disturbances. [106]	Disrupted circadian regulation impairs cholinergic recycling	Diurnal AChE variation	Decreased	Circadian clock genes (e.g., BMAL1) regulate AChE expression; dysregulation worsens AD.
STUDY 40	Curcumin's Dual Effects Curcumin inhibits AChE and reduces amyloid plaques in AD models.	Natural polyphenols offer multi-target benefits for cholinergic and amyloid pathways.	Amyloid burden, AChE activity	Decreased	Curcumin competitively binds AChE's active site while suppressing Aβ aggregation. [107]

Discussion

Since the identification of cholinergic deficiency in Alzheimer's disease (AD), acetylcholinesterase (AChE) has been extensively investigated in tissues.[43] Acetylcholinesterase is an enzyme associated with senile plaques.[44] Alzheimer's disease characterized by significant decline in components of cholinergic system.[45] It was first discovered by Dr. Alois Alzheimer in 1901, a German psychiatrist who described its typical symptoms in one of his patients [46] This disease is one of the most widespread single cause of dementia in our ageing society [47] occurring with advancing age. [48] The basic symptom of this disease i.e. Define in cognitive abilities caused by disrupted cholinergic neurotransmission. [49] One important factor in neurological illness is acetylcholinesterase [50] Its clarification will improve our knowledge of the pathophysiology and etiology of these conditions. [51]This enzyme performs a significant roles that are shared by majority of illness that have been characterized,

including involvement in oxidative stress and inflammation, apoptosis and abnormal protein adhesion. [52] Besides inhibiting the enzyme, the available AChE inhibitors possess several collaborative effects that will perhaps help delay the disease progress.[53] It is thought generally that they act symptomatically and not via causes. [41] Some of these, however, may make possible their causal use. [54] AchE contains a uniquely designed active site and a PAS. The majority of these MTDLs are designed with AChE as their target. [55] Because this enzyme is crucial to the pathogenesis of the majority of neurodegenerative disorders, effort to develop and find new medications that target it may result in medication that is helpful in treating other brain diseases [50]

Phytochemical variability tends to be the problem when plant extracts are used to try and treat these diseases. [56] The extracts exhibit variable degrees of potency in AChE inhibition as well as other antioxidant or anti-inflammatory activities so that the results of individual extracts tend to be inconsistent. [57]

Conclusion

Alzheimer disease is major contributor for dementia. [58] It is responsible for up to 75% of all neuro-dysfunction cases. [59] For two decades, rigorous investigation aiming to establish the casual factor of this disease have been carried out, hoping to produce safe and efficacious drug therapies. [60]. The acetylcholinesterase is among the important mediate the function and response of nerves.[61].It is main enzyme responsible for neurotransmitter acetylcholine hydrolysis and the primary target of almost all clinical drugs for AD [62] [63] AChE overexpression among cells do not trigger apoptosis, and those expressing an AChE at basal level normally develop. Needs research work based on human settings to be addressed for authentication of Alzheimer's Disease. Thus, published data in this review shows AChE which hydrolyses acetylated esters involved in the physiology of conditions like Alzheimer diseases which cause Dementia, Cognitive impairment, and Behavioral and Psychological symptoms through it. It is clear that the cholinergic signaling is significant in AD. But further research is needed to develop more effective therapeutic agents. The enzyme has several important functions that are common to most of the described disorders. Studies should be directed towards understanding the cause of the differential response to AChE inhibitors in depressive disorders. In these whole studies, a substantial progress has been observed. The evolution of acetylcholinesterase inhibitors (AChEIs) from 1990 to 2024 reflects a transformative journey in Alzheimer's disease (AD) therapeutics, marked by both significant achievements and ongoing challenges. Initially grounded in the cholinergic hypothesis which identified acetylcholine depletion as a core driver of cognitive decline early AChEIs like tacrine provided foundational proof-of-concept but faced limitations due to hepatotoxicity and poor tolerability. Future research must prioritize precision targeting of peripheral vs. central AChE isoforms, combination therapies that bridge symptomatic and disease-modifying effects, and real-world efficacy studies in diverse populations. Ultimately, while AChEIs remain indispensable for symptomatic management, their convergence with novel AD therapeutics heralds a new era of holistic intervention strategies aimed at not just mitigating decline but altering AD's trajectory.

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