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Interaction study of two diterpenes, cryptotanshinone and dihydrotanshinone, to human acetylcholinesterase and butyrylcholinesterase by molecular docking and kinetic analysis

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ABSTRACT

Alzhemier's disease (AD) is a common form of dementia in the ageing population which is characterized by depositions of amyloids and a cholinergic neurotransmission deficit in the brain. Current therapeutic intervention for AD is primarily based on the inhibition of brain acetylcholinesterase (AChE) to restore the brain acetylcholine level. Cryptotanshinone (CT) and dihydrotanshinone (DT) were diterpenoids extracted from *Salvia miltiorrhiza* Bge. having anti-cholinesterase activity. Here we characterized the inhibition property of these two diterpenoids towards human AChE and butyrylcholinesterase (BChE). Both CT and DT were found to be mixed non-competitive inhibitors for human AChE and an uncompetitive inhibitor for human BChE. The docking analyses of CT and DT into the active sites of both cholinesterases indicate that they interact with the allosteric site inside the active-site gorge mainly by hydrophobic interactions.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which is characterized by the loss of cholinergic activity in patient's brain [1–3]. Natural compound from plant which posses anti-AChE activity has been a source of anti-AD drug. The majority of them are alkaloids. Examples like huperzine-A and galantamine, which are a quinolizidine alkaloid and a steroidal alkaloid respectively [4]. So far, there are few terpene-type AChE inhibitors ever reported, which are of relatively low potency, with IC_{50} in milli-molar level [5,6]. However, two diterpenoid, dihydrotanshinone (DT) and cryptotanshinone (CT), which extracted from *Salvia miltiorrhiza* Bge. (Danshen), showed potent anti-AChE activity [7–9]. In order to have a better understanding on the mode of action of CT and DT on AChE inhibition, we report herein our effort to determine how CT and DT interact with AChE and its peripheral homologue, butyrylcholinesterase (BChE) through detailed analyses of their enzyme kinetics and molecular docking.

2. Materials and methods

CT and DT were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, State Drug Administration, China. The purity (>98%) was confirmed by HPLC method. Activity of recombinant human AChE (hAChE) (Sigma, USA) or human BChE (hBChE) (Sigma, USA) was measured using the Ellman colorimetric method [10] using acetylthiocholine iodide (ACTI) (Sigma, USA) or butyrylcholine thio (BuTI) (Sigma, USA) as substrate.

Docking calculations were performed by the Autodock 4.0 software. The coordinates of hAChE (PDB ID: 1B41) and hBChE complexed with choline (PDB ID: 1P0I) and butyrate (PDB ID: 1P0M) were obtained from the Protein Data Bank. The structures were edited using the software from the ADT package to remove all water molecules and add hydrogen atoms. Non-polar hydrogens and lone pairs were then merged and each atom within the macromolecule was assigned a Gasteiger partial charge. A grid box of $41 \times 53 \times 41$ points, with a spacing of 0.375 Å, was positioned at the active-site gorge. The Lamarckian genetic algorithm (LGA) was employed with the settings of 70 runs per simulation, population size of 150 individuals, maximum number of generations and energy evaluations of 27,000 and 1.7 million respectively. The top dockings were examined further by clustering with an RMSD tolerance of 0.7 Å using the ADT software. The representative conformations of the ligands in

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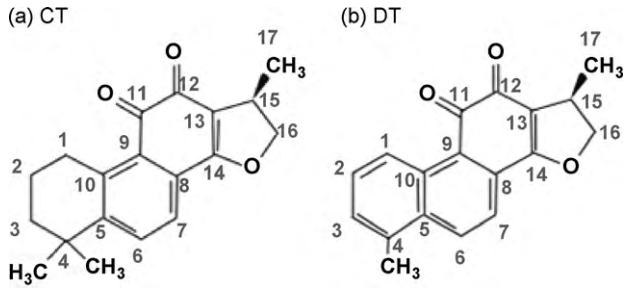

Fig. 1. Structures of (a) cryptotanshinone (CT) and (b) dihydrotanshinone (DT).

the lowest energy clusters were inspected visually and evaluated based on their interactions with the receptors (Fig. 1).

3. Results and discussion

3.1. Inhibition of CT and DT towards AChE and BChE

The inhibitory effect of CT and DT on hAChE and hBChE was studied with purified enzymes. hAChE or hBChE was incubated with increasing concentrations of CT/DT. The cholinesterase activities were determined by Ellman colormetric assay. The IC_{50} values of CT and DT for hAChE/hBChE were calculated by regression analysis (Table 1). The IC_{50} calculated for hAChE were in a close match with previous report [7]. From the ratio of their IC_{50} to hAChE and hBChE, DT was found to be more specific to hAChE (Table 1).

The mode of inhibition of CT and DT towards hAChE and hBChE, enzyme kinetic experiments were carried out in the substrate range where both enzymes obey Michaelis–Menten kinetics [11,12]. Results are illustrated in the form of Lineweaver–Burk plots in Fig. 2. Lineweaver–Burk plots show that with increasing concentration of CT and DT, there is an increase in K_m and decrease in V_{max}. This is the characteristic of a mixed non-competitive inhibition (Fig. 2a and b). Inhibition constant with enzyme (K_i) and enzyme–substrate complex (K_{is}) of CT and DT for hAChE was determined by secondary plots of the Lineweaver–Burk plot. Both CT and DT were found to be uncompetitive inhibitors against BChE (Fig. 2c and d), The apparent K_i, as K'_i, for the inhibitor was calculated by graphical analysis. The kinetic parameters are summarized in Table 1.

3.2. Molecular docking study

In order to gain functional and structural insight into the mechanism of inhibition, molecular docking simulation of CT/DT to cholinesterases were performed. Fig. 3a illustrates the most energetically favorable binding modes of CT and DT at the active site of hAChE (pdb: 1B41). These representative binding modes of CT and DT suggest free binding energies of −7.55 and −8.18 kcal/mol respectively. The computed K_i for the above binding models of CT and DT are 2.94 and 1.01 μM respectively, which are similar with the experimental K_i.

CT and DT were accommodated within the central hydrophobic region of the active-site gorge (Fig. 3a), arranged by Trp86, Tyr124 and Tyr337 [13], with occupation to the choline-binding site and the oxyanion hole region [14,15]. The interactions of CT and DT with these allosteric sites explain their non-competitive inhibiting property. In the meantime they also directly interact with the catalytic triad residues Ser203 and His447. The simultaneous occupation of the subsites where substrate should be bound explains their mixed-type inhibiting property.

Despite both CT and DT accommodate the same position, they are oriented differently. The penta ring of DT is facing the bottom of the gorge where the penta ring of CT is facing in the opposite direction and positioned towards the gorge mouth. The orientation of oxygen atom of C11 of DT favors hydrogen bond with the phenol-OH group of Tyr337. Moreover the oxygen at the penta ring of DT was at a position that likely to form hydrogen interaction with the backbone NH of Tyr133. These H-bonds were believed to contribute to the higher affinity of DT towards hAChE, and made it a more potent inhibitor than CT.

Comparison with other AChE structures that are bound with other polycyclic compounds of similar sizes shows that the bent hinge-shaped huperzine-A (PDB ID: 1VOT) and galanthamine (PDB ID: 1DX6) along with the relatively planar tacrine (PDB ID: 1ACJ) all bind at the bottom of the active-site gorge. Despite occupying different binding sites inside the gorge due to the different shapes and sizes, all three compounds occupy the choline-binding site, interact with the residues of the catalytic triad and stack against the indole ring of Trp86 like the predicted models of our diterpenoids (Fig. 3b).

The inhibitors were also docked to the active site of hBChE. According to the result of enzyme kinetic study they were both uncompetitive inhibitor towards hBChE. Therefore in order to simulate the binding of uncompetitive ligands to hBChE, the reported positions of butanoic acid (PDB ID:1P0I) and choline (PDB ID:1P0M) in native BChE were used to mimic the product intermediates-bound form during the docking studies due to the lack of native BChE–substrate structure. Fig. 3c shows the position of CT and DT at the active site of hBChE. The best-fitted models of CT and DT have estimated free binding energies of −7.03 and −7.17 kcal/mol respectively. The estimated inhibition constants of CT and DT to BChE in the presence of product analogues are 7.08 and 3.63 μM respectively, which are close approximations to the experimental K_i. Similar with the case in hAChE, the computed docking positions are surrounded by aromatic residues, Trp430, Phe329 and Tyr332. In both docking models of hBChE, CT and DT are located at a position close enough to directly interact with the product analogues, which might further stabilize the enzyme–product intermediates. This explains why they were uncompetitive inhibitors to hBChE in our assays. However, Tyr337 of hAChE, which suppose to form hydrogen interaction with DT, is replaced to alanine in hBChE [16]. Several other aromatic groups lining the gorge and the acyl-binding pocket of hAChE are also replaced by smaller or flexible side-chains in hBChE, namely Tyr72, Tyr124, Trp286, Phe295, Phe297, and Tyr337 of the former enzyme to Asp68, Gln119, Ala277, Leu286, Val288 and Ala328 of the latter respectively. These replacements result in an enlarged gorge in hBChE and may account for the significant drop in DT's binding specificity and thus potency on inhibiting BChE. Interestingly, several known uncompetitive hBChE inhibitors are also mixed non-competitive inhibitor for hAChE [17,18]. Further investigation on the interactions of these inhibitors to hAChE and hBChE may provide insight for designing of a new class of AChE inhibitor.

Table 1
A summary of inhibitory action of CT and DT towards hAChE and hBChE.

	Enzyme	Type of inhibition	IC_{50} (μM)	Selectivity IC_{50} (hAChE/hBChE)	K_i (μM)	K_{is} (μM)	K_i' (μM)
CT	hAChE	Mixed non-competitive	4.67 ± 0.41	0.70	6.73	7.65	
	hBChE	Uncompetitive	6.66 ± 0.42				2.17
DT	hAChE	Mixed non-competitive	0.89 ± 0.28	0.16	0.64	1.44	
	hBChE	Uncompetitive	5.51 ± 1.12				6.31