Published in final edited form as:

CNS Neurol Disord Drug Targets. 2011 November ; 10(7): 845–848.

Interaction of Human Brain Acetylcholinesterase with Cyclophosphamide: A Molecular Modeling and Docking Study

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Abstract

This study describes the interaction between human acetylcholinesterase (AChE), a key regulator of central and peripheral cholinergic function, and the widely used nitrogen mustard alkylating agent, cyclophosphamide (CP). Modeling of the AChE sequence (NCBI Accession No: AAI05061.1) was performed using 'Swiss Model Workspace'. The protein-model was submitted to the Protein Model Database and was assigned accession number PM0077393. A plot showing normalized QMEAN scores versus protein size was made to compare the model with a non-redundant set of Protein Data Bank structures, which gave a Z-score QMEAN as -0.58 . The predicted local error for the modeled structure was found to be well within tolerable limits. Z-score values for C β interaction, all atom interaction, solvation and torsion were found to be -1.10 , -0.90 , -0.06 and -0.40 , respectively. Docking between CP and AChE was performed using 'Autodock4.2'. Apart from other interaction-types, six carbon atoms of CP (C1, C2, C3, C4, C6 and C7) were determined to be involved in hydrophobic interactions with amino acid residues Y121, W233, L323, F331, F335 and Y338 of the 'acyl pocket' within AChE. Five carbon atoms of CP (C2, C4, C5, C6 and C7) were involved in hydrophobic interactions with 3 amino acid residues within the enzyme's 'catalytic site'. In conclusion, hydrophobic interactions play a major role in the appropriate positioning of CP within the 'acyl pocket' as well as 'catalytic site' of AChE to permit suitable orientation and allow docking. This information may aid the design of more potent and versatile AChE-inhibitors as pharmacologic tools and drugs to characterize and treat neurological disorders, and additionally provides a model whose value can be quantitatively assessed by X-ray crystallographic analysis of the AChE-CP three-dimensional structure.

Keywords

Cyclophosphamide; docking; enzyme-inhibition; human brain acetylcholinesterase; hydrophobic interactions; modeling

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BACKGROUND

Acetylcholinesterase (AChE, EC 3.1.1.7), a serine hydrolase, derives from a large family of proteins that, jointly, share a common α/β fold [1]. Such proteins comprise enzymes that are esterases, lipases and proteases, along with non-enzymatic proteins that operate as adhesion molecules and pro-hormones [1-3]. As an enzyme, the primary physiological role of AChE involves the termination of chemical transmission at cholinergic synapses and secretory organs by catalyzing the hydrolysis of the neurotransmitter acetylcholine, ACh, at a high turnover rate that approaches 2.5×10^4 molecules per second. Numerous roles have been attributed to AChE in diseases of high priority research, as in cancer [4] and Alzheimer's disease (AD) [5], to name a few. Inhibitors of AChE have been demonstrated to have efficacy (e.g., donepezil, rivastigmine and galantamine in AD; and pyridostigmine in myasthenia gravis) [6, 7] as well as toxicity (e.g., organophosphate and carbamate pesticides in health) [8], depending on their time-dependent concentration, mechanism of binding and use.

The nitrogen mustard derivative, cyclophosphamide (CP) monohydrate {2-[bis(2-chloroethyl) amino tetrahydro-2H-1, 3, 2-oxaza-phosphorine 2-oxide]}, is a widely used anticancer drug of the alkylating group class. It is routinely used in the treatment of lymphomas, some forms of leukemia and in specific solid tumors as well as in various autoimmune diseases, such as rheumatoid arthritis [9-11]. CP has additionally been used as an immunomodulatory treatment in myasthenia gravis [12, 13]. In addition, mutations at specific AChE genetic loci are associated with leukaemia and myelodysplastic syndromes [14].

CP has been reported to inhibit brain and retinal AChE enzymatic activity in several animal models [15-17]. The relevance of this to human enzyme remains to be elucidated, and hence the identification of the amino acid residues crucial to the interaction between human AChE and CP is of scientific interest. Such information may not only aid in optimizing the safe and efficacious clinical use of CP in a wide number of diseases but, additionally, provide drug-design insight in the search and development of more potent and versatile AChE-inhibitors. Currently, no X-ray crystallographic structural data is available within the Protein Data Bank to aid in characterizing the basis for interaction between AChE and CP. Our study therefore centered on defining the mode of potential interactions that may underpin CP-induced inhibition of human brain AChE, which could provide the focus of future confirmatory structural analyses.

METHODS

The amino acid sequence of human brain AChE was retrieved from the Pubmed Protein database (Accession No: AAI05061.1). Modeling was performed using 'Swiss Model Workspace'. The modeled structure (prior to docking) was verified using the 'Structure Assessment' function of the Swiss Model Workspace, which incorporates 'Procheck', 'DFIRE' and 'QMEAN' programmes. The Protein Data Base (PDB) structure of CP was retrieved from Pubchem (CID: 2907). Thereafter, the ligand (CP) was docked to modeled human brain AChE enzyme using 'Autodock4.2'. The MMFF94 force field was used for

energy minimization of the ligand molecule. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on the protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 30×30×30 Å grid points and 0.375 Å spacing were generated using the Autogrid program aimed to target grid co-ordinates in proximity with the acyl pocket and the anionic sub site of the catalytic site (CAS) of AChE. The values of x, y and z co-ordinates used for targeting the 'acyl pocket' were 94.00, 89.00 and 3.75, respectively. The x, y and z values used in docking calculations to target the CAS were 90.81, 83.98 and -8.04, respectively. To target the peripheral anionic site (PAS), several docking experiments were performed by placing the center of the grid at different well-recognized amino acid residues known to constitute the PAS. The grid dimensions for targeting the PAS used in this study varied from 20×20×20 Å to 60×60×60 Å. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the 'Lamarckian genetic algorithm' and the 'Solis & Wets local search method'. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 100 different runs that were set to terminate after a maximum of 2,500,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. All Figures were generated using Discovery Studio2.5 (Accelrys).

RESULTS AND DISCUSSION

The modeled protein structure described in this study was submitted to Protein Model Database and was assigned accession number PM0077393. The model was constructed using PDB crystal structure 3liiA (3.20 Å), sequence identity was 99% and final total energy was -26477.338 KJ/mol (Modeling Log). A density plot for QMEAN Scores of the reference set is shown in Fig. (1). A plot showing normalized QMEAN scores versus protein size was made to compare the model with a non-redundant set of PDB structures, which gave a Z-score QMEAN as -0.58 (Fig. 2). The predicted local error for the modeled structure was found to be well within tolerable limits (Fig. 3). DFIRE stands for 'Distance-scaled Finite Ideal-gas Reference' [18]. 'DFIRE-statistical energy' is a function which provides an accurate loop prediction at a fraction of computing cost required for more complicate physical-based energy functions [19]. Modeled-structures with lower DFIRE energy are ranked higher (i.e. regarded as better models) in molecular modeling studies. The DFIRE Energy for the constructed model was found to be -845.15 KJ/mol. High resolution X-ray structures on average have a Z-score as 0. Accordingly, the Z-score values for C β interaction, all atom interaction, solvation and torsion were found to be -1.10, -0.90, -0.06 and -0.40, respectively. This further confirms the accuracy of the protein structure constructed by homology modeling from its amino acid sequence (Fig. 4).

The original characterization of the crystal structure of AChE uncovered features that were initially unexpected but are now largely confirmed. First, the catalytic amino acid triad, S200, H440 and E327 in *Torpedo californica* AChE (human AChE: S203, H447 and E334), fundamental to the hydrolysis of ACh, rather than being localized on the surface, was

located at the bottom of a deep and narrow gorge of some 20 Å length within the enzyme [1, 3, 20]. The three-dimensional structure of AChE is somewhat comparable to two hemispheres that sandwich the catalytic center between two loops (the acyl- and omega-loops) that provide the sidewalls of the active site gorge that is approximately 300 Å³ in dimension [1, 3]. A combination of studies have aided the characterization of four different sub-sites within the gorge that include, (i) a PAS at the gorge mouth that is the initial binding domain encountered by a substrate or inhibitor, (ii) a deeper cationic- π site (CAS), where the quaternary ammonium of choline of ACh interacts, (iii) a still deeper acylation site that is the active center of the enzyme in which the catalytic triad resides, and, (iv) the acyl-binding pocket located at the base of the gorge [1, 3].

The acyl pocket of human brain AChE was predicted to interact with CP through the amino acid residues Y70, Y121, W233, V287, F288, R289, F290, Y334, F408 and Y442 (Fig. 5). The free energy of binding and estimated inhibition constant (K_i) for the 'CP-AChE acyl pocket-interaction' were determined to be -28.93 KJ/mol, and 8.49 μ M, respectively. Six carbon atoms of CP, namely C1, C2, C3, C4, C6 and C7 were predicted to be involved in hydrophobic interactions with amino acid residues Y121, W233, L323, F331, F335 and Y338 of human AChE enzyme. The hydrogen atom (H9) of CP was involved in cation- π interaction with F331 of AChE enzyme.

The CAS site of human brain AChE enzyme was determined to interact with CP through residues W84, N85, G116, G117, Y121, S122, G123, L127, Y130, E198, Y334, H443 and G444 (Fig. 6). The free energy of binding and estimated K_i for the 'CP-AChE CAS-interaction' were determined to be -30.56 KJ/mol and 4.42 μ M, respectively. It is germane to elaborate this interaction as it could aid in the design of new AChE inhibitors, as none are currently focused on the backbone of CP. Atoms N2 and H9 of CP formed hydrogen bonds with amino acid residues Y334 and W84 of the study enzyme, respectively. CP also formed a polar bond with residue W84 through its atom N2. Five carbon atoms of CP, namely C2, C4, C5, C6 and C7 were involved in hydrophobic interactions with 3 amino acid residues of human brain AChE, namely W84, Y334 and H443. The predicted K_i values for interaction within the acyl pocket and CAS can be compared to those of compounds known to interact and inhibit AChE, such as physostigmine (K_i 0.056 μ M), phenserine (K_i 0.048 μ M), the AD drugs donepezil (K_i 0.112 μ M), galantamine (K_i 0.44 μ M), rivastigmine (K_i 37.6 μ M) and tacrine (K_i 0.43 μ M) [21, 22], all of which are reported to inhibit AChE in humans and elevate ACh levels in the brain of animal models [23].

Figs. (5, 6) provide a close-up view of CP docked to the 'acyl pocket' and 'CAS' sites of modeled human brain AChE enzyme, respectively. The docking position and interacting amino acid residues for the described sites are in strong agreement with X-ray crystallographic structures of known AChE complexes. For instance, the acyl pocket of human brain AChE was found to interact with CP through the amino acid residues Y70, Y121, W233, F288, F290, Y334, F408 and Y442. These residues are known to be highly conserved, and most have been assigned functional roles [24]. A higher (negative) free energy of binding is an indicator of efficient interaction between an enzyme and inhibitor [25]. Accordingly, the free energy of binding for the complexes shown in Figs. (5, 6) were found to be -28.93 KJ/mol and -30.56 KJ/mol, respectively. These values fall within the

same approximate range as a series of donepezil analogues [26], which suggests that CP is an efficient inhibitor of human brain AChE. In the case of a third docking experiment (CP to PAS of human brain AChE), it was observed that minor changes in the grid co-ordinates resulted in a different docking pose and even changes in interacting residues, indicative of a partial-mixed inhibition system. In this regard, this is in accord with a preliminary but as yet unpublished study of the mode of inhibition of human platelet-derived AChE by CP (Abdulaziz A. Al-Jafari *et al.*, personal communication).

Whether or not sufficiently high concentrations of CP could readily enter the brain of humans to impact AChE and cholinergic transmission remains to be determined. The brain entry of CP, a water-soluble compound, is limited with a brain/plasma ratio of 0.2 [27]. Nevertheless, high-dose treatment or blood-brain barrier breakdown could elevate brain drug levels or result in the achievement of high systemic levels in the realm of CP-inducible AChE inhibition. Furthermore, the described binding interactions between CP and AChE highlight the numerous ways that structurally diverse pharmacological compounds utilized in the focused treatment of one disorder can occasionally dock as a non-covalent inhibitor at an unrelated target, such as with AChE [28], to not only potentially afford useful as well as adverse actions but also provide new directions to aid future drug design.

CONCLUSION

This study explores the interaction of human brain AChE with CP, a widely used anticancer alkylating agent. Hydrophobic interactions play a key role in the correct positioning of CP within the 'acyl pocket' as well as 'catalytic site' of AChE to permit docking. Such information may aid in the design of versatile AChE-inhibitors based on the oxazaphosphorine pharmacophore, as well as aid in the most efficacious and safe clinical use of CP. Scope remains in the determination of the three-dimensional structure of an AChE-CP complex by X-ray crystallography to validate the described data.

ABBREVIATIONS

AChE	Acetylcholinesterase
AD	Alzheimer's disease
CAS	The anionic sub-site of catalytic site of human AChE
CP	Cyclophosphamide
DFIRE	Distance-scaled Finite Ideal-gas Reference
K_i	Inhibition constant
PAS	The peripheral anionic site of human AChE
PDB	Protein Data Bank

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Fig. (1).

Density plot for QMEAN Scores of the reference set used in constructing the protein model of human brain AChE. The model was constructed using the crystal structure of PDB entry 3liiA.

Fig. (2).

Plot showing normalized QMEAN scores versus protein size to compare the constructed AChE model with a non-redundant set of PDB structures.

Fig. (3).
Plot showing predicted local error for the AChE protein model (predicted error versus residue number).

Fig. (4).
'Slider Plot' showing Z-score values for C β interaction, all atom interaction, solvation and torsion with reference to the constructed AChE model.

Fig. (5).

Interaction of cyclophosphamide (CP) docked to the “acyl pocket” of human brain acetylcholinesterase (AChE). Human brain AChE was modeled from amino acid sequence Accession No: AAI05061.1 of the Pubmed Protein database.

Fig. (6).
Interaction of cyclophosphamide (CP) docked to the “catalytic site” or “CAS” of the human brain acetylcholinesterase (AChE) model.