(RESEARCH ARTICLE)

Computational study on the molecular interactions of tramadol with selected antioxidant and detoxification enzymes in *Drosophila melanogaster*

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Abstract

Tramadol is a potent analgesic medication prescribed worldwide for treatment of acute and chronic pains. Its relative tendency to be abused has become a public health concern. This study was designed to evaluate the molecular interactions of tramadol with selected antioxidant and detoxification enzymes of *Drosophila melanogaster*. The structures of CYP2D6, catalase and defense repressor 1 were retrieved from protein database (PubMed, Swiss model) while tramadol 2D structure was obtained from PubChem repository and prepared using LigPrep scripts as implemented in Small-Molecule Drug Discovery Suite of Schrödinger. 2D structure of tramadol was docked into the protein model binding site using Glide software from Schrodinger. The result revealed that, on the binding pocket of CYP2D6, tramadol and CYP2D6 inhibitor bind the protein pocket via hydrogen bond. The hydroxyl group of tramadol and the inhibitor interacts with the C=O groups of the residues Glu 128 and Lys 264 on the protein pocket respectively. Tramadol binds with higher affinity with a docking score of -4.581 kcal/mol when compared with the inhibitor which gave a docking score of -1.865 kcal/mol. With catalase, tramadol and the crystalized ligands were observed to exhibit hydrogen bonding with Ala 64 residue on the protein binding pocket with docking score of -2.348 kcal/mol and -3.431 kcal/mol respectively. The ability of tramadol to interact with these enzymes, strongly suggests that tramadol treatment may induce oxidative stress and at high dose might result in cellular toxicity. Therefore, the toxic effects of tramadol should be of concern despite the important role it plays in pain management.

Keywords: Tramadol; Antioxidant enzyme; Molecular docking; Catalase; Cytochrome P450 2D6

1. Introduction

Addiction is an increasing social and health problem worldwide despite all efforts to prevent and control it. Analgesics are among the most popular drugs which are being abused [1]. The abuse liability of naturally occurring opiates (e.g., morphine, codeine) and synthetic opioids (e.g tramadol, heroin, oxycodone, and buprenorphine) are well documented in the literatures [2, 3]. Tramadol is a centrally acting analgesic agent that is structurally related to morphine and codeine that is believed to naturally occur in *Nauclea latifolia* [4]. L-lysine, L-arginine, L-tyrosine, L-phenylalanine and L-tryptophan are the biosynthetic amino acids precursor of tramadol [5].

Addicts abuse tramadol every day by using the drug without doctor prescriptions. In the USA and Europe, according to Hawton *et al*. [6], there have been an upsurge in tramadol use following withdrawal of dextropropoxyphene from the market thus raising the risk of increased poisonings and deaths attributed to this drug. Similarly, in Nigeria, the rate of tramadol abuse has been on the increase among Nigerian youths in recent time [7]. However, wide-spread use of tramadol is associated with toxicity and a recent study showed that tramadol cause brain, heart and lung toxicity [8]. The central role of liver and kidney in drug metabolism predisposes them to toxic injury. Tramadol in the liver is converted to O-desmethyl-tramadol by cytochrome P450 which itself is an active substance and is two to four times

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more potent than tramadol. Further, biotransformation results in inactive metabolites, which are excreted through kidneys [9]. Persistent tramadol administration might lead to the accumulation of toxic metabolites in the body, increase the risk for its toxic kinetics effects and/or lower the clearance of tramadol, thus increasing its potential for toxicity [10]. Neurotoxicity of tramadol has been reported in patients receiving tramadol both at the recommended dosage and the high dosage ranges in animal and human studies [11]. The neurotoxicity of tramadol commonly manifests as generalized tonic-clonic seizures. Chronic use of tramadol in increasing doses causes neuronal degeneration in the rat brain, which probably contributes to cerebral dysfunction [12].

Reactive Oxygen Species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA. The shift in balance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress." Oxidative stress contributes to many pathological conditions, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma. Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed. Cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, and to regulate redox sensitive signaling pathways. Three of the primary antioxidant enzymes contained in mammalian cells that are believed to be necessary for life in all oxygen metabolizing cells are Super Oxide Dismutase (SOD), catalase and a substrate specific peroxidase, glutathione peroxidase (GPx). The SODs convert superoxide radical into hydrogen peroxide and molecular oxygen (O_2), whereas the catalase and peroxidases convert hydrogen peroxide into water and, in the case of catalase, into oxygen and water. The net result is that two potentially harmful species, superoxide and hydrogen peroxide, are converted to water.

The relevance of the *D. melanogaster* model for understanding the human condition under stress of toxicants has largely been accepted as more insight into the abundance of highly conserved genes and pathways controlling development, stress response, and xenobiotic metabolism across these divergent species is now available [13]. The cytochrome P450 superfamily, of which Drosophila Cyp2d6 is a member, is a group of metabolic enzymes with an unusually wide range of substrates [14]. They are conserved throughout evolution [15] and are involved in the metabolism of many endogenous and exogenous compounds.

This study is aimed at investigating the possible toxicity *in-situ* as a result of interaction of tramadol with antioxidant and detoxification enzymes using *drosophila melanogaster* as a model.

2. Material and methods

All molecules under study were docked into the binding site of the receptor using Glide (Grid-Based Ligand Docking with Energetics) software from Schrodinger. The three-dimensional structures of the proteins were retrieved from the Protein Data Bank. Tramadol 2D structure was obtained from PubChem repository [16]

2.1. Protein preparation

Maestro was used as graphical user interface. Proteins were prepared using protein preparation wizard as described by Sastry *et al* [17], module in maestro 11.5 was used to prepare each protein complex. Protein structure's missing hydrogen atoms, missing loop, and missing side chains were fixed while the added hydrogen atoms were optimized at pH 7.0. Optimized structures were then minimized using the OPLS3 force field by converging heavy atoms to RMSD of 0.3Å.

The protein binding site was identified by utilizing the receptor grid generation tool in maestro 11.5. Receptor grid defines the region of interaction between the protein and the ligand. The co-crystal ligands of each protein were used to identify the binding cavity employing default parameters of van der Waals scaling factor 1.00 and charge cutoff of 0.25 around the binding site residues of the protein structures.

2.2. Ligand preparation Receptor glide generation and protein-ligand docking

After successful generation of the grids, the ligand library generated were imported to Maestro and prepared using the Schrodinger suite version 2018-1 [18]. Utilizing Ligprep v4.5 [19], Epik v4.3 [20] with OPLS3 force field, for protonation, stereo-isomerization, tautomers generation, and to attain biological conformer. Energy minimization was achieved for all tautomeric state at pH of 7±2. Ligands were kept flexible, while the proteins were rigid and docking started with

extra precision mode (XP mode). The docking calculation generated few poses for each ligand. The selection of the best pose was done on the interaction energy between the ligand and the protein as well as on the interactions the ligand shows with experimentally proved important residues.

3. Results

Table 1 The docking result of tramadol and selected proteins

Proteins	Catalase	CYP2D6	Defense repressor 1
Standards (kcal/mol)	-3.431	-1.865	-4.920
Tramadol (kcal/mol)	-2.348	-4.581	-4.985

Figure 1 Binding pose and amino acid residues around tramadol and crystalized ligand at the binding pocket of D. melanogaster catalase

The interaction profile reveals that the crystalized ligand (violet) binds to a pocket of the protein defined by Ala 64, Phe 68, and Lys 67 via H-bond. The compound, tramadol (green) on the other hand binds the pocket defined by Ala 64, Asp 91, Ser 95 and Gln 96.

It was observed to exhibit H-bond with the amino acid residues Ala 64. The crystallized ligand and tramadol binds to the protein with a docking score -3.431 kcal/mol and -2.348 kcal/mol respectively. Compared to tramadol, crystallized ligand has more binding affinity for the enzyme and it binds with more of the pocket residues. Ala 64 is the only common bond residue.

Figure 2 Binding pose and amino acid residues around tramadol and inhibitor at the binding pocket of D. melanogaster CYP2D6

From the interaction profiling of tramadol (green) compared to cyp2d6 inhibitor (violet), camptothecin, both ligands bind the protein pocket via H-bond. The hydroxyl group of tramadol and the inhibitor interact with the C=O groups of the residue Glu 128 and Lys 264 on the protein pocket respectively.

Camptothecin gave a docking score of -1.865 kcal/mol and tramadol -4.581 kcal/mol when docked to the protein pocket.

The crystalized ligand (violet), Dredd, interact with Asp 111 and Leu 329 of the active site. Tramadol (green) interact with the amino acid residues Glu 46, Asp 111, Leu 329 and Glu 372 of the protein's binding pocket. On comparison, both ligands form salt bridge via a Nitrogen atom (NH_3^+ for the crystalized ligand and NH^+- for tramadol) with the C=O of the carboxylic group of Asp 111 and another between tramadol and Glu 46. Meanwhile, visual screening reveal H-bond interaction between the ligands and C=O of Leu 329 for the crystalized ligand and Glu 372 for tramadol on the protein's active site respectively.

The ligands show little difference in their affinity for the protein. Tramadol binds with a docking score of -4.985 kcal/mol while the crystalized ligand binds with a docking score -4.920 kcal/mol respectively.

Figure 3 Binding pose and amino acid residues around tramadol and crystalized ligand at the active site of *D. melanogaster* Defense repressor 1

4. Discussion

The complexities of human genetic disorders often require model systems to provide a better understanding of the disease mechanism. *Drosophila melanogaster* provides an excellent system for human disease research because of its genetic tractability and the presence of many homologs of human disease genes in the fly genome [21]. *Drosophila* also has proven to be an invaluable tool to research the effects of chemotherapeutic drugs [22, 23, 24, 25, 26] and the effects of mutations in key DNA repair genes [26].

4.1. Tramadol detoxification potential of CYP2D6

Tramadol is metabolized to M1 by the CYP2d6 p450 isoenzyme. Camptothecin is a competitive inhibitor of this enzyme. Camptothecin, the crystalized ligand of cyp2d6 and its derivatives stabilize the normally transient covalent link between DNA and Top1, thereby interfering with the relaxation of supercoiling that occurs during events requiring DNA unwinding, such as replication or transcription [27, 28, 29]. Previous finding proposes that the accumulation of regressed forks or supercoiled DNA is responsible for the toxic effects of camptothecin [30].

The tramadol sensitivity observed in fruit flies would suggest that Cyp2d6-mediated breakdown or removal of tramadol prevents its toxicity from reaching lethal levels in flies at normal dose, this results from the fact that active site of Cyp2d6 can accommodate tramadol. Compared to camptothecin, the most likely explanation for such specificity would be the chemical properties of the drugs. Both ligands are lipophilic molecule, whereas the human P450 enzymes most critical

for drug detoxification, including Cyp3a4, tend to favor lipophilic substrates [31]. This study shows structural similarity between the molecules as both molecules bind to the Gln 165 and Lys 264 of the active site of the protein.

Another hypothesis posits that lipophilic molecule may accumulate in the fat body [32], which functions analogously to the liver in fruit flies and other insects [33, 34]. The observation that Cyp2d6 is highly expressed in the fat body [35], supports this model [36]. Coupled with the binding potential of tramadol to Cyp2d6 observed in this study (docking score -4.581), all of these hypotheses assume that Cyp2d6 is involved in tramadol detoxification, but other possible explanations may exist.

4.2. Tramadol interferes with the free radical scavenging prowess of Catalase

Catalase (CAT) is a heme-containing enzyme that catalyses the dismutation of H_2O_2 to molecular oxygen and water, thereby lessening the risk of hydroxyl radical formation [37]. Several studies have pointed to the relationship between the chronic opioid administration and increased production of reactive oxygen species (ROS) [38, 39, 40]. Oxidative stress is an imbalance between ROS production of and the ability of antioxidant defenses to neutralize them. Tramadol is evident in the inhibition of antioxidant enzymes, catalase and reduced glutathione (GSH) in liver and kidney of rats in research studies [41]. CAT is an important antioxidant enzyme which played a pivotal role in scavenging of oxidative free radicals [42]. The inhibition of these antioxidant enzymes observed in this study could be linked to exhaustion of these enzymes as a result of oxidative stress caused by tramadol administration.

In this study, the interaction profile of tramadol in the binding pocket is defined by Ala 64, Asp 91, Ser 95 and Gln 96. It was observed to exhibit H-bond with the amino acid residues Ala 64. Compared to tramadol, the ligand has Ala 64 as a common bond residue. Resveratrol is a natural polyphenol and a phytoalexin with anti-oxidative and anti-inflammatory properties whose neuroprotective activity has been linked with its ability to cross the blood-brain barrier to enhance the activity of neural catalase [43]. This study reveals that the crystalized ligand binds to a pocket of the protein defined by Ala 64, Phe 68, Lys 67 via H-bond. From the result obtained in this study, it can hypothesize that tramadol-Ala 64 H-bond interaction in the catalase binding pocket plays a pivotal role in oxidative stress induction of tramadol.

4.3. Defense repressor1 (Dnr 1) activity on tramadol

Studies demonstrate that Dnr1 forms a complex with Dredd in S2 cells, and that overexpression of Dnr1 significantly decreases Dredd proteins levels [44].

Dredd interact with Asp 111 and Leu 329 of the active site. Tramadol interacts with the amino acid residues Glu 46, Asp 111 and Glu 372 of the protein's binding pocket. On comparison, both ligands form salt bridge via a Nitrogen atom (NH_3^+ for the crystalized ligand and NH^+- for tramadol) with the C=O of the carboxylic group of Asp 111 and another between tramadol and Glu 46. Meanwhile, visual screening reveal H-bond interaction between the ligands and C=O of Leu 329 for the crystalized ligand and Glu 372 for tramadol on the protein's active site respectively. Salt bridges is believed to contribute to stability, resisting denaturation by high temperature [45, 46]. Tramadol binds with a docking score of -4.985 while the crystalized ligand binds with a docking score -4.920.

From the interaction profile obtained from this study, coupled with the structural similarity of tramadol and dredd, we can assume that tramadol will undergo similar interaction with defense receptor as dredd.

5. Conclusion

Tramadol antioxidant enzyme inhibition property combine with its binding metabolism rate which is significantly high indicating that tramadol is harmful at the cellular level and can enhance oxidative stress cause by reactive oxygen species and toxic effects which could lead to changes in neural and muscular tissues. Therefore, the toxic effects of tramadol should keep in mind, in spite of the important role it plays as powerful pain manager.

Compliance with ethical standards

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