

# Pharmacological Overview and Future Perspectives of Cholinergic Therapy in Alzheimer's Disease

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**Abstract:** Since damage to cholinergic neurons was found widely and severely in the brain of Alzheimer's disease (AD) patients in 1970s, many clinical trials of several kinds of cholinomimetics have been actively undertaken to stimulate the central cholinergic system which might be involved in cognitive function. Although there are various drug targets to activate the cholinergic system, only cholinesterase inhibitors prevailed in the clinical trials with regard to AD. At present, three cholinesterase inhibitors donepezil, galantamine, and rivastigmine are prescribed as a symptomatic treatment for AD. This class of drugs inhibits cholinesterase which breaks down acetylcholine, thus raising the level of acetylcholine in the synaptic clefts, activating central cholinergic function and finally leading to improvement of cognitive function, as well as other symptoms associated with AD. Clinical studies revealed that cholinesterase inhibitors reliably improved clinical rating scales of the two co-primary endpoints, typical cognition measured by ADAS-cog (Alzheimer's Disease Assessment Scale cognitive subscale) and global function assessed by CIBIC-plus (Clinician's Interview-Based Impression of Change), in mild-to-moderate AD patients. Furthermore, clinical evidence has accumulated to show efficacy in other states of dementia as well, namely dementia with Lewy bodies and severe AD. In addition to their symptomatic effects in AD, recent pharmacological studies showed that some cholinesterase inhibitors displayed certain disease modifying characteristics, namely interaction with the amyloid processing pathway, neuroprotection and enhancement of adult brain neurogenesis.

## 1. HISTORICAL BACKGROUND: INVOLVEMENT OF CHOLINERGIC SYSTEM IN AD PATHOLOGY

Acetylcholine was the first chemical to be identified as a neurotransmitter. In 1921, Otto Loewi [1] placed an isolated, beating frog heart in a saline-filled chamber connected to another chamber with a second isolated frog heart. He stimulated the vagus nerve and observed slowing down of the beating rate. After a short delay, he noticed that the pace of the other connected heart also slowed down. Thus, he proved the existence of a humoral substance, which was released by vagus stimulation and with the ability to slow down the other heart. He called it "Vagusstoff" and it was chemically identical to "acetylcholine", as it turned out later. This was the founding period in the history of modern neurotransmission research. At that time, nobody could imagine that this discovery would become related to the most common form of dementia, Alzheimer's disease (AD), which was first described by Dr. Alois Alzheimer [2].

In the 1970s, neurotransmitter research was thoroughly established, and many other classical neurotransmitters had already been discovered. With progress in development and establishment of neurochemical technologies to measure neurotransmitters themselves and activities of related enzymes, precise analysis of the dynamics of neurotransmitters in the synaptic cleft became possible. These technological advances backed up basic neurotransmitter research and led to the creation of many centrally-acting drugs like neuroleptics and antidepressants. In the stream of this neuroscience research trends, neurochemical researchers made efforts to

unveil what kind of changes in neurotransmitter system happened in the brain of AD patients. In 1976 and 1977, the abnormality in the brain neurotransmitter system was revealed by three different research groups. Bowen *et al.* [3] and other groups [4, 5] investigated the cerebral cortex and the hippocampus of AD patients and found a profound decrease in the activity of choline acetyltransferase (ChAT) when compared with age-matched control subjects. ChAT is an enzyme that biosynthesizes ACh from choline and acetylCoA at nerve terminals and which was found to be depleted in specific parts of the brain of AD patients. This meant that AD was described as a deinervating disorder of central cholinergic neurons projecting to cerebral cortex and limbic system. This neurochemical pathology was confirmed later by many other researchers [6-8]. The affected sites of AD brain showing cholinergic damage are relatively limited to the cerebral cortex and hippocampus, but not in sub-cortical regions like basal ganglia. Therefore, clearly not all of the cholinergic neurons are impaired, but the cerebral cortex, in particular temporal cortex and adjacent limbic areas, are heavily affected. Interestingly, there are two clinical studies demonstrating a correlation between ChAT activity and cognitive function, showing that the central cholinergic deficit might lead to some cognitive symptoms of AD: Perry *et al.* [9] described the correlation of ChAT activity in the cerebral cortex with cognitive function scores in mental test. Bowen *et al.* [10] also found a relationship between ChAT activity of brain samples and choice reaction time results in AD patients.

There are two major groups of cholinergic neurons in the central nervous system [11, 12]. One group resides in the basal forebrain area between the rostral portion of caudate and the anterior perforated substance, which innervates the cerebral cortex, hippocampus and amygdala. The other group exists in the pons sending its projections ascendingly to the

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thalamus and other nuclei in the diencephalon, and descending to the pons/medulla oblongata, cerebellar nuclei and cranial nerve nuclei. In 1982, Whitehouse *et al.* [13] reported the presence of lesions to magnocellular cholinergic neurons in the nucleus basalis of Meynert in AD. This group quantitatively investigated neurons in the Meynert nuclei in five individual brain samples of AD patients and noticed a remarkable 73% reduction in the number of neurons in the nuclei as compared to age-matched controls. The nucleus basalis of Meynert is enriched in magnocellular cholinergic neurons projecting afferently to the cerebral cortex. This was a milestone discovery meaning that sub-cortical neuronal loss was found and relatively selective groups of cholinergic neurons were damaged in AD. With this as a turning point, the focus was turned to the basal forebrain cholinergic system as being significant in AD neurochemical pathology.

Pharmacological evidence revealed that the central cholinergic system is involved in cognitive function. Antimuscarinic drugs that block cholinergic neurotransmission at the receptor level caused memory loss like that often seen in elderly people [14, 15]. It is well known nowadays that drugs having anti-muscarinic property have a risk to elicit impairment of cognitive function in elderly people. Experimentally, the anti-muscarinic drug scopolamine induces learning deficits in many species from mice and rats [16] to monkeys [17]. These findings suggest that the central cholinergic neurons are deeply involved in cognitive functions including learning and memory.

Although other neurotransmitter deficits or abnormalities besides cholinergic system were noticed in AD patients' brain (Table 1), cholinergic damage had gained a firm position in AD research among these impairments, since it was commonly and constantly found among a wide variety of AD cases. In the process of the historical background mentioned above, the "choline hypothesis in AD" was proposed by Bartus [18] and Coyle [19].

## 2. CLINICAL CHALLENGE WITH CHOLINOMIMETICS TO INTERVENE SYMPTOMS OF ALZHEIMER'S DISEASE

Activation of the brain cholinergic system seemed one of the promising therapeutic ways to alleviate symptoms of AD patients on the understanding that central cholinergic lesions are related to many aspects of AD symptoms. There is a good analogy with a therapy in Parkinson disease, where the central dopaminergic system is relatively selectively damaged. L-dopa was a breakthrough drug in Parkinson disease, which penetrates the brain, is converted into dopamine and compensates for dopamine in the brain. In early studies, choline, a precursor of acetylcholine, or lecithin, which is rich in choline, had been clinically tried [20, 21]. These clinical trials failed because of insufficient efficacy. One might speculate that not enough of choline could reach the brain in order to elevate central acetylcholine levels, although large amounts of choline or lecithin were ingested.

In another approach, agonists of the muscarinic cholinergic receptor directly stimulate the receptor and possibly facilitate central cholinergic activity. In fact, in some of the early small-scale clinical trials, muscarinic agonists like arecholine [22, 23] and RS-86 [24] showed some effectiveness

in cognitive function in AD patients. However, these clinical efforts eventually did not come to fruition, assuming that the main reason to fail might be the difficulty to balance between target central nervous system (CNS) pharmacology and peripheral side effects. Among these trials of cholinomimetics, the only strategy that proved successful was cholinesterase inhibitors. Cholinesterase inhibitors have long been used clinically as a therapy for myasthenia gravis and glaucoma [25], and centrally acting cholinesterase inhibitors were tried next to alleviate symptoms of AD.

## 3. SUCCESS OF CHOLINESTERASE INHIBITORS

Cholinesterases are the enzymes that hydrolyze acetylcholine into choline and acetic acid and inactivate acetylcholine. There are two kinds of genetically different cholinesterases [26], namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is the dominant type of cholinesterase in nerve cells and has the crucial role in cholinergic transmission to inactivate acetylcholine in the synaptic cleft. On the contrary, BuChE is distributed in peripheral tissues, serum and glial cells in the CNS. Its physiological role is still unknown, but interestingly, the level of this enzyme increases in the process of AD [27].

Physostigmine is a potent and competitive cholinesterase inhibitor that preferentially inhibits AChE compared with BuChE [28]. In an early study, physostigmine was observed to improve long-term memory processes in healthy volunteers [29], showing the possibility that physostigmine may enhance human memory function. In AD, physostigmine alone and its combination with lecithin showed improvement of cognitive function in small-scale clinical studies [30-34], but sometimes the dose-response had an inverted-U-shaped curve, and the observed effect was small. These clinical findings of physostigmine were confirmed later by other investigators [35-38]. Overall, results of studies are mixed. Many are positive, while some of them are somewhat negative [39-41]. Physostigmine has an extremely short half-life (15~30 min) and relatively potent peripheral side effects, which might be the reason why physostigmine did not show consistent clinical results.

Tacrine is a conventional cholinesterase inhibitor and was reported early as an antidote against barbiturate coma and morphine narcosis in experimental animals [42]. Summers *et al.* [43] gave tacrine intravenously to 12 probable AD patients and observed improvement in memory tests in half of the patients. Based on this finding, Summers and colleagues [44] conducted a placebo-controlled double-blind study and demonstrated a dramatic symptomatic improvement by oral tacrine. This striking result indicated that cholinesterase inhibitors might be a symptomatic treatment for AD and accelerated the developmental competition of cholinesterase inhibitors later on as a symptomatic therapy for AD. Multi-centered clinical studies of tacrine were conducted to confirm Summers' results [45, 46]. Results were positive, but mild, and not as dramatic as expected. FDA approved tacrine as the first drug for AD in 1993. At the same time, clinical studies revealed that tacrine often caused elevation of transaminase (ALP/SGT), suggesting hepatotoxicity.

**Table 1. Change in Neurotransmitter System in the Brain of Alzheimer's Disease**

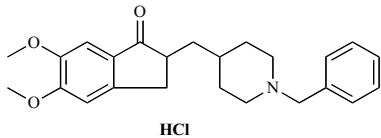
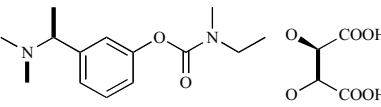
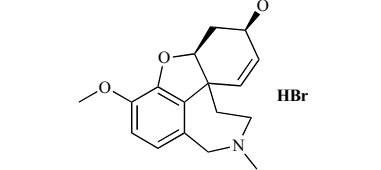
Neurotransmitter System	Changes
Cholinergic	basal forbrain cholinergic neurons ↓↓
	choline acetyltransferase activity ↓↓
	acetylcholinesterase activity ↓↓
	m1 muscarinic receptor →
	m2 muscarinic receptor ↓
	nicotinic receptor ↓
Noradrenergic	locus ceruleus noradrenergic neurons ↓
	noradrenaline ↓
	MHPG ↓
	dopamine β hydroxylase ↓
	α1, α2, β receptor →
Serotonergic	raphe nucleus serotonergic neurons ↓
	serotonin ↓
	5-HIAA ↓
Dopaminergic	dopamine →
	HVA →
	monoamine oxidase B ↑
Gabaergic	γ-aminobutyric acid →/↓
	glutamate decarboxylase →
Neuropeptides	
somatostatin	immunoreactivity ↓↓
CRF	immunoreactivity ↓↓
neurotensin	immunoreactivity ↓
opioid peptides	immunoreactivity →
substance P	immunoreactivity →
cholecystokinin	immunoreactivity →
VIP	immunoreactivity →
TRH	immunoreactivity →

→:no change, ↓:decrease, ↑:increase, ↓↓:marked decrease.

Later, pharmaceutical efforts were stepped up in order to overcome the issues that conventional cholinesterase inhibitors like physostigmine and tacrine had and new classes of cholinesterase inhibitors emerged. Eisai, Ltd had developed a piperidine class of cholinesterase inhibitor, donepezil (Aricept<sup>®</sup>; Eisai/Pfizer), which is a long-acting, potent and selective AChE inhibitor [28]. Now only three cholinesterase inhibitors, donepezil, rivastigmine (Exelon<sup>®</sup>; Novartis), and galantamine (Razadyne<sup>®</sup>; Johnson & Johnson) are prescribed in the U.S. (Table 2).

Early clinical studies of cholinesterase inhibitors had been conducted mostly in mild-to-moderate stages of AD. It was assumed that central cholinergic system had been thoroughly damaged in severe cases of AD, and thus the pharmacological intervention of cholinesterase inhibitors focused on mild-to-moderate AD. However, in reality, even in the severe stages of AD, the central cholinergic system, while being damaged, still remains active enough for cholinergic treatment to be effective. It therefore seems possible for cholinesterase inhibitor therapy to improve the symptoms of

**Table 2. Chemical Structures of Cholinesterase Inhibitors**

Brand Name/Marker	Structure	Selectivity	Dosages	Indication
Aricept Eisai/Pfizer	 HCl	AChE>>BuChE	5-10mg 3,5,10mg in Japan	mild to moderate AD severe AD in the U.S. & Japan
Exelon Novartis		AChE>=BuChE	6-12mg	mild to moderate AD
Razadyne, Razadyne ER Johnson & Johnson	 HBr	AChE>BuChE	30mg	mild to moderate AD

even severe AD, related to central cholinergic changes. Recently, two clinical studies of donepezil suggested some benefits in severe AD patients: Winblad and the severe AD study group [47] showed in a double-blind, placebo-controlled clinical trial that donepezil improves cognition and preserves function in severe AD patients who live in nursing homes. A second, multinational study [48] conducted with severe AD patients demonstrated greater efficacy of donepezil compared to placebo on measures of cognition and global function. Formally approved for severe AD in the U.S. and Japan (among other countries), donepezil became the only cholinesterase inhibitor indicated for all stages, from mild to severe AD.

Evidence showing effectiveness of cholinesterase inhibitors in other types of dementia has also been accumulating. Dementia with Lewy bodies (DLB) is considered one of the common forms of dementia and the core symptoms of DLB are cognitive deficits accompanied with visual hallucinations and/or parkinsonism-like motor disorders [49]. Parkinson's disease associated with simultaneous symptoms of dementia is defined as Parkinson's disease dementia; etiology and symptomatology significantly overlaps with DLB. In these diseases, central cholinergic depletion has been demonstrated [50, 51] and cholinesterase inhibitors are thought to be a rational treatment for symptoms of these neuropathological conditions. Generally, cholinesterase inhibitors have been shown to be effective not only in improving cognitive deficits, but also in regard to neuropsychiatric symptoms in several small clinical studies. Some long-term open label trials showed that treatment with cholinesterase inhibitors such as rivastigmine, galantamine or donepezil in patients with DLB improved cognitive function and neuropsychiatric symptoms [52-55]. Rivastigmine is approved for use in Parkinson's disease dementia in the EU and US [56, 57].

Although competition in clinical development of cholinesterase inhibitors seems to be settled now for the time being, pharmaceutical efforts in clinical development are nevertheless made to prove efficacy of some other types of cho-

linesterase inhibitors in AD. For instance, phenserine (TorreyPines Therapeutics) [58, 59], which is a derivative of physostigmine, may have an effect to decrease amyloid  $\beta$  ( $A\beta$ ) levels in plasma and cerebrospinal fluid (CSF). However, the clinical program for phenserine has been halted due to lack of efficacy in the Phase III studies. Huperzine A (NIA/Georgetown Univ; Phase II) [60] is a potent and selective AChE inhibitor with putative neuroprotective characteristics against glutamate-induced neuronal injury. It is being developed as a once-a-week patch formulation.

#### 4. DISEASE MODIFYING ASPECTS OF CHOLINESTERASE INHIBITORS

Cholinesterase inhibitors are basically a symptomatic therapy for AD, but there are a couple of clinical findings which make it tempting to consider a disease modifying action of cholinesterase inhibitors. An open-label study of donepezil for long-term efficacy and safety in AD [61] showed that the overall decline of ADAS-cog (Alzheimer disease assessment scale cognitive sub-scale) score of treated patients was less than estimated if this cohort of patients had not been treated. As another example, in a randomized, placebo-controlled pilot trial of donepezil in AD, Krishnan *et al.* [62] described that the donepezil-treated patients had significantly smaller average decreases in total hippocampal volume compared with the placebo-treated patients. Recent basic experimental research has also thrown light on the possibility that some of cholinesterase inhibitors might possess the property to intervene in the progress of the disease besides symptomatic effects.

##### 4.1. Cholinergic Hypothesis and $A\beta$ Hypothesis

Although there is no concrete evidence that the central cholinergic deficit directly causes accumulation of senile plaques or neurofibrillary tangles, it seems that some interaction exists between cholinergic transmission and amyloid plaque formation. Nitsh *et al.* [63] first demonstrated that the non-selective muscarinic agonist carbachol increased the release of soluble  $A\beta$  fragments, sAPP $\alpha$ , from m1 muscarinic

receptor-transfected HEK293 cells. Furthermore, the m1 muscarinic acetylcholine receptor agonist AF102B lowered A $\beta$  levels in CSF [64]. These basic and clinical findings suggest that stimulation of m1 muscarinic receptors prompts APP processing *via* the  $\alpha$ -secretase pathway.

Interestingly, the brains of Parkinson disease patients who had received anti-cholinergic medications for more than two years displayed 2.5 times higher amyloid plaque density and were also abundant in neurofibrillary tangle densities [65]. Conversely, patients with dementia with Lewy bodies who had taken cholinesterase inhibitors had significantly less parenchymal A $\beta$  deposition in the brain compared with matched untreated patients [66]. Although these two clinical studies are small-scaled, they suggest that long-term cholinergic medication might modulate neuropathological changes in AD brain.

#### 4.2. Neuroprotection

*In vitro* studies unveiled that not all, but some cholinesterase inhibitors possessed neuroprotective effects on neurons from various kinds of insults. Takada *et al.* [67] reported that donepezil, tacrine and galantamine, but not physostigmine, showed protective action against glutamate-induced neurotoxicity in rat cortical neurons. Another study [68] indicated that galantamine, donepezil and rivastigmine prevented cell death of SY5Y cells caused by A $\beta$  and okadaic acid. Our laboratory also independently conducted neuroprotection studies [69] showing that donepezil, but not tacrine and galantamine, alleviated cell damage in rat primary cultured cortical neurons caused by oxygen and glucose deprivation. Main study results of donepezil are summarized in Table 3, indicating that donepezil exhibits a broad effect profile in regard to neuroprotection from various kinds of insults [70-76].

The neuroprotection of cholinesterase inhibitors appears to be mediated *via* a mechanism that is independent of its

ability to inhibit cholinesterase activity. The concentration at which donepezil causes neuroprotective effects ranges from 0.1 to 1.0  $\mu$ M, which is much higher than the concentration needed for cholinesterase inhibition (IC<sub>50</sub>: 6.7 nM). Also physostigmine, which is a very potent cholinesterase inhibitor, gave no apparent protection against glutamate insult. Maurice and his colleagues [77, 78] had conducted *in vivo* neuroprotection studies which suggested that donepezil showed its protective effect *via* pharmacological interaction with the sigma 1 receptor. The nicotinic acetylcholine receptor has also been proposed to be involved in neuroprotective mechanisms by the finding that nicotinic receptor antagonists counteracted the protective effects of cholinesterase inhibitors. Using the permanent middle cerebral artery occlusion model, Fujiki *et al.* [79] demonstrated in rats that donepezil could prevent the resulting infarction from expanding, an effect which was counteracted by mecamylamine, a nicotinic acetylcholine receptor antagonist. Recently, Lorrio *et al.* [80] reported similar results in a gerbil ischemia model, the neuroprotective effects of galantamine being transduced *via* nicotinic receptor. These two *in vivo* studies suggest that a nicotinic mechanism is one of the candidates to explain the neuroprotective action of cholinesterase inhibitors.

#### 4.3. Neurogenesis

Neural progenitor cells give rise to neurons in the adult hippocampus throughout life. This hippocampal neurogenesis is reported to be modulated by the central cholinergic system. Lesion of the central cholinergic system after an injection of the immunotoxin 192IgG-saporin into the lateral cerebroventricles suppressed the survival of newly generated cells in the dentate gyrus of the hippocampus [81]. A cholinesterase inhibitor, physostigmine, enhanced neurogenesis in the dentate gyrus [82]. We found that four weeks oral treatment of rats with donepezil caused an increase in BrdU-labeled cells in the hippocampus, suggesting enhancement of adult hippocampal neurogenesis [83, 84]. Since the mus-

**Table 3. Summary of the Neuroprotective Effects of Donepezil**

Insult	Cell	Donepezil Effective Concentration ( $\mu$ M)	Reference
A $\beta$	PC12	0.1-1	Svensson <i>et al.</i> [70]
	culture neurons	0.1-10	Kimura <i>et al.</i> [71]
	culture neurons	1-10	Kimura <i>et al.</i> [72]
A $\beta$	SH-SY5Y	1	Arias <i>et al.</i> [68]
Okadaic acid		0.3	
Hydrogen peroxide	PC12	1-10	Zhang <i>et al.</i> [73]
Oxygen-glucose deprivation	PC12	0.1-10	Zhou <i>et al.</i> [74]
	culture neurons	0.1-10	Akasofu <i>et al.</i> [69]
Glutamate	culture neurons	0.1-10	Takada <i>et al.</i> [67]
	culture neurons	10	Takada-Takatori <i>et al.</i> [75]
NMDA	culture neurons	0.1-10	Akasofu <i>et al.</i> [76]

carinic acetylcholine receptor antagonist scopolamine decreased BrdU-positive cells, we suggest that the enhancing effect for neurogenesis is due to central cholinergic activation by inhibition of cholinesterase. The effective doses (0.5 & 2mg/kg, p.o.) of donepezil are in the same range of the doses to alleviate behavioral impairment in rat hypocholinergic models [85], and it is tempting to assume that the same effects might happen in the hippocampus in daily clinical treatment of AD patients.

## 5. FUTURE PERSPECTIVES OF CHOLINESTERASE INHIBITORS

Based on the cholinergic deficit in AD, cholinesterase inhibitors have been clinically developed and are now prescribed world-wide as a standard symptomatic therapy for AD. In recent years, appropriate use and limitations of cholinesterase inhibitors as well as possible application to other diseases have been clarified. Numerous phytoconstituents including rivastigmine and galantamine are reported as cholinesterase inhibitors [86] and continuous efforts are made to discover new chemical structures of the inhibitors in plants. In this review, I tried to outline the historical background and the current status of cholinesterase inhibitors and introduced some new pharmacological evidence of cholinesterase inhibitors. This category of AD drugs is thought to be symptomatic; however some of the inhibitors were proven to share disease-modifying properties which are neuroprotection, enhancement of neurogenesis and modulation of A $\beta$  processing. These are intriguing pharmacological characteristics and might not only contribute at least partly to AD therapy, but expand the clinical application to other CNS diseases beside AD.

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