Salbutamol May Inhibit Monoamine Oxidase B (MAO-B) Enzyme: A Computational Study

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Abstract:

Background: Parkinson's disease is a progressive degenerative illness that mainly affect dopaminergic neurons in elderly individuals. Treatment of Parkinson's disease is focused toward symptoms alleviation but with no neuroprotection capacity. As such, the search for new therapeutic agents is still ongoing to introduce new drugs against Parkinson's disease with neuroprotection ability. In this trend, some epidemiological studies had suggested a potential negative association between exposure to β_2 -adrenoreceptor agonists and risk of Parkinson's disease. Also, small clinical trials had concluded that the use of β_2 -adrenoreceptor agonist, salbutamol, may be beneficial in Parkinson's disease patients treated with levodopa. In this computational study, we evaluated the ability of salbutamol to selectively inhibit MAO-B enzyme as an additional beneficial effect in Parkinson's disease patients under levodopa therapy. **Methods:** Both molecular docking and dynamics simulation were employed to assess the binding affinity of salbutamol against MAO-A and MAO-B crystals. **Results:** This study predicted that docking orientation, ligand movement root-mean-square deviation and MM-PBSA binding energy throughout simulation interval are more favorable toward salbutamol capacity to bind to MAO-B as compared to MAO-A. **Conclusion:** Salbutamol may be able to selectively inhibit MAO-B enzyme. However, these findings must be further validated both in vitro and in vivo.

Graphical abstract

Keywords: Salbutamol; MAO-B; Parkinson's disease; docking; molecular dynamics simulation.

1. Introduction:

Parkinson's disease (PD) is a neurodegenerative illness that primarily influence elderly people between the age of 55 and 65 years [1]. PD is mainly characterized by the progressive damage and loss of dopaminergic nerve cells in the *substantia nigra pars compacta* area of midbrain. When 60% -70% of these dopaminergic neurons are lost, four motor related symptoms will emerged and these are: resting tremor, bradykinesia, muscles rigidity and postural imbalance [2,3]. As PD develop over time, non-motor manifestations like depression may also arise [4]. PD is also characterized by the buildup of Lewy bodies within dopaminergic neurons, these Lewy bodies are considered proteinaceous inclusions made up mainly of α -synuclein that is misfolded [5]. Although no precise cause of PD has been specified, several factors are reported to have positive association with development of PD like pesticides and genetic factors. On the other hand, it is believed that both smoking and consumption of coffee may lower the risk of PD [6]. The pathology of PD seems to be complex and involves numerous etiological pathways. As such, designing a drug molecule for PD treatment that target a single pathway appear to be ineffectual therapeutic approach. Currently, there is an urgent need to introduce a drug candidate that can target multiple pathways within PD pathogenesis network [7,8]. The available treatment options for PD, like levodopa and monoamine oxidase B (MAO-B) inhibitors, focus mainly on the mitigation of symptoms but with no ability to modify disease course [9,10].

As PD disease course advances over time, both Lewy bodies and α -synuclein are increased in the brain of affected patients [11]. It is believed that targeting α -synuclein metabolism may reduce the accumulation of this protein in affected neurons and thereby halt the progression of PD disease course. In this direction, several cell line studies showed that β_2 -adrenoceptor agonists like salbutamol can reduce the expression of α -synuclein by modulating the acetylation of lysine 27 of histone H3. Treatment of human neuroblastoma cell model with propranolol, a non-selective β -adrenoceptor antagonist, was found to increase the concentration of α -synuclein in these cells [12]. Additionally, two epidemiological studies have suggested a positive association between PD risk and administration of β -adrenoceptor antagonists. These two studies also proposed that exposure to β_2 -adrenoceptor agonists may decrease the risk of PD [12,13]. However, this epidemiological link between PD risk and β -adrenoceptor agonists

or antagonists was abolished when association was adjusted to confounding factors like essential tremor and smoking [14]. Moreover, three small clinical trials had observed a beneficial effect when salbutamol was added to levodopa in PD patients [15–17].

The beneficial effect of salbutamol in PD patients may not be only due to α -synuclein lowering effect. Animals studies suggested that administration β -adrenoceptor agonists can enhance brain extraction of levodopa and leucine, possibly through peripheral mechanisms that decrease the concentration of other competing large neutral amino acids (LNAAs) for transport. Thus, the adjunct use of β -adrenoceptor agonists in PD patients may enhance delivery of levodopa to brain, reduce daily requirement of levodopa and improve clinical symptoms [18]. In this computational study, we proposed that the adjunct use of salbutamol may have an additional useful effect in PD patients treated with levodopa. We hypothesized that salbutamol may be able to selectively inhibit monoamine oxidase B (MAO-B) and thereby decreases the degradation of dopamine in central nervous system. It is well known that selective inhibition of MAO-B enzyme can decrease daily requirement of levodopa in PD patients by elevating the level of both endogenous and exogenous dopamine [19]. Also, inhibition of MAO-B can reduce oxidative stress by lowering the production of hydrogen peroxide, a byproduct of oxidative deamination reaction [20].

2. Results and Discussion:

2.1. Molecular docking:

According to docking results, the energy of binding for salbutamol against MAO-A and MAO-B monomers were 7.1 and 7.2 Kcal/ mol respectively. As reported here, the energy of binding for salbutamol with active site of MAO-A and MAO-B isozymes are almost equal. However, two-dimensional images for docking results in Figure 1 showed that salbutamol can exhibit a more preferred orientation within MAO-B active site as compared to MAO-A. As seen in Figure 1 (A), salbutamol is involved in unfavorable donor-donor interaction with Lysine 286 residue of chain A for MAO-A crystal. The formation of unfavorable interactions in docking results may indicate the presence of repulsive forces between ligand and target. Therefore, the generation of these unfavorable interactions can adversely influence the stability of ligand-target complex in docking studies [21]. Despite the fact that salbutamol docking energy against MAO-A and MAO-B are almost identical but the binding orientation of salbutamol within

MAO-B active site may be more favorable due to absence of unfavorable interactions in two-dimensional docking image, as seen in Figure 1 (B).

Figure 1: Visualization of docking results for salbutamol against (A) MAO-A and (B) MAO-B in three-dimensional and two-dimensional views.

2.2.Molecular dynamics (MD) simulation:

Docking results were then further validated by molecular dynamics (MD) simulation for 50 nanoseconds. By superposing the complex between salbutamol and enzyme on its reference structure, the proximity of salbutamol to enzyme active site was reported in Figure 2 as a function of simulation time. As can be noted in Figure 2, salbutamol was able to maintain a closer proximity to MAO-B active site as compared to MAO-A. Throughout simulation period, the mean ligand movement RMSD for salbutamol against MAO-A and MAO-B was 9.55 and 2.94 Angstrom respectively. As such, the closer proximity of salbutamol to MAO-B active site throughout simulation time may indicate a stronger interaction as compared to MAO-A.

Figure 2: Ligand movement RMSD for salbutamol against the active site for MAO-A and MAO-B throughout simulation interval.

Finally, calculations of MM-PBSA binding energy indicate that salbutamol may have a better interaction with MAO-B active site than MAO-A. According to YASARA guide, the more positive binding energy refers to better interaction between ligand and target. And in this regard, the reported average MM-PBSA binding energy for salbutamol against MAO-A and MAO-B was -12.09 and -6.03 Kcal/ mol respectively. So, MM-PBSA binding energy for salbutamol against MAO-B active site is more positive than that against MAO-A.

3. Materials and methods:

In this study, we used molecular docking to assess the binding affinity and selectivity of salbutamol against monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B)

crystals. Then, the docking results were further validated by employing molecular dynamics (MD) simulation.

3.1. Molecular docking:

To predict both binding orientation and affinity of salbutamol against MAO-A and MAO-B crystals, we have used 1-CLICK DOCKING tool available in Mcule.com platform for drug discovery [22]. This online platform has an embedded version of AutoDock Vina to carry out docking operations [23]. At first, the two-dimensional structure of salbutamol was drawn in the board of 1-CLICK DOCKING tool. Then, docking was performed against only chain A of MAO-A (PDB: 2BXR) and MAO-B (PDB: 2BK3) crystals [24,25]. The platform of Mcule.com uses AutoDock tools to prepare both ligand and target for docking process [26]. For MAO-A crystal, the employed docking coordinates were (X: 21.0, Y: 2.0, Z: 6.0). While for MAO-B crystal, we applied the following coordinates (X: 56.0, Y: 153.0, Z: 23.0). The binding site area used for both MAO-A and MAO-B was (22*22*22) Angstrom. Then, the ligand-enzyme complex with least energy of binding pose were then evaluated and visualized by using both PyMOL v2.4.1 and Discovery Studio Visualizer v21.1.0.20298 [27,28].

3.2. Molecular dynamics (MD) simulation:

The docking results were then further validated through molecular dynamics (MD) simulation for 50 nanoseconds. In this MD study, the docking complex between salbutamol and each enzyme with the minimum energy of binding pose was submitted for simulation by YASARA Dynamics v20.12.24 [29]. For this simulation study, the followed steps are similar to what we have used in our previous research articles [30–33]. As a summary, the simulation includes an optimization of hydrogen bonds network with a prediction of pKa value to fine-tune amino acid residues protonation at pH 7.4 [34]. Also, sodium chloride was added in a concentration of 0.9% and an excess of sodium or chloride were applied to neutralize the complex of salbutamol and enzyme. In addition, steepest descent and simulated annealing minimizations were used for simulation to remove any possibility of clashes. The following force fields were applied during MD simulation: AMBER14 for solute, TIP3P for water, GAFF2 and AM1BCC for ligand [35–37]. For this MD study, van der Waals forces cutoff value was 8 Angstrom and default parameters were used for AMBER [38]. On the other hand, no cutoff value was applied for electrostatic forces as Particle Mesh Ewald algorithm was used for this simulation [39]. At a pressure of 1 atm and a temperature of 298K, motions equations were applied as a multiple timestep of 1.25 and 2.5 femtoseconds for bonded and non-bonded interactions respectively

[40]. After evaluation of the root-mean-square deviation (RMSD) for solute as a function of simulation interval, the first 50 nanoseconds were considered as equilibrium time and excluded from any additional analysis. And lastly, GraphPad Prism version 8.0.2 was employed to plot and evaluate ligand movement RMSD of salbutamol throughout simulation interval.

Then, Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) binding energy was calculated for each docking complex of salbutamol and enzyme by employing AMBER14 force field. A built-in macro within YASARA Dynamics software has the capacity to carry out calculations of MM-PBSA binding energy both easily and automatically. As mentioned in YASARA guideline, the more positive binding energy refers to better interactions between ligand and target [41,42]. The following equation was used by YASARA Dynamics to calculate binding energy:

$$\text{Binding Energy} = \text{EpotRecept} + \text{EsolvRecept} + \text{EpotLigand} + \text{EsolvLigand} - \text{EpotComplex} - \text{EsolvComplex}$$

4. Conclusions:

In this computation study, we report that salbutamol may be able to bind and inhibit MAO-B enzyme. Docking study showed that salbutamol can exhibit a more preferred orientation within active site of MAO-B as compared to MAO-A. This may be due to absence of any unfavorable interactions between salbutamol and MAO-B active site, unlike MAO-A in docking images. Moreover, molecular dynamics simulation showed that salbutamol can have a closer proximity to the active site of MAO-B monomer when compared to MAO-A throughout simulation interval. Also, the average MM-PBSA binding energy for salbutamol against MAO-B was stronger than that calculated against MAO-A throughout simulation time. All these results refer to the potential selective ability of salbutamol to bind and inhibit MAO-B enzyme. However, in vitro and in vivo evaluation of salbutamol effect against MAO-B enzyme is required to validate these computational findings.

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personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the manuscript.

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References:

1. Rizek P, Kumar N, Jog MS. An update on the diagnosis and treatment of Parkinson disease. *CMAJ*. 2016;188(16):1157-1165. doi:10.1503/CMAJ.151179/-/DC1
2. Arkinson ' P, Isease SD, Nthony A, Ang EL, Ozano ML. Parkinson's disease. First of two parts. *N Engl J Med*. 1998;339(15):1044-1053. doi:10.1056/NEJM199810083391506
3. Sian-Hülsmann J, Mandel S, Youdim MBH, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. *J Neurochem*. 2011;118(6):939-957. doi:10.1111/J.1471-4159.2010.07132.X
4. Barbosa ER. Nonmotor manifestations of Parkinson's disease. *Ann Neurol*. 2008;64 Suppl 2(SUPPL. 1). doi:10.1002/ANA.21472
5. Mehra S, Sahay S, Maji SK. α -Synuclein misfolding and aggregation: Implications in Parkinson's disease pathogenesis. *Biochim Biophys acta Proteins proteomics*. 2019;1867(10):890-908. doi:10.1016/J.BBAPAP.2019.03.001
6. Georgiou A, Demetriou CA, Christou YP, et al. Genetic and Environmental Factors Contributing to Parkinson's Disease: A Case-Control Study in the Cypriot Population. *Front Neurol*. 2019;10(OCT). doi:10.3389/FNEUR.2019.01047
7. Van Der Schyf CJ, Geldenhuys WJ, Youdim MBH. Multifunctional drugs with different CNS targets for neuropsychiatric disorders. *J Neurochem*. 2006;99(4):1033-1048. doi:10.1111/J.1471-4159.2006.04141.X
8. Odhar H. Identification of novel scaffolds for Monoamine oxidase B inhibitors.

Published online 2014.

9. Meissner WG, Frasier M, Gasser T, et al. Priorities in Parkinson's disease research. *Nat Rev Drug Discov*. 2011;10(5):377-393. doi:10.1038/NRD3430
10. Odhar HA, Kareem SM, Alhaideri MRA, Hasan MA, Geldenhuys WJ. The Design and Evaluation of a Novel Monoamine Oxidase B Inhibitor Through in Silico Approach. 2019;10(2):349-354. doi:10.25258/ijpqa.10.2.23
11. Braak H, Bohl JR, Müller CM, Rüb U, de Vos RAI, Del Tredici K. Stanley Fahn Lecture 2005: The staging procedure for the inclusion body pathology associated with sporadic Parkinson's disease reconsidered. *Mov Disord*. 2006;21(12):2042-2051. doi:10.1002/MDS.21065
12. Mittal S, Bjørnevik K, Im DS, et al. β 2-Adrenoreceptor is a regulator of the α -synuclein gene driving risk of Parkinson's disease. *Science*. 2017;357(6354):891-898. doi:10.1126/SCIENCE.AAF3934
13. Gronich N, Abernethy DR, Auriel E, Lavi I, Rennert G, Saliba W. β 2-adrenoceptor agonists and antagonists and risk of Parkinson's disease. *Mov Disord*. 2018;33(9):1465-1471. doi:10.1002/MDS.108
14. Searles Nielsen S, Gross A, Camacho-Soto A, Willis AW, Racette BA. β 2-adrenoreceptor medications and risk of Parkinson disease. *Ann Neurol*. 2018;84(5):683-693. doi:10.1002/ANA.25341
15. Hishida R, Kurahashi K, Narita S, Baba T, Matsunaga M. "Wearing-off" and beta 2-adrenoceptor agonist in Parkinson's disease. *Lancet (London, England)*. 1992;339(8797):870. doi:10.1016/0140-6736(92)90313-R
16. Alexander GM, Schwartzman RJ, Nukes TA, Grothusen JR, Hooker MD. Beta 2-adrenergic agonist as adjunct therapy to levodopa in Parkinson's disease. *Neurology*. 1994;44(8):1511-1513. doi:10.1212/WNL.44.8.1511
17. Uc EY, Lambert CP, Harik SI, Rodnitzky RL, Evans WJ. Albuterol improves response to levodopa and increases skeletal muscle mass in patients with fluctuating Parkinson disease. *Clin Neuropharmacol*. 2003;26(4):207-212. doi:10.1097/00002826-200307000-00011
18. Uc EY, Dienel GA, Cruz NF, Harik SI. β -Adrenergics enhance brain extraction of

- levodopa. *Mov Disord.* 2002;17(1):54-59. doi:10.1002/MDS.10002
19. Finberg JPM, Wang J, Bankiewicz K, Harvey-White J, Kopin IJ, Goldstein DS. Increased striatal dopamine production from L-DOPA following selective inhibition of monoamine oxidase B by R(+)-N-propargyl-1-aminoindan (rasagiline) in the monkey. *J Neural Transm Suppl.* 1998;52(52):279-285. doi:10.1007/978-3-7091-6499-0_28
 20. Youdim MBH, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br J Pharmacol.* 2006;147 Suppl 1(Suppl 1). doi:10.1038/SJ.BJP.0706464
 21. Dhorajiwala TM, Halder ST, Samant L. Comparative In Silico Molecular Docking Analysis of L-Threonine-3-Dehydrogenase, a Protein Target Against African Trypanosomiasis Using Selected Phytochemicals. *J Appl Biotechnol Reports.* 2019;6(3):101-108. doi:10.29252/JABR.06.03.04
 22. 1-Click Docking. Accessed December 26, 2021. <https://mcule.com/apps/1-click-docking/>
 23. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2009;31(2):NA-NA. doi:10.1002/jcc.21334
 24. De Colibus L, Li M, Binda C, Lustig A, Edmondson DE, Mattevi A. Three-dimensional structure of human monoamine oxidase A (MAO A): Relation to the structures of rat MAO A and human MAO B. *Proc Natl Acad Sci U S A.* 2005;102(36):12684-12689. doi:10.1073/PNAS.0505975102
 25. Hubálek F, Binda C, Khalil A, et al. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. *J Biol Chem.* 2005;280(16):15761-15766. doi:10.1074/JBC.M500949200
 26. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem.* 2009;30(16):2785-2791. doi:10.1002/jcc.21256
 27. PyMOL | pymol.org. Accessed December 26, 2021. <https://pymol.org/2/>
 28. Free Download: BIOVIA Discovery Studio Visualizer - Dassault Systèmes. Accessed

- December 26, 2021. <https://discover.3ds.com/discovery-studio-visualizer-download>
29. Krieger E, Vriend G. YASARA View - molecular graphics for all devices - from smartphones to workstations. *Bioinformatics*. 2014;30(20):2981-2982. doi:10.1093/bioinformatics/btu426
 30. Odhar HA, Ahjel SW, Albeer AAMA, Hashim AF, Rayshan AM, Humadi SS. Molecular docking and dynamics simulation of FDA approved drugs with the main protease from 2019 novel coronavirus. *Bioinformation*. 2020;16(3):236-244. doi:10.6026/97320630016236
 31. Odhar HA, Ahjel SW, Humadi SS. Towards the design of multiepitope-based peptide vaccine candidate against SARS-CoV-2. *BioRxiv*. Published online 2020:1-17. doi:10.1101/2020.07.07.186122
 32. Odhar HA, Ahjel SW, Odhar ZA. Computational Screening of FDA Approved Drugs from ZINC Database for Potential Inhibitors of Zika Virus NS2B/NS3 Protease: A Molecular Docking and Dynamics Simulation Study. *J Pharm Res Int*. 2021;33(39B):308-319. doi:10.9734/JPRI/2021/V33I39B32208
 33. Odhar HA, Ahjel SW, Albeer AAMA, Hashim AF, Humadi SS. Virtual Screening of FDA Approved Drugs Library to Identify a Potential Inhibitor against NS2B-NS3 Protease of Yellow Fever Virus. *J Pharm Res Int*. 2021;33(51B):177-186. doi:10.9734/JPRI/2021/V33I51B33528
 34. Krieger E, Dunbrack RL, Hooft RWW, Krieger B. Assignment of protonation states in proteins and ligands: Combining pK_a prediction with hydrogen bonding network optimization. *Methods Mol Biol*. 2012;819:405-421. doi:10.1007/978-1-61779-465-0_25
 35. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general Amber force field. *J Comput Chem*. 2004;25(9):1157-1174. doi:10.1002/jcc.20035
 36. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C. ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. *J Chem Theory Comput*. 2015;11(8):3696-3713. doi:10.1021/acs.jctc.5b00255
 37. Jakalian A, Jack DB, Bayly CI. Fast, efficient generation of high-quality atomic

- charges. AM1-BCC model: II. Parameterization and validation. *J Comput Chem.* 2002;23(16):1623-1641. doi:10.1002/jcc.10128
38. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple amber force fields and development of improved protein backbone parameters. *Proteins Struct Funct Genet.* 2006;65(3):712-725. doi:10.1002/prot.21123
 39. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald method. *J Chem Phys.* 1995;103(19):8577-8593. doi:10.1063/1.470117
 40. Krieger E, Vriend G. New ways to boost molecular dynamics simulations. *J Comput Chem.* 2015;36(13):996-1007. doi:10.1002/jcc.23899
 41. JM S, RH H, JA M. Revisiting free energy calculations: a theoretical connection to MM/PBSA and direct calculation of the association free energy. *Biophys J.* 2004;86(1 Pt 1):67-74. doi:10.1016/S0006-3495(04)74084-9
 42. BindEnergyObj - Calculate binding energies. Accessed July 16, 2021. <http://shaker.umh.es/yas/20.10.4/BindEnergyObj.html>