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A molecular approach in drug development for Alzheimer's disease

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ABSTRACT

An increase in dementia numbers and global trends in population aging across the world prompts the need for new medications to treat the complex biological dysfunctions, such as neurodegeneration associated with dementia. Alzheimer's disease (AD) is the most common form of dementia. Cholinergic signaling, which is important in cognition, is slowly lost in AD, so the first line therapy is to treat symptoms with acetylcholinesterase inhibitors to increase levels of acetylcholine. Out of five available FDA-approved AD medications, donepezil, galantamine and rivastigmine are cholinesterase inhibitors while memantine, a *N*-methyl D-aspartate (NMDA) receptor antagonist, blocks the effects of high glutamate levels. The fifth medication consists of a combination of donepezil and memantine. Although these medications can reduce and temporarily slow down the symptoms of AD, they cannot stop the damage to the brain from progressing. For a superior therapeutic effect, multi-target drugs are required. Thus, a Multi-Target-Directed Ligand (MTDL) strategy has received more attention by scientists who are attempting to develop hybrid molecules that simultaneously modulate multiple biological targets. This review highlights recent examples of the MTDL approach and fragment based strategy in the rational design of new potential AD medications.

1. Introduction

Dementia is a progressive neurodegenerative disease that results in a gradual decline in cognitive function. The main causes of dementia are Alzheimer's disease (AD), frontotemporal dementia, Lewy body dementia, and vascular dementia [1]. AD, the most common form of dementia [2,3], is a result of a neurodegeneration, due to extracellular deposition of β-amyloid plaques and intracellular deposition of neurofibrillary tangles, which cause neurotoxicity and synaptic loss [4]. Dementia is also observed in the later stages of Parkinson's disease (PD) in 25–30% of cases [5].

At present, there are five prescription drugs approved by the U.S. FDA for the symptomatic treatment of AD [6]. These are: (i) donepezil, (ii) galantamine, (iii) memantine, (iv) rivastigmine, and (iv) donepezil and memantine (Fig. 1). In China, huperzine A, an alkaloid extracted from the native toothed clubmoss (*Huperzia serrata*) [7], is approved for use as a drug to treat AD, whereas in the United States it is marketed as a dietary supplement [8]. Donepezil, galantamine, rivastigmine, and huperzine A are competitive or reversible acetylcholinesterase inhibitors (AChEI), while memantine is a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. Irreversible inhibitors of AChE, especially organophosphates, have been widely used as insecticides and chemical weapon agents [9,10]. However, none of the FDA approved drugs have shown good efficacy and tolerability over a wide range of patients. They only provide symptomatic treatments and temporarily delay cognitive decline in approximately 50% patients with AD, while having very little effect in severe, advanced cases of AD [11].

The cholinergic hypothesis suggests that low levels of the acetylcholine (ACh) neurotransmitter in the body, due to degeneration of cholinergic neurons, leads to the cognitive, functional and behavioral symptoms seen in AD patients [12]. Inhibition of the enzyme acetylcholinesterase (AChE), which catalyzes the breakdown of ACh to choline and acetate [13], increases the concentration of ACh at the synapse, boosting cholinergic transmission [14]. Memantine, a NMDA receptor antagonist alters the brain's response to glutamate, a chemical messenger involved in all brain functions, such as learning and memory [15]. Excessive stimulation of the NMDA receptor results in more calcium ions entering the cell through the ion channel on the receptor, which at high enough levels results in neuronal injury or death. Although memantine is approved for the symptomatic treatment of more advanced AD it has only a modest effect on the disease [16].

Many drugs approved for use in AD have limited therapeutic efficacy and long-term tolerability, due to poor bioavailability, hepatotoxicity, and non-selectivity, which leads to adverse effects such as dizziness, diarrhea, nausea and vomiting. Tacrine, the first AChEI drug approved to treat AD, is no longer recommended for use due to its

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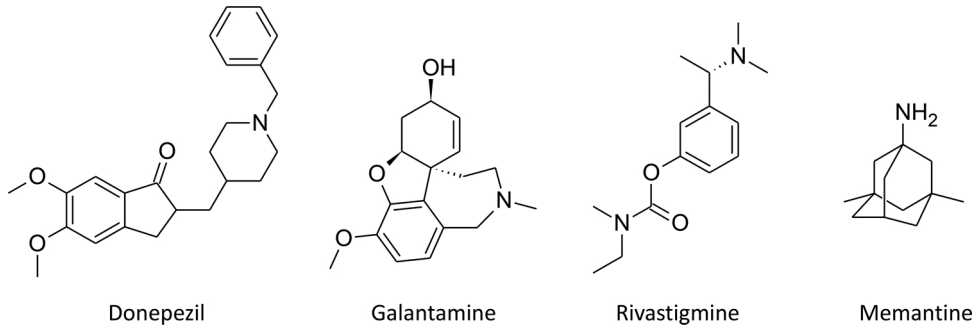

Fig. 1. Alzheimer drugs approved for use.

hepatotoxicity [17,18]. Currently, there are no medications that can cure, delay or stop the progression of AD.

2. The physiological roles of cholinesterases

Mammalian brains contain two cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Within a given species they have similar tertiary structures as they share a 65% amino acid sequence homology [19], though they differ in affinity for substrates, catalytic activity, and distribution within the brain. Both enzymes have a special affinity for methyl groups. However, BuChE is more specific for choline than AChE. Thus, the carbon analogue of ACh (carbamate) is hydrolyzed by AChE at 60% of the ACh rate, but only at 35% the ACh rate by BuChE [20].

AChE is the predominant ACh hydrolyzing enzyme in the nervous system and erythrocytes of most species, while BuChE is present in the blood plasma at high concentrations. The physiological role of BuChE is not clear since it doesn't have an endogenous substrate [21]. However, many workers believe that BuChE may have a role in neuronal development [22], since its expression varies during different developmental stages. It may also have an auxiliary role in modulating synaptic transmission and act as a backup for AChE [22,23]. High concentrations of BuChE in plasma also provides protection for AChE by acting as a scavenger of cholinergic toxins [24] such as succinylcholine and cocaine [25]. Conversely, BuChE plays a compensatory role in hydrolysis of ACh in the brain that is affected by degenerative changes. In the normal brain BuChE can hydrolyze ACh if AChE is completely inhibited [26]. While a decrease in AChE activity is observed in a number of areas of the brain, BuChE activity is not compromised and may even increase, making BuChE available in neuritic plaques.

3. Comparison of AChE and BuChE

The AChE molecule is ellipsoidal in shape and is approximately 45 Å × 60 Å × 65 Å in size. The substrate accesses the active site through a deep groove (gorge) in the surface of the molecule, which is approximately 20 Å deep and less than 5 Å wide at its narrowest point. The gorge widens out close to its base, leading to the catalytic active site (CAS) of the enzyme, which is near the bottom of the gorge (Fig. 2) [27]. The gorge is lined with 14 aromatic side chains (*e.g.*, Tyr-70, Trp-84, Tyr-121, Trp-279, Phe-288, Phe-290, Phe-330, and Tyr-334) which help to form 40% of the surface of the enzyme, and also the shape the gorge [28]. The amino acids also form a peripheral anionic binding site (PAS) around the rim of the gorge. At this site, "cation-π" interactions with the positively charged end of the ACh molecule, help to guide it further down to the active site at bottom of the gorge, where hydrolysis of ACh takes place [27]. In contrast, the volume of the gorge in BuChE is approximately 200 $Å^3$ larger, because it lacks six of the aromatic amino acids (out of the fourteen) that line the gorge in AChE [29,30]. This results in BuChE being less stereoselective for substrates than AChE [31]. The peripheral site is responsible for allosteric regulation of ACh

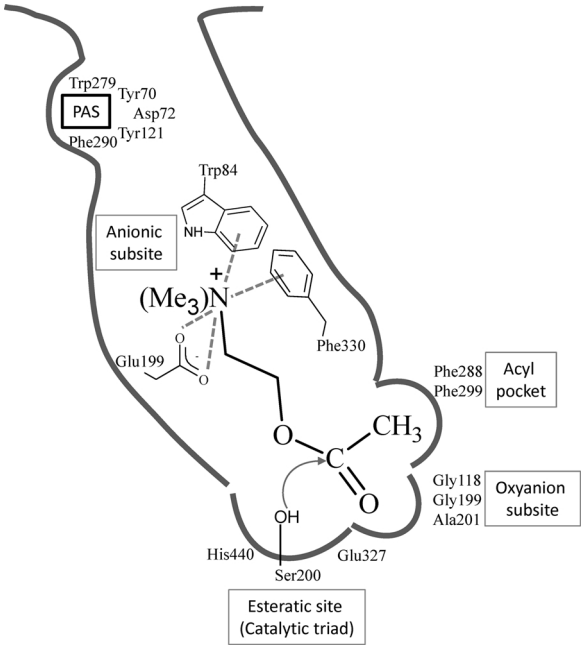

Fig. 2. ACh docked at the binding sites in the AChE gorge.

hydrolysis, *i.e.* it assists in bringing and aligning ligands to the binding site. Binding to the PAS at higher substrate concentrations may result in substrate inhibition [32], while at lower substrate concentrations it may accelerate the rate of hydrolysis [33]. It is interesting to note that recent evidence suggests that the PAS is involved in the amyloid-β (Aβ) aggregation process observed in AD. Both enzymes, AChE and BuChE, interact with the brain fibrils of Aβ peptides. However it is only AChE that causes an increase in the final yield of Aβ fibrils [34]. BuChE, lacks three of the aromatic amino acids (Tyr-70, Tyr-121, Trp-279) present in the PAS of AChE, resulting in no observable effect on amyloid formation [35–38]. Hence, the PAS of BuChE, which mediates substrate activation, has a weaker affinity than the PAS on AChE.

Piazzi et al. were the first to report an inhibitor able to bind at both the catalytic and the peripheral sites of AChE [39]. As AChE can induce β-amyloid fibril formation [40], peripheral site inhibitors may be used to decrease the rate of senile plaques formation. BuChE does not affect amyloid formation. Replacement of two phenylalanine residues, with the smaller valine and leucine amino acids in the peripheral site of BuChE, makes the acyl pocket larger and enables larger molecules to enter, resulting in lower enzyme specificity. Ligands that bind to the PAS will partially hinder the entrance to the enzyme's gorge, partially precluding the β-amyloid peptide from interacting with AChE. If PAS inhibitors are able to significantly reduce the rate of Aβ peptide binding with AChE, they may be able to reduce the rate of progression of AD [41–43]. Studies with propidium, a selective peripheral site ligand,