



ORIGINAL ARTICLE

In Silico Analysis of Ethanol Binding Activity in Neuronal Nicotinic Acetylcholine Receptors

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Received: 20/04/2020, Accepted: 22/04/2020, Published: 30/04/2020

Abstract

Ethanol and nicotine are two common substances that are often linked to complications in alcoholic smokers. The high number of the co-consumptions in alcoholic smokers suggested a possible interaction between ethanol and nicotine in the central nervous system and a potential similar mechanism of action. Both ethanol and nicotine are shown to bind with neuronal nicotinic acetylcholine receptors (nAChRs), a ligand gated cation channel specifically targeted by the endogenous acetylcholine. Ethanol has a much less specific binding capability to modulate the receptors, however, emerging reports indicates that ethanol can interact with nAChRs both directly and indirectly. This study focuses on the analysis of ethanol binding sites with nAChRs using molecular docking techniques obtained from the Protein Data Bank. The obtained data showed a possible binding site for ethanol in nAChRs, however, upon validation, result is not substantial. Nevertheless, the obtained data should be useful for future reference for the basis of ethanol interactions with the human nAChRs proteins.

Keywords: Alcoholism, Acetylcholine, Binding site, Ethanol, Nicotine Receptors

Introduction

Ailments due to alcohol consumption lead to 3 million deaths per year and is shown to have causal relationships with a range of mental and behavioural disorders (World Health Organisation, 2018). In addition to alcohol consumption, more than 80% of alcoholics are smokers with around 60% of smokers consumed a high amount of alcohol (Batel et al., 2006; Friend & Pagano, 2005). The high number of alcoholic smokers suggested a possible interaction or similar mode of action between ethanol in alcohol and tobacco. Moreover, this hypothesis has been supported by an FDA approved drug used as a smoking cessation aid named varenicline, which targets neuronal nicotinic acetylcholine receptors (nAChRs), was reported to also reduce alcohol consumption (McKee et al., 2009).

Ethanol is a relatively small molecule that is also commonly used in the production of alcoholic beverages. Ethanol structure consists of two parts: a short hydrophobic region and a hydroxyl group that is rather hydrophilic whose chemical formula is C₂H₅O (Khrustalev, Khrustaleva, & Lelevich, 2017). The chemical structure makes ethanol amphiphilic in nature

and allows it to easily participate in different types of functions with proteins. Another interesting thing to note is that due to its small and simple structure, ethanol is able to pass through numerous body tissues, binding to both cellular and intracellular surfaces (Marin & Morais-Silva, 2017). This includes tissues such as the blood-brain barrier, making ethanol one of the many substances which can alter neuronal brain activities by a variety of measures.

Neuronal nAChR are a ligand-gated cation channels that are specifically activated by either the neurotransmitter acetylcholine (ACh) or the tertiary alkaloid nicotine (Hendrickson, Guildford, & Tapper, 2013; Liu et al., 2013). Several studies have reported that ethanol can interact with nAChR, acting as a co-agonist and increases the affinity of the receptor towards nicotine and acetylcholine, act as a stabilizer for open channel state while also increasing the rate of opening (Bradley, Peper, & Sterz, 1980; Linder, Pennefather, & Quastel, 1984; Marszalec, Aistrup, & Narahashi, 1999).

Molecular docking has been used since the early 1980 and are a well-known model to explain molecule specificity which can give insights towards interactions between two-molecules at the atomic scale (Kuntz, Blaney, Oatley, Langridge, & Ferrin, 1982). Molecular docking processes generally consists of two phases: the prediction of the ligand conformation and the assessment of the ligand binding affinity (Meng, Zhang, Mezei, & Cui, 2011). In this regard, as a proteomics-based method, molecular docking is the most reliable instrument to assess the biochemical repertoire of the neurotransmitter physiological expression (Leonard, 2014; Ravna, Sylte, & Dahl, 2009; Zaheer-ul-Haq, Halim, Uddin, & Madura, 2010).

To further analyse the interaction between ethanol and neuronal nAChR and how ethanol increase the affinity towards nicotine and acetylcholine, this study employs a molecular docking approach to model the interaction between ethanol and neuronal nAChR. To validate the result from the docking protocol, two software are used and each output from these software are compared and analysed.

Materials and Methods

The pipeline for this research was inspired from the existing structural bioinformatics methods with some indicators and tools modifications as stated in this section (Parikesit, 2018; Parikesit & Nurdiansyah, 2020; Valeska et al., 2019).

For the current study, structure of nAChR receptor protein along with ethanol molecule was taken from the Protein Data Bank (PDB) managed by Research Collaboratory for Structural Bioinformatics (RCSB) (<https://www.rcsb.org/>). The nAChR receptor protein was downloaded in the form of PDB file format while the ethanol molecule was downloaded with SDF format. The specific format is important as an input file and should the format be different from the previously mentioned ones, the files were converted to the mentioned format using OPENBABEL software (<http://openbabel.org/wiki/Category:Installation>) (O'Boyle et al., 2011).

Both the nAChR protein and ethanol has been subjected to RosettaLigand webserver docking protocol (<https://rosie.graylab.jhu.edu/>) (DeLuca, Khar, & Meiler, 2015). RosettaLigand has been a reliable tool to predict binding poses in protein-small molecules complexes, however, the long running time could be a problem for High Throughput Screening (HTS) analysis. All the available parameters were left as default.

For further validation, the MTiAutoDock software for protein-small molecule docking from the MTiOpenScreen web server (<http://bioserv.rpbs.univ-paris-diderot.fr/services/MTiOpenScreen/#references>) was used with the same file as input (Labbé et al., 2015). All the parameters were left as default.

The output files from the docking software are still in PDB file format. To visualize the PDB file, the software Yet Another Scientific Artificial Reality Application (YASARA) was used (Krieger & Vriend, 2014). The illustration of the complete pipeline could be observed in the Figure 1.

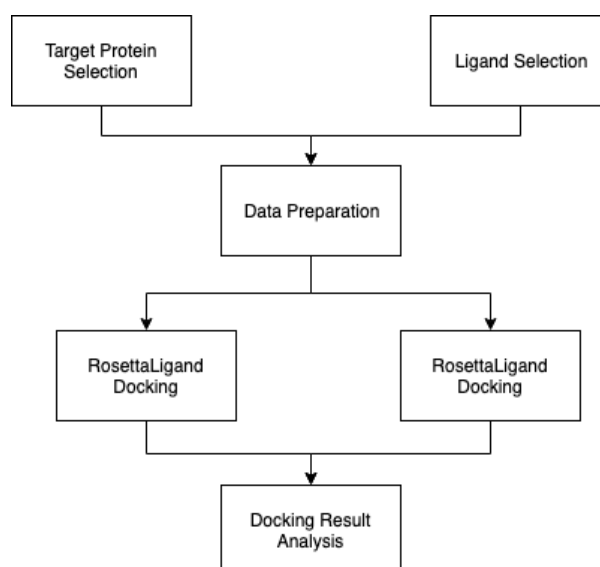


Figure 1: Molecular docking protocol

Results and Discussion

Both RosettaLigand and MTiAutoDock showed a staggering difference in the runtime. This difference is possibly due to the Monte Carlo Minimization (MCM) refinement for the binding position and scoring.

Table 1: Total runtime of RosettaLigand and MTiAutoDock

Software	Time
RosettaLigand	7 hours 0 minutes
MTiAutoDock	0 hours 9 minutes

In the Table 1, the output from RosettaLigand showed a graphical output for Interface to Total score calculation. The range of the interface score spans from -1.0 to 1.0. A high interface score indicates that the specific ligand might be enriched in that region and a low interface score indicates otherwise (Hwang, Petrey, & Honig, 2016).

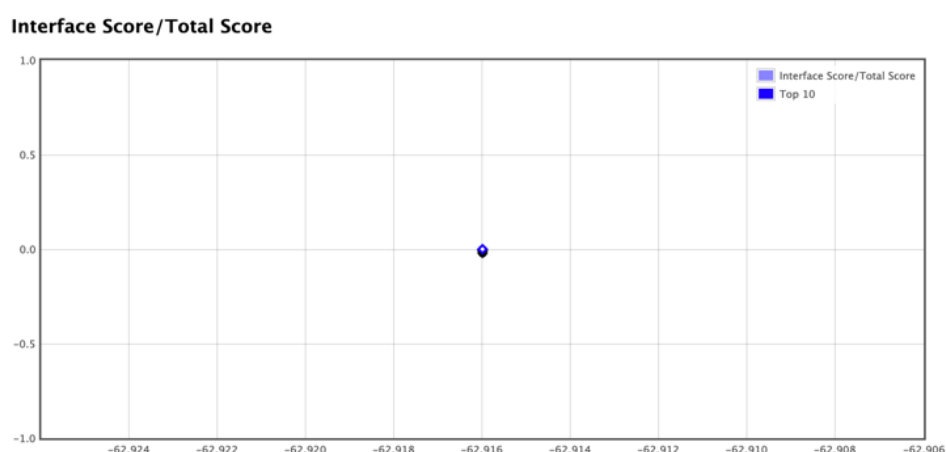


Figure 2: Graph depicting interface score to total score. Interface score is shown by the y-axis while the total score is shown by the x-axis.

RosettaLigand returned 10 possible ligand binding site, however, Figure 2 showed that there was no interaction at all between the protein and the ligand. This is indicated by an

interface score of 0. Surprisingly, this score are persistent across all 10 of the predicted binding sites.

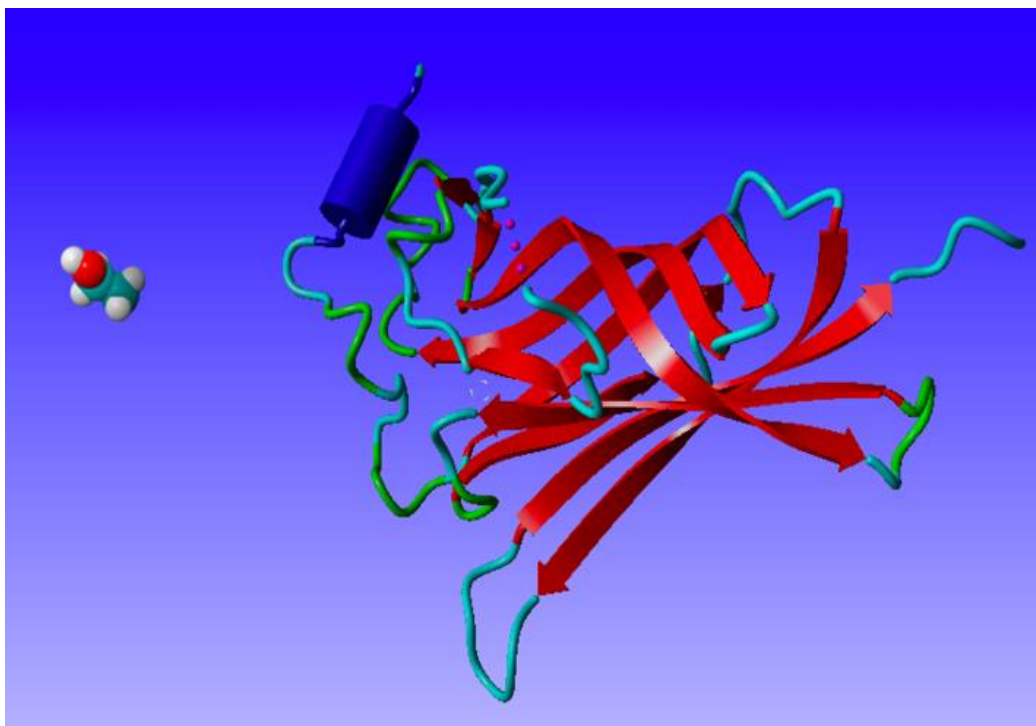


Figure 3: PDB file output from RosettaLigand visualized using YASARA

Upon subjecting the result from RosettaLigand to YASARA, it is easier to see why the interface score returned a null value. Based on Figure 3, the ethanol did not bind to the protein at all. Furthermore, the result from RosettaLigand stated that there are no ligand conformers to be found from the study. This could be due to some possible runtime error considering that the protein used has been check for ligand or even to the algorithm the software used to imply rigid docking. Compared to flexible docking, rigid docking can reduce pose prediction drastically (Lexa & Carlson, 2012).

Compared to RosettaLigand, however, MTiAutoDock seemed to return a higher number of ligand conformation. MTiAutoDock returned 10 possible ligand conformation with varying energy score requirements along with the number of possible torsion angle for each rotatable bond (Alsafi & Al-Shaikhli, 2012). In total, 100 ligand was generated with varying energy scores.

Table 2: List of 5 best ligand conformation along with the respective energy score

Ligand	Energy Score
ligands_in3_1.pdbqt	-8.610000
ligands_in3_2.pdbqt	-8.610000
ligands_in3_3.pdbqt	-8.470000
ligands_in3_4.pdbqt	-8.360000
ligands_in3_5.pdbqt	-8.280000

Referring to Table 2, the best ligand conformation seemed to be the third predicted structure with an energy score of -8.610000. Compared to RosettaLigand, the MTiAutoDock was able to bind the ethanol to the protein structure as seen on Figure 4.

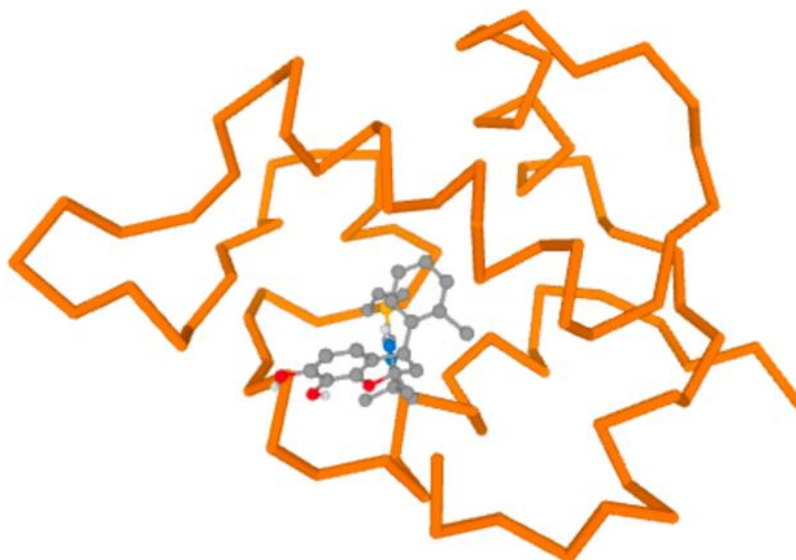


Figure 4: Visualization of ligand binding position by MTiAutoDock.

The molecular simulation analysis of the neuronal nAChRs has been conducted in extensive basis and involved various research groups. The nAChRs are acted as target for the snake toxins in order to induce paralysis to human, as proven by the molecular simulation study (Gunasekaran, Sridhar, Suryanarayanan, Manimaran, & Singh, 2017). Thus, how the peptide-based snake toxin binding conformation to nAChRs have been elucidated with online tools as well (Leffler et al., 2017).

The molecular simulation study of nAChRs also established not only with snake venom, but also with venom of the marine cone snail (Wen & Hung, 2019). The ongoing research for establishing correlation between nAChRs and venoms are crucial as the means for antidote design, and eventually comprehend the mechanism of the neurological disorders (Kalkman & Feuerbach, 2016). The utilization of natural products as agonist for nAChRs receptor has been devised as the Alzheimer treatment candidate (Remya, Dileep, Variayr, & Sadasivan, 2016). In this end, the study nAChRs also devised to design biopesticide as well (Tian et al., 2019).

The aforementioned research has shown that the potential information gathered from the molecular simulation studies of the nAChRs receptor have so many potential application. Our finding that there is interactions between ethanol and nAChRs is consistent with the trend that shown previously, if alcoholic addiction is definitely related to neurological disorder. Interference of alcohol, or any other substances like animal venom, with the nAChRs receptor could disrupt the physiological order of the central nervous system (Kulbatskii, Bychkov, & Lyukmanova, 2018). In this regard, the software benchmarking effort in this research has shown that the nAChRs receptor interaction with alcohol as binding ligand definitely occurred as possible precursor of the alcoholism.

Conclusion

In this study, the molecular interactions of ethanol with neuronal nAChRs was explored using molecular docking software and validation analysis in order to confirm the interactions. Through the study, there seemed to a ligand interaction between the molecule and protein, however, there are some insignificant discrepancy on the interaction details between the two software in the analysis pipeline. Further analysis should be conducted to validate the study using a molecular dynamics simulation.

Acknowledgments

This research is fully supported by the Institute of Research and Community Services of the Indonesia International Institute for Life Sciences (LPPM I3L). The authors fully acknowledged LPPM I3L for the approved fund that makes this important research viable and effective.

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How to cite this paper: Angganararas Lungidningtyas & Arli Aditya Parikesit (2020). In Silico Analysis of Ethanol Binding Activity in Neuronal Nicotinic Acetylcholine Receptors. *Malaysian Journal of Applied Sciences*, 5(1), 54-61.