Journal of
Food and Pharmaceutical Sciences

Original Article

Molecular Docking Study of Caffeic Acid as An Acetylcholinesterase Inhibitor

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Received: 3 April 2023; Revised: 12 May 2023; Accepted: 05 July 2023; Published: 21 December 2023

Abstract: Acetylcholinesterase (AChE) receptor is a receptor that has been widely used as a potential drug target for Alzheimer's disease. Caffeic acid is a phenolic compound that had been experimentally proven to be an inhibitor of AChE. In this study, 100 molecular docking simulations were performed to study the interaction of caffeic acid in inhibiting AChE. The molecular docking simulations were performed using YASARA software with an in-house developed plug-ins. Redocking results showed that there were 99 out of 100 docking poses had an RMSD value of ⩽ 2.000 Å, which indicated that the molecular docking procedure could be used for further processes. The molecular docking of caffeic acid showed that all docking poses had an RMSD value of ⩽ 2.000 Å relative to the best pose of the first simulation, revealing that there was only one dominant docking pose in the AChE active site. Caffeic acid interacted favorably in the AChE active site with binding energy of about -8.022 kcal/mol. Its interactions were stabilized by hydrophobic and pi-anion interactions, in which some of the interactions resembled the same interaction of the native ligand.

Keywords: acetylcholinesterase, Alzheimer's disease, caffeic acid, molecular docking

1. INTRODUCTION

Acetylcholinesterase (AChE), a cholinergic enzyme of serine hydrolase family, is an enzyme which hydrolyzes acetylcholine into choline and acetic acid in a synaptic cleft [1]. It was found that inhibitory of AChE activity caused acetylcholine accumulation and interference neurotransmission, which could be a prominent strategy for the treatment of Alzheimer's disease [1], [2]. There are many strategies to overcome Alzheimer's disease, such as the utilization of natural products which has been widely used to develop more potent Alzheimer's disease drugs [3], [4]. In fact, natural products have played a vital role in many drug developments. Along with time, natural products-based drug discovery has been an interesting research topic due to their ability to interact with various biological targets [5].

Caffeic acid, a natural product which belongs to the phenolic family, has a wide range of biological activities such as antidiabetic, antioxidant, anti-inflammatory, and anti-carcinogenic [6], [7]. Caffeic acid could be synthesized and extracted from plant tissues such as fruits, potatoes, coffee beans, and even spent coffee grounds [8]–[10]. Previous research found that caffeic acid could potentially be an inhibitor of dipeptidyl peptidase IV (DPP4), a well-known target in drug discovery for treating type-2 diabetes mellitus [11]. In addition, caffeic acid showed a potent inhibitory activity on AChE experimentally [12].

Molecular docking and dynamics studies of caffeic acid targeting DPP4 in the previous research reported that caffeic acid could probably be a non-competitive inhibitor of DPP4 [11], showing that with the same method, researchers could conduct a study of the mechanism of action of a drug against its receptor. This study aimed to comprehend the potential mechanism of caffeic acid in inhibiting AChE based on molecular docking study. This research hopefully would also provide insight into designing dual inhibitors targeting DPP4 and AChE for the therapy of diabetes and Alzheimer's disease.

2. MATERIALS AND METHODS

2.1. Protein-ligand Preparation

The crystallographic structure of acetylcholinesterase complexed with its native ligand i.e., huprine X (PDB: 1E66) was downloaded from the Protein Data Bank using YASARA software [13] with a command of "LoadPDB 1e66, Center=Yes, Download=latest, SeqRes=Yes, Contour =1.0 sigma. This structure was prepared by correcting the amino acids lost from the crystallographic structure based on the SEQRES data from the PDB file assisted with the software module of "Build > N-terminal loop" and "Build > C-terminal loop". In addition, solvent molecules (Hoh) and other unimportant residues (Nag) were omitted. The system was totally corrected using "CleanAll" command and the terminal cap was added with a command of "AddCapObj 1,Type=ACE+NME,Location=all".

In this preparation, we found that YASARA cannot identify the single bond of the ethyl group in the native ligand. Therefore, we corrected it manually by deleting all hydrogen atoms with a command of "DelAtom Element H" and changed the atom sequence of 8522 and 5626 to 4345 and 4347, respectively. Furthermore, we edited the bond from double bond to single bond with certain commands of "DelBond 4345, 4347", "AddBond 4345, 4347,1", and "AddHydAll" respectively thereby representing the correct structure of huprine X. YASARA-Structure identifies double bonds from a PDB file based on bond angle and bond length. Hence, after manually corrected, the native ligand should be energy minimized to anticipate the bond-detecting error with commands of "Options > Choose Experiment > Energy minimization". Afterwards, the system pH was set to 7.4 with commands "pH value=7.4,update=Yes" and the structure was automatically checked using commands of "Edit > Clean > All". The energy minimization of the acetylcholinesterase-huprine X complex was then conducted using commands of "Options > Choose experiment > Energy minimization". This prepared and corrected structure was saved as a YASARA-Object file with a file name of "1e66-corr.yob" in the working directory.

2.2. Redocking of the Native Ligand using YASARA Plug-in

The prepared and corrected AChE-huprine X file, "1e66-corr.yob", was set as the MacroTarget for our in-house developed YASARA plug-in with commands of "MacroTarget {WorkingDirectory}\1e66-corr.yob,Remove=Extension" and "molmod – Penambatan ulang 100 kali" on YASARA-Structure, respectively. Processes happening in that in-house developed YASARA plug-in were: (1) AChE-huprine X structure were separated into two different objects, i.e., object 1 and object 2, respectively. A cubic simulation cell was added with a distance at least 5 Å from the outer atom. This simulation cell was defined as object 3. (2) Object 2 was saved separately as YASARA-Object with a file name of "(MacroTarget)_ref.yob" and "(MacroTarget)_ligand.yob" and then object 2 was deleted from the YASARA-Structure. (3) The other object was saved as YASARA-Scene with a file name of "(MacroTarget)_receptor.sce" continuing with 100 redocking simulations of "(MacroTarget)_ligand.yob" on "(MacroTarget)_receptor.sce" using AutoDock Vina in YASARA-Structure, employing the default setting in every simulation. The RMSD value of each best-redocking huprine X in every simulation compared to the co-crystalized huprine X i.e., "((MacroTarget)_ref.yob)" was calculated. The results were saved in the working directory namely "rmsd_bestpose_all.txt.". The RMSD values were used to evaluate the validity of the proposed molecular docking procedure [14], [15]. In addition, there was a file named "(MacroTarget)_config.mcr" containing molecular docking configurations which could be used as

the configurations for the proposed compound. This file is also useful for the molecular docking configuration optimization if the molecular docking procedure is still not valid based on the resulted RMSD values. The molecular docking configuration optimization could be conducted by modifying the "config.mcr" file in the directory of "(YASARADir)\mcr\epi" to obtained the valid molecular docking procedure.

2.3. Molecular Docking of Caffeic Acid

Molecular docking of caffeic acid was performed using the same procedure as the redocking. In the different working directory, "1e66-corr_receptor.sce" and "1e66-corr_config.mcr" files were copied as the previous validation results. The three-dimensional structure of caffeic acid was built using YASARA-Structure from its SMILES code i.e., C1=CC(=C(C=C1C=CC(=O)O)O)O with a module of "Edit > Build > Molecule from SMILES string" and the hydrogen atoms were added in a pH of 7.4 with a command of "pH value=7.4,update=Yes". Afterwards, structure checking and AM1 geometry optimization were conducted using module of "Edit > Clean > All" and "Options > Choose experiment > Energy minimization", respectively. This structure was saved as "1e66-corr_ligand.yob". MacroTarget was set to perform our in-house developed plug-in i.e., "molmod – Penambatan senyawa 100 kali Tahap 1" with commands of "MacroTarget {Direktori Kerja}\1e66-corr.yob,Remove=Extension" and "molmod – Penambatan senyawa 100 kali Tahap 1" on YASARA-Structure, respectively. This our in-house developed plug-in aimed to set a setting with the same molecular docking procedure as "(MacroTarget)_config.mcr." file.

Subsequently, our next in-house developed plug-in i.e., "molmod – Penambatan senyawa 100 kali Tahap 2" was executed to perform 100 molecular docking simulations with the same configuration resulted from the redocking configuration. To calculate the RMSD values of the best-docking pose of caffeic acid compared to the best-docking pose from the first molecular docking simulation, we used our other in-house developed plug-in i.e., "molmod – Penambatan senyawa 100 kali Tahap 3". The results were saved as "rmsd_bestpose_all.txt" file in the working directory. The best pose indicates the most stable docked conformation, which its binding energy was calculated using AutoDock Vina scoring function [16]. The docked caffeic acid having RMSD > 2.000 Å indicates that docked caffeic acid conformation is distinguishable from the best-docking pose of caffeic acid conformation in the first simulation. All ligand-protein interactions were visualized using YASARA and LigPlot software [17].

3. RESULTS AND DISCUSSION

Preparation of ligand-receptor i.e., acetylcholinesterase-huprine X complex was succesfully conducted by omitting the water molecules and other unimportant residues in order to avoid the unnecessary interaction between ligand and the receptor. The molecular docking validation based on redocking results showed that 99 out of 100 docked native ligands had an RMSD value of $\leqslant$ 2.000 Å. These results also denoted that all redocked huprine X having RMSD value $\leqslant$ 2.000 Å had nearly the same docked conformation and binding energy, ranging from -13.168 to -13.201 kcal/mol. There was only one redocked huprine X having a highly RMSD value of 11.783 Å with a binding energy of -8.045 kcal/mol. This could be noted that this redocked conformation was greatly different from those redocked huprine-X conformations having an RMSD value $\leqslant$ 2.000 Å, resulting in a high binding energy difference.

As shown in the redocking results, more than 95% of the redocking results could produce nearly similar native ligand co-crystalized conformation. These redocking results also indicated that molecular docking procedure was valid and reliable because the RMSD value of 99 out of 100 redocking poses was $\leqslant$ 2.000 Å [15]. Therefore, the redocking procedure and parameters could be used to perform molecular docking of caffeic acid. Redocked huprine X having the lowest RMSD value of 0.2748 Å had a binding energy of -13.18 kcal/mol, as visualized in Figure 1a. As shown in

Figure 1b, hurpine X was stabilized by hydrophobic interactions such as Phe331, Tyr121, Gly118, Ser122, Trp84, Phe330, Trp432, Tyr442, Gly117, Phe290, Gly119, and two of the three catalytic triad i.e., His440 and Ser200.

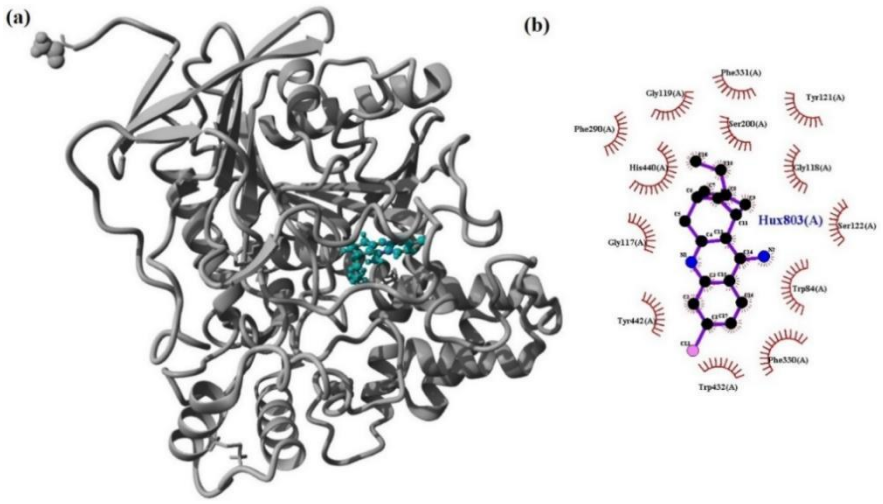

Figure 1. Molecular interactions of the native ligand i.e., huprine X in the active site of AChE showed in the three-dimensional (huprine X and AChE is visualized in a cyan and grey color, respectively) (a) and two-dimensional visualization (b).

In molecular docking of caffeic acid, every best-docking pose resulted from 100 molecular docking simulations had an RMSD value $\leqslant$ 2.000 Å with maximum RMSD value of 0.0934 Å. These results indicated that there was only one dominant pose in the interaction of caffeic acid with AChE, also suggesting that all docked caffeic acid had nearly the same conformation. It was found that the average binding energy of the best-docking caffeic acid was -8.022 kcal/mol, having a value ranging from -7.975 to -8.033 kcal/mol. Each best-docking caffeic acid pose had an almost similar binding energy value, suggesting that each best-docking caffeic acid pose had relatively the same conformation compared to the best-docking conformation from the first molecular docking simulation. Visualization of docked caffeic acid interacting in the AChE active site from the first molecular docking simulation, having a binding energy of -8.024 kcal/mol, is depicted in Figure 2a.

Caffeic acid interacted nicely in the active site of AChE via hydrophobic interactions with certain amino acid residues such as Gly117, Tyr334, Phe330, Trp432, Tyr442, Ile439, Trp84, Gly441, and His440 as visualized in Figure 2b. Caffeic acid formed the same hydrophobic interactions as the native ligand, which were Gly117, Phe330, Trp432, Tyr442, Trp84, and His440. In forming the hydrophobic interactions, aromatic ring-containing amino acids such as Phe330 and Trp84 interacted via pi-pi stacked interactions to the phenyl ring of the caffeic acid. These interactions were also observed in the native ligand interactions, showing that the quinoline ring of huprine X could form pi-pi stacked interactions to Phe330 and Trp84. As expected, a carboxyl group in caffeic acid formed an electrostatic interaction (pi-anion) with aromatic ring of Trp432, which also contributed in stabilizing the interactions.

According to our results, the existence of the aromatic ring and carboxyl group played an essential role in forming ligand-receptor interactions. Studies have found that the inhibitory effect of acetylcholinesterase inhibitors related to the phenyl ring and carboxyl group, which could promote the inhibitory activity [18], [19]. This finding indicated that those interactions could support and was

in good agreement with the previous research that showed caffeic acid could inhibit AChE experimentally [12].

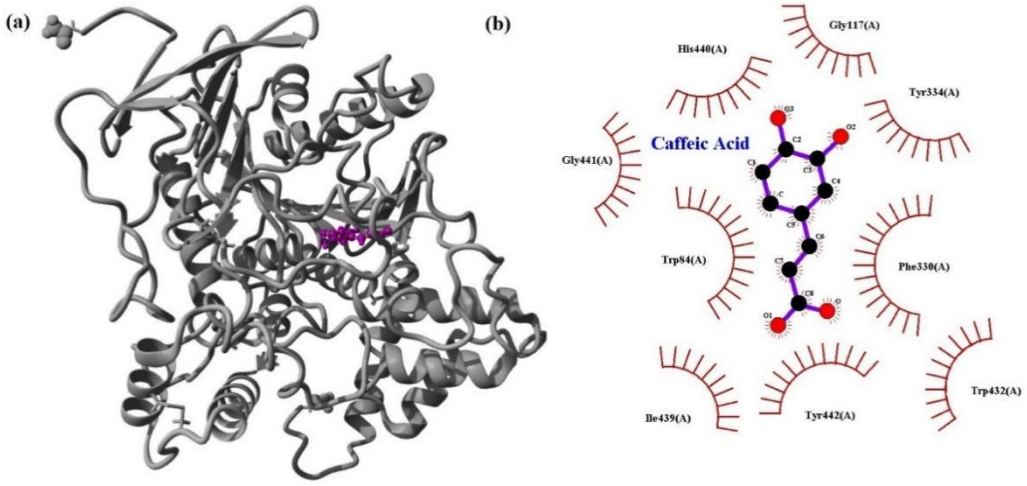

Figure 2. Molecular interactions of caffeic acid in the active site of AChE showed in the three-dimensional (caffeic acid and AChE is visualized in a purple and grey color, respectively) (a) and two-dimensional visualization (b).

This finding was also correlated to research conducted by Istyastono and Riswanto (2022), showing that caffeic acid could potentially be an allosteric DPP4 inhibitor by also forming pi-pi stacked interactions and additional interaction such as hydrogen bond. From molecular dynamics simulation performed by Istyastono and Riswanto (2022), the carboxyl group could form hydrogen bonds to the amine group of Lys71 and the hydroxyl group of Ser59 whereas the phenyl ring could form a pi-pi stacked interaction with the phenyl ring of Tyr105 in the allosteric site of DPP4 [11]. Therefore, we believe that the carboxyl group and phenyl ring play crucial role in inhibiting DPP4 and also AChE. Concluding to our results, caffeic acid bound favorably in the AChE active site by forming hydrophobic and pi-anion interactions which stabilized the AChE-caffeic acid interaction. Nevertheless, molecular dynamics simulations of AChE-caffeic acid in the active site should be carried out in order to asses the ligand-protein stability in a solvation system and in a certain simulation time.

4. CONCLUSION

This study conducted 100 molecular docking simulations to comprehend the mechanism of caffeic acid in inhibiting acetylcholinesterase (AChE). The results showed that caffeic acid favorably interacted in the AChE active site by forming hydrophobic and pi-anion interactions, with average binding energy of -8.022 kcal/mol. Our findings revealed that caffeic acid could form the same hydrophobic interactions as the native ligand i.e., Gly117, Phe330, Trp432, Tyr442, Trp84, and His440, showing the nearly similar inhibition mechanism. We also found that the existence of the carboxyl and phenyl ring plays a crucial role in the interaction stabilization. Our findings suggest that a molecular dynamic simulation is needed to explore and evaluate the dynamics stability of caffeic acid and acetylcholinesterase interactions.

Funding: This research received no external funding.

Acknowledgments: -

Conflicts of interest: The authors declare no conflict of interest.

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