WHY IS CLOZAPINE MORE EFFECTIVE THAN OTHER ATYPICAL ANTIPSYCHOTICS? DENSITY FUNCTIONAL THEORY AND MOLECULAR DOCKING APPROACH

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Abstract

In order to find some clues as to why clozapine is a more effective drug than the sertrindole and quetiapine, we investigated the molecular structures of these three molecules and calculated their structural properties by Density Functional Theory (DFT) technique. In this article, we suggested that clozapine has different binding possibilities depending on the dopamine level in the medium. If so, it can be understood why clozapine is more effective in resolving clinical problems caused by different dopamine levels in the frontal and subcortical regions. To test this hypothesis, we measured the binding of three drugs to D₁ and D₂ dopamine receptors by molecular docking. We found that clozapine had a greater potential for binding with D₁ and D₂ receptors. We suggested that this feature might give clozapine a higher therapeutic effect.

Keywords: Dopamine, molecular docking, clozapine, schizophrenia.

1. Introduction

Schizophrenia is a severe mental disorder involving brain dopamine signaling. This disorder is characterized by profound derangements in thinking, speech, perception, affect and self-image of the patient. It is often characterized by psychotic experiences, such as auditory hallucinations or delusions. The cognitive impairment associated with the executive functions of the prefrontal cortex is the core component of this disease. Dopamine (DA) has a strong modulating effect on working memory (WM). This effect is known to be predominantly via the activation of D₁ and D₂ receptors. According to the dopamine hypothesis of schizophrenia, extra- or extra-high extracellular DA concentrations in the prefrontal cortex (PFC) can severely impair the functioning of WM (de Keyser et al., 1990; Qin et al., 2009). Working memory problems also cause negative, positive and cognitive symptoms of schizophrenia.

Therefore, it is necessary to intervene in both D₁ and D₂ receptors in the treatment of schizophrenia. The dual nature of schizophrenia requires the blocking of D₂ receptors for the treatment of positive symptoms, as well as the release of D₁ for the treatment of negative and cognitive symptoms.

Ideally, antipsychotic drugs must exhibit antagonism at D₂ receptors and agonism at D₁ receptors for efficacious treatment (Arnt et al., 1992). Clozapine is successful in the treatment of schizophrenia by exerting both of these effects. According to a meta-analysis on antipsychotic medications, clozapine is the most effective antipsychotic drug (Siskind et al., 2016). Clozapine’s unique clinical effect may in part involve the release of dopamine from the prefrontal cortex (Devoto et al., 2003). Because of modulation of the subcortical mesolimbic dopamine system by the prefrontal cortex, the release of dopamine induced by clozapine in the prefrontal cortex may result in a reduction in the amount of mesolimbic dopamine (Khokhar et al., 2018).

DFT calculations are a unique method for obtaining information about the molecular docking with quantum mechanical methods. However, there have been previous studies on the molecular docking of dopamine receptors such as D₂ and D₁ with drugs by DFT (Thomas et al., 2016; Aranda et al., 2008). Salmas et al. have investigated the molecular docking of drugs such as risperidone, clozapine, aripiprazole, olanzapine, ziprasidone, and quetiapine with active sites of the D₂ receptor in comparison with quantum mechanical approaches (Salmas et al., 2018).

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We have considered that it would be an important guide in the production of new drugs for the treatment of schizophrenia if we could determine the mechanism of the different efficacy of clozapine from other antipsychotic drugs. We thought that structural and electronic configurations of clozapine may have a special feature of binding to D₁ and D₂ receptors. In order to find some clues as to why clozapine is a more effective drug, docking of the dopamine, clozapine, quetiapine and sertindole in D₁ and D₂ receptors have been calculated in this study.

Our hypothesis was that the high therapeutic efficacy of the clozapine due to the dual-action mode of binding to D₁ and D₂ receptors.

2. Methodology
In order to investigate the molecular properties of clozapine, which gives high therapeutic effect, sertindole and quetiapine, which have some similarities to clozapine, were selected. Sertindole was chosen because of its high affinity for D₂ receptors and its effect on cognitive and negative symptoms. The reason for the selection of quetiapine was that its molecular structure was similar to clozapine but the therapeutic effect in schizophrenia was not as high. All molecule structure file downloaded from National Center for Biotechnology Information web site (URL-1, 2019) as initial molecule structures for DFT calculations.

In order to find the best structure and electronic configuration, a DFT study has been carried out. For all these studies we have used one of the most used functional B3LYP, which is introduced by Becke (Becke, 1993) and improved by Lee et al. (Lee et al., 1988) and Yang et al. (Yang et al., 1986) Full geometry optimizations and energy level of these four molecules have been performed at the B3LYP/6-311++G** level of carrying out with Gaussian 09W package program, in the gas phase. Using this basis set, the calculated energies of HOMO and LUMO together with some low-lying bound states are given in Table 1. The HOMO and LUMO energies and the corresponding optimized geometry of these four molecules are demonstrated.

Table 1. The HOMO, LUMO and some low-lying excited state energies of considered molecules.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>HOMO (eV)</th>
<th>LUMO (eV)</th>
<th>Difference (eV)</th>
<th>L1 (eV)</th>
<th>L2 (eV)</th>
<th>L3 (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quetiapine</td>
<td>-5.679</td>
<td>-1.342</td>
<td>-4.337</td>
<td>-0.827</td>
<td>-0.337</td>
<td>-0.304</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-4.908</td>
<td>-2.481</td>
<td>-2.426</td>
<td>-0.719</td>
<td>-0.386</td>
<td>-0.263</td>
</tr>
<tr>
<td>Sertindole</td>
<td>-5.805</td>
<td>-1.132</td>
<td>-4.673</td>
<td>-1.132</td>
<td>-0.869</td>
<td>-0.296</td>
</tr>
<tr>
<td>Dopamine</td>
<td>-5.568</td>
<td>-0.168</td>
<td>-5.399</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In this study, docking scores of dopamine, clozapine, quetiapine and sertindole related to their binding to D₁ and D₂ receptors have been calculated and compared with each other. The ligands docking with 5AER (PDB ID: 2YOU) protein were assessed using AutoDock4 and AutoDock-Vina software programs. Before the docking calculations were carried out, blind docking with Autodock-Vina revealed binding pockets on the entire surface of the 5AER receptors. The most common region of interaction of the residues was selected by determining the optimum docking results with Auto Dock4. Four ligands docked to a receptor within this grid region of 40x40x40 points and 0.375 Å grid spacing were detected.

3. Results and Discussion

3.1 Geometry Optimization and Molecular Orbital Approach
Dopamine molecule (3, 4-dihydroxyphenylethylamine) is an endogenous compound containing a benzene ring with two hydroxyl substituents and an amino-ethyl group. Its ball and stick structure are shown in Figure 1a. The structural and electronic configuration of clozapine, quetiapine and sertindole, which are bound to D₁ receptor molecules, are studied and compared with each other. The ball and stick structure of these three drugs clozapine, quetiapine and sertindole are shown in Figure 1b, Figure 1c and Figure 1d, respectively.
Using basis set, as defined above, the calculated energies of HOMO and LUMO together with some low-lying bound states are given in Table 1. These results are quite close to the results obtained by other researchers (Bayri et al., 2016).

Since the recognition between biomolecules relies on the formation of very specific interactions, one must have some information not only related to the electronic configuration but also electronic surface distribution since the interactions between these molecules are generally controlled by surface distribution. When the electronic configurations of dopamine and these drugs are compared, it becomes obvious that clozapine has some similarities with dopamine. The same similarities may easily be figured out from the electron density plots of the HOMO of these molecules.

The HOMO and LUMO energies and the corresponding optimized geometry of dopamine, clozapine, quetiapine, and sertindole molecules are shown in Figure 2a, Figure 2b, Figure 2c and Figure 2d, respectively.
Figure 2. Electron density of HOMO and LUMO for (a) and (b) dopamine, (c) and (d) clozapine, (e) and (f) quetiapine, (g) and (h) sertindole, respectively.
3.2 ESP of the Molecules

The ESP of the molecules was given in Figure 3 and the potential change depending on the positions of the atoms in molecules was indicated before. When the ESP results of 4 molecules were compared, we found that they all have generally different structures. The most similar ESP results among the molecules were obtained between dopamine and clozapine. The surface potential of the molecules is important for the interaction with the receptors, which will be discussed in the molecular docking section.

According to ESP calculation of the molecules, we can say that the red region on the molecules exhibits the partially negative charge, which is the bonding region of the receptors. So, the drugs in this study were bound to the positive region of the D1 and D2 receptors. In this case, it should be noted that the strength of the electrostatic force of the receptors and the drugs has crucial importance for the interaction between the drug and its receptor. The thermal and chemical fluctuations in the receptor regions due to the change of the cell potential, temperature, etc. affect the bonding time and probability of each molecule. Although they need a statistical study, it can be said that the molecular electrostatic force may show individual differences, which may affect receptor-drug interactions.

The ESP of the molecules give information about the negative and positive electric field regions on the surface and it has a crucial role in the interactions among other molecules, proteins, receptors, etc. Figures 3 show the ESP results of the dopamine, clozapine, quetiapine and sertindole.
Figure 3 shows the ESP results of the dopamine, clozapine, quetiapine and sertindole. The blue, green and red regions on the ESP graph show the positive, neutral and negative electric fields on the surface, respectively. The dopamine molecule in Figure 3a has a circular negative region on the H-N-H part of the molecule and the benzene ring and O-H also have a negative potential surface as seen in the red region. According to the ESP results of the clozapine molecule (Figure 3b), a positive potential surface does not exist, and the negative potential surface was observed at the region of benzene rings, Cl and N parts of the molecule. Similarly, quetiapine also has a negative region at the O and N parts and one of the benzene rings of the molecule (Figure 3c). Sertindole exhibited a negative region on one of the benzene rings, F and Cl parts of the molecule (Figure 3d).

3.3 Molecular Docking Consideration

Surface potentials of the molecules are important for the interaction with the receptors, which will be discussed in the molecular docking section.

The molecular docking is an important tool for drug studies since it can determine the region of interaction between the molecule and its receptor, and the statistical probability of the bonding region, etc. Docking of dopamine, clozapine, sertindole and quetiapine by D1 and D2 receptors shown in Figure 4 and minimum binding energies, inhibition constants (Ki), and best position scores are given in Table 2 and Table 3. It is clear from these calculations that there are not so many differences between the binding energies. However, there is an obvious difference between the number of good poses. From the calculations, it is evident that clozapine has greater advantages when compared with the sertindole and quetiapine. Probably this is one of the reasons why clozapine is better than the other atypical antipsychotics quetiapine and sertindole.

Based on molecular dynamics calculations, the binding energies of dopamine, clozapine, quetiapine and sertindole with their receptors are given in Table 2 and Table 3. It is well known from the molecular orbital theory calculations that the strength of the bonding energy is related to the stability of the molecular structure and thus higher bonding energy may lead to an increase in the lifetime of the unified structure. If the D1 receptor is considered, it is quite clear that the strength of the stability is given as Sertindole> Clozapine> Quetiapine> dopamine.

When the D2-receptor is considered, the sequence of binding energy becomes as Clozapine> Sertindole> Quetiapine> dopamine and surely the lifetime would be changed in the same sequence.
From the calculations, it is obvious that the clozapine molecule has the highest binding energy compared with the others.

Table 2. Results of docking with AutoDock (100 runs) and AutoDock-Vina (20 runs) for D₁ receptor.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Clozapine</th>
<th>Dopamine</th>
<th>Quetiapine</th>
<th>Sertindole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kᵦ= Estimated Inhibition Constant, Kᵢ = uM</td>
<td>0.041</td>
<td>2.83</td>
<td>0.116</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of good poses after 100 runs</td>
<td>13</td>
<td>15</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Number of multi-member conf. cluster</td>
<td>9</td>
<td>13</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Number of distinct conformational clusters</td>
<td>11</td>
<td>25</td>
<td>42</td>
<td>54</td>
</tr>
</tbody>
</table>

RMSD-tolerance; 2.0 Å, Temperature = 298.15 K.

Table 3. Results of docking with AutoDock (100 runs) and AutoDock-Vina (20 runs) for D₂ receptor.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Clozapine</th>
<th>Dopamine</th>
<th>Sertindole</th>
<th>Quetiapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Energy of Binding (AutoDock), kcal/mol</td>
<td>-7.73</td>
<td>-6.38</td>
<td>-7.51</td>
<td>-6.70</td>
</tr>
<tr>
<td>Free Energy of Binding (Vina), kcal/mol</td>
<td>-6.50</td>
<td>-5.30</td>
<td>-6.90</td>
<td>-5.70</td>
</tr>
<tr>
<td>Kᵦ= Estimated Inhibition Constant, Kᵢ = uM</td>
<td>2.16</td>
<td>20.99</td>
<td>3.11</td>
<td>12.21</td>
</tr>
<tr>
<td>Number of good poses after 100 runs</td>
<td>56</td>
<td>17</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Number of multi-member conf. cluster</td>
<td>11</td>
<td>22</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Number of distinct conformational clusters</td>
<td>23</td>
<td>40</td>
<td>76</td>
<td>73</td>
</tr>
</tbody>
</table>

RMSD-tolerance; 2.0 Å, Temperature = 298.15 K.

So, based on the calculations of binding energy between D₁ and D₂ receptors and their ligands, the D₁ receptor-ligand bond has a crucial lifetime limit and the D₂ needs more lifetime as in the case with clozapine.

The molecular docking study was performed 100 times to increase the accuracy of the results and the data obtained for D₁ and D₂ receptors are presented in Table 2 and Table 3. It is found that the dopamine and atypical antipsychotic drugs have different bounding surfaces on the D₁ and D₂ receptors. At 13 different binding sites dopamine showed an affinity for D₁ receptors, but quetiapine and sertindole had a greater number of binding sites than dopamine as seen in Table 2. The minimum number of binding sites for D₁ was detected for clozapine molecule that may be explained by more selectivity of the molecule when compared with the others. Based on
D₂ molecular docking study (Table 3), similar results were observed namely clozapine molecule was again more selective than the others.

In addition to these data, we also calculated the statistical data of the molecules for binding to the D₁ and D₂ receptors and the obtained results are presented in Table 2 and Table 3. After 100 runs, the highest binding probability for D₁ was obtained in the dopamine molecule and quetiapine and sertindole exhibited very low binding properties when compared to the others. The clozapine molecules have a probability that closes to dopamine molecule for D₁ receptors. The calculation for D₂ receptor revealed that dopamine has 0.15 probability and quetiapine and sertindole have 0.03 and 0.01, respectively. The highest probability was obtained for the clozapine molecules.

4. Conclusion
We theoretically investigated the dopamine, clozapine, quetiapine and sertindole molecules by Gaussian suit and their structural properties were determined, HOMO/LUMO energies were calculated, and their ESP analyses were performed. Additionally, docking of the dopamine, clozapine, quetiapine, and sertindole in D₁ and D₂ receptors has been calculated. It was found that clozapine has higher mobility than the others due to its lowest energy gap. The HOMO-LUMO energy gap seems to be a disadvantage of clozapine, however, when the receptor-ligand relationship is concerned it turns into an advantageous position compared with the molecular structures of other studied antipsychotics. As is already known, the relationship between the receptor and its agonist should not be considered in an isolated environment and the synaptic region, which involves so many ingredients, should be taken into consideration. Synaptic interactions in the region seem to be most easily tolerated by the clozapine molecule. The most similar ESP results among the molecules were obtained between dopamine and clozapine. This similarity may cause clozapine to have a dopamine-like effect on cognitive functions. According to the results of docking calculations, clozapine is more advantageous than the other studied antipsychotics. It may be easily predicted that the clozapine-receptor bond occupies its optimal position in the configuration when compared with the other two drugs. According to binding energy calculations of D₁ and D₂ receptors and their ligands, we found that the D₁ receptor has a crucial effective lifetime limit with ligands and the D₂ needs more lifetimes as provided by clozapine. This longer lifetime of the clozapine may play a crucial role in the therapy of schizophrenia. Probably this lifetime approach may be used in order to measure the effectiveness of the drugs since it is directly related to the unified ligand-receptor structure. It is seen that clozapine is more strongly attached to D₁ when dopamine decreased in the medium, whereas it is more bound to D₂ in higher dopamine concentrations. These two characteristic features of clozapine are superior when compared with other antipsychotic drugs according to the dopamine hypothesis of schizophrenia. In particular, thanks to its effects on D₂ receptors clozapine suppresses positive symptoms mediated by D₁ receptors and through this mechanism, it treats the impairment in cognitive and negative symptoms. Due to this heteromeric activity clozapine is superior to other antipsychotics. In recent years, studies that draw attention to the heteromeric structure of dopamine receptors have provided new insights into the efficacy of clozapine. Because of the remarkable potential of dopamine receptor heteromers to access diverse signaling cascades or to modulate the nature of the transduced signal, these heteromeric complexes represent likely candidates in the search for new drug therapies (Perreault et al., 2011). The heteromerization of dopamine receptors provides an important benefit to the treatment of schizophrenia because it adds a new diversity to the dopamine receptor structure. Tauscher et al. (Tauscher et al., 2004) studied the in vivo D₁ and D₂ receptor profile of clozapine compared with other atypical antipsychotics such as olanzapine, quetiapine, or risperidone. Among the atypical antipsychotics, clozapine appears to have a simultaneous and equivalent occupancy of dopamine D₁ and D₂ receptors. The ratio of striatal D₁/D₂ occupancy was significantly higher for clozapine (0.88) relative to olanzapine (0.54), quetiapine (0.41), or risperidone (0.31). The efficacy of clozapine seems to be related to having the low energy gap, have a dopamine-like EPS and have more effective binding to D₁ and D₂. Dual-action mode of clozapine may lead to the discovery of new therapeutic solutions for schizophrenic disorders. So, we can say that the binding of the clozapine molecule to D₁ and D₂ receptors is more effective when compared with the other molecules studied, which should carry crucial importance for the clinical results of the treatment.

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