Bioorganic & Medicinal Chemistry Letters 23 (2013) 239–243

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Investigating the binding interactions of galantamine with β-amyloid peptide

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ARTICLE INFO

Article history:
Received 3 October 2012
Accepted 26 October 2012
Available online 10 November 2012

Keywords:
Alzheimer's disease (AD)
Cholinesterase inhibitors (ChEIs)
Acetylcholinesterase (AChE)
Butyrylcholinesterase (BuChE)
Disease-modifying agents (DMA)
Nuclear magnetic resonance (NMR)
β-Amyloid peptide (Aβ)
Molecular dynamics (MD)
Root mean square deviation (RMSD)

ABSTRACT

The anti-Alzheimer's agent galantamine is known to possess anti-amyloid properties. However the exact mechanisms are not clear. We studied the binding interactions of galantamine with amyloid peptide dimer ($A\beta_{1-40}$) through molecular docking and molecular dynamics simulations. Galantamine's binding site within the amyloid peptide dimer was identified by docking experiments and the most stable complex was analyzed by molecular dynamics simulation. These studies show that galantamine was interacting with the central region of the amyloid dimer (Lys16–Ala21) and the C-terminal region (Ile31–Val36) with minimum structural drift of Cα atom in those regions. Strikingly, a significant drift was observed at the turn region from Asp23-Gly29 (Cα atom RMSD = 9.2 Å and 11.6 Å at 50 fs and 100 fs respectively). Furthermore, galantamine's binding mode disrupts the key pi–pi stacking interaction between aromatic rings of Phe19 (chain A) and Phe19 (chain B) and intermolecular hydrogen bonds seen in unbound peptide dimer. Noticeably, the azepine tertiary nitrogen of galantamine was in close proximity to backbone C=O of Leu34 (distance <3.5 Å) to stabilize the dimer conformation. In summary, the results indicate that galantamine binding to amyloid peptide dimer leads to a significant conformational change at the turn region (Asp23–Gly29) that disrupts interactions between individual β-strands and promotes a nontoxic conformation of $A\beta_{1-40}$ to prevent the formation of neurotoxic oligomers.

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by loss of memory, cognitive decline and dementia. The pathological hallmarks of AD include cholinergic deficits, β-amyloid peptide (Aβ) aggregation and neurofibrillary tangle (NFT) deposition postmortem.[1–3] Worldwide the prevalence of AD is on the rise due to an increase in elderly population and increased lifespan. The health, social and economic consequences are going to be a major concern for many nations in the coming years.[4–6] The pathophysiology of AD is complex and is described by one of the earliest theories based on cholinergic dysfunction due to reduced levels of the neurotransmitter acetylcholine (ACh) in the central nervous system (CNS).[3,7] Later studies focused on the amyloid hypothesis of AD which describes the formation of neurotoxic amyloid aggregates, oligomers, protofibrils and fibrils due to amyloid-precursor protein (APP) misprocessing as a major event.[8] These studies show that both the cholinergic and amyloid hypotheses play a critical role in AD pathophysiology. The current pharmacotherapy of AD is mainly dependent on cholinesterase inhibitors (ChEIs) such as donepezil (Aricept® **1**, Fig. 1), galantamine (Razadyne® **2**) and rivastigmine (Exelon® **3**).[9–11] These agents are known to inhibit both acetyl- and butyrylcholinesterase (AChE and BuChE) enzymes respectively and decrease the rate of ACh degradation in the CNS. Pharmacotherapy of AD with ChEIs provides symptomatic relief and is not considered as long term treatment for advanced stages of AD.[12] The multifactorial nature of AD suggests that development of novel therapies as 'disease-modifying agents (DMAs)' will go a long way to treat both early onset as well as advanced stages of AD.

Among the marketed ChEIs, recent studies have indicated that galantamine is a potent inhibitor of amyloid aggregation suggesting its multiple modes of action as a DMA.[13,14]

Galantamine is a natural product derived from the bulbs and flowers of plant species such as *Lycoris radiata* and *Galanthus nivalis* belonging to the *Amaryllidaceae* family. Chemically it is a tertiary amine base with a tetracyclic fused ring system consisting of a benzazepine and a benzofuran rings along with three stereocenters (**2**, Fig. 1). It was approved by the US Food and Drug Administration (FDA) as a treatment option for mild-to-moderate dementia associated with AD in 2001.[15–17] Galantamine is known to inhibit the aggregation of both $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides as well as rescue human neuroblastoma cells exposed to $A\beta_{1-40}$ indicating its neuroprotective activity.[13,14] These studies suggest that the anti-amyloid action of galantamine is primarily due to inhibition of neurotoxic Aβ-oligomerization and weak inhibition of fibril formation. The exact mechanism of amyloid inhibition by galantamine is unclear. Current literature indicates that no investigations have been carried out till date to understand the potential binding modes of galantamine with Aβ-peptide. Here we use $A\beta_{1-40}$ peptide as a model to probe the binding interactions of galantamine using molecular

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http://dx.doi.org/10.1016/j.bmcl.2012.10.111

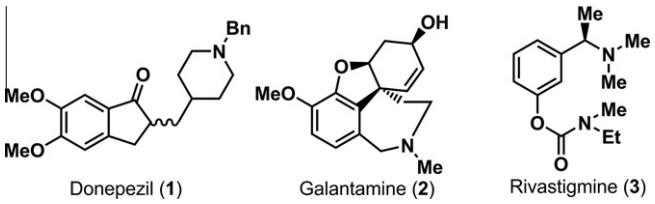

Figure 1. Structures of some cholinesterase inhibitors (**1–3**).

docking and molecular dynamics simulation investigations. The results add a new dimension in understanding the disease-modifying effects of galantamine and have implications in the design of small molecule inhibitors of amyloid oligomerization.

Previous studies with galantamine indicate its ability to prevent Aβ-oligomerization.[14] The initial step in the Aβ-oligomerization process is the formation of dimers.[18] Therefore we decided to study its interaction with $A\beta_{1-40}$ peptide dimer. Docking experiments were carried out to determine potential binding sites of galantamine. The coordinates of NMR structure of amyloid fibrils ($A\beta_{1-40}$) from protein data bank were obtained (pdb code: 2LMN, Tycko model).[19] Amyloid peptide consisting of chains A and B was extracted from the pdb file and used as antiparallel β-sheet (dimer) peptide model. The N-terminal octapeptide region consisting of amino acid residues 1–8 ($A\beta_{1-8}$) was not considered for modeling since those residues are not involved in amyloid aggregation.[20,21] Initially, three different sites of the dimer were considered to define a 20 Å sphere as binding site. A total of 1000 individual galantamine conformations were generated and docked on $A\beta_{1-40}$ peptide dimer (rigid receptor docking) and subjected to simulated annealing with 2000 heating steps and 5000 cooling steps. The electrostatic and nonpolar contribution to solvation energy was approximated by an implicit solvent model generalized Born with a simple switching function (GBSW) in Discovery Studio.[22,23] Galantamine was docked at three different sites of $A\beta_{1-40}$ peptide dimer. The first site consisting of His13–His14 (N-terminal) and third site consisting of Met35 and Val36 (C-terminal) did not yield any docked poses whereas the second site consisting of Phe19 and Phe20 (part of KLVFF segment) provided 10-ligand poses. Each pose was ranked according to their dock score function using CHARMm forcefield present in CDOCKER docking program. The ligand pose with the highest score (Fig. 2a, CDOCKER interaction energy = −18.79 kcal/mol) was investigated further by monitoring polar and nonpolar contacts with $A\beta_{1-40}$ peptide dimer. The rigid galantamine ring was oriented such that the cyclohexenol O*H* was forming a hydrogen bond with the backbone C=*O* of Val18 of chain B (distance = 2.6 Å). Additional contact with chain B was seen with another hydrogen bond to backbone N*H* of Val18. The cyclohexene carbon-carbon double bond (C=C) stacks up against the pi-system of Phe19 (chain B, distance <4.4 Å). The seven-membered nitrogen-containing azepine ring was oriented toward chain A (Fig. 2a). The *N*-methyl group exhibited equatorial geometry and underwent van der Waal's interaction with methyl groups of Leu34 and Val36 (chain A, distance <4.3 Å). One of the methylenes (CH_2) of azepine was in contact with methyl group of Leu34 (chain A, distance <3.5 Å). The aromatic benzene ring in galantamine underwent pi-stacking interaction with Phe19 (chain B, distance <3.6 Å). The methoxy substituent formed a weak hydrogen bond with Phe20 backbone N*H* (chain B, distance <3.7 Å). It is known that the tertiary amine of galantamine is protonated at physiological pH. In this regard, the closest contact to a positively charged quaternary nitrogen was backbone C=*O* of Leu34 (chain A, distance <6.4 Å). A hydrophobicity map of $A\beta_{1-40}$ peptide dimer clearly indicates that galantamine binds in hydrophobic regions of chains A and B, respectively (Fig. 2b). These investigations suggest that

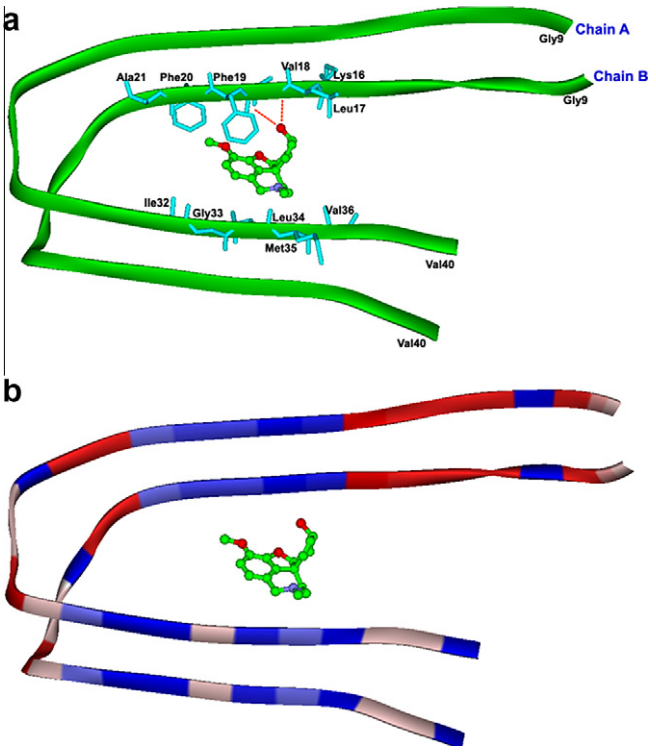

Figure 2. (a) The binding mode of galantamine (ball and stick) with $A\beta_{1-40}$ peptide dimer (ribbon diagram: CDOCKER interaction energy = −18.79 kcal/mol). Key amino acid residues of the peptide are rendered in stick model. (b) Hydrophobicity map of $A\beta_{1-40}$ peptide dimer showing polar and nonpolar regions (color code: red—polar and blue—nonpolar). Red dotted lines indicate hydrogen bonding interactions. Hydrogen atoms are removed to increase clarity.

galantamine primarily interacts with the KLVFF segment of $A\beta_{1-40}$ peptide dimer to stabilize the ligand-peptide complex and prevent its propagation to form oligomers, protofibrils and fibrils.

In order to determine the stability of galantamine-$A\beta_{1-40}$ peptide dimer binding, molecular dynamics (MD) simulation was carried out on the ligand-bound peptide and compared with the free peptide dimer. Standard dynamics cascade protocol in Discovery Studio was applied to both free and ligand-bound peptide. Energy minimization was done using CHARMm forcefield with 500 steps each of steepest descent (RMS gradient 0.1 kcal/mol Å) and conjugate gradient minimization, respectively (RMS gradient 0.0001/mol Å). The system temperature was raised from 50 K to 300 K with 1000 steps of equilibration (time step = 1 fs) and 5000 steps of production (time step = 2 fs) on NVT ensemble using an implicit solvent model GBSW along with SHAKE constraint. Coordinates were saved every 100 steps. The non-bonded cut-off radius was 14 Å whereas electrostatic interactions were calculated using spherical cut-off.

The MD trajectories were analyzed for both free $A\beta_{1-40}$ peptide and galantamine-$A\beta_{1-40}$ peptide complex (Figs. 3 and 4). The trajectory investigation on free $A\beta_{1-40}$ peptide dimer shows a considerable shift in the Cα atom root mean square deviation (RMSD) as a function of time indicating the flexibility of peptide dimer (Table 1). In order to analyze this further, RMSD in specific regions were measured. At the N-terminal region of Glu11–Gln15 after 50 fs the RMSD was 3.4 Å and after 100 fs RMSD was 5.1 Å compared with the initial conformation at time zero (Table 1). The central segment consisting of Lys16–Ala21 exhibited similar structural shift as the N-terminal segment with RMSD of 3.9 Å and 5.6 Å respectively at 50 and 100 fs (Fig. 3a). A major structural shift was observed in the turn region consisting of Glu22–Gly29 with 7.2 Å and 7.1 Å drifts seen at 50 and 100 fs respectively (Fig. 4a,