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Research Article

Investigating the Effect of Dexamethasone on CYP3A4 and Glucocorticoid Receptor by *in silico* Analysis

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Abstract

Cytochromes are enzymes of the dehydrogenase class with a hemoprotein structure in which the iron in these compounds undergoes oxidation and reduction reactions upon receiving or losing electrons. Quantitatively speaking, CYP3A4 is the most important isoenzyme of cytochrome P450, oxidizing foreign organic molecules such as drugs or toxins to cause them to leave the body. Many drugs and antibiotics can induce or inhibit the activity of cytochrome P450, including dexamethasone. Dexamethasone is a steroidal anti-inflammatory drug used to treat of inflammatory diseases and chronic autoimmunity. This study aimed to investigate the induction effect of dexamethasone in biotransformation pathways by in silico tools. Molegro Virtual Docker software was used to investigate the molecular docking of the enzyme and dexamethasone, which indicated the binding of the drug to the enzyme. The molecular simulation was performed in Linux with the GROMACS program. Root-mean-square distance (RMSD), and radius of gyration (Rg), were evaluated. The results were analyzed with Pymol and VMD software, and the obtained curve was plotted with GRACE software. Docking results show that a cluster with a bond energy of -60.81 was the best cluster, and the bond size between ligand and internal atoms was -23.191in the complex. In addition, the amount of bond between the ligand and water for this pose was zero. The stability of the enzyme-ligand complex and the induction effect of dexamethasone on CYP3A4 were indicated by RMSD and RG results. Results of RMSD and RG of CYP3A4 glucocorticoid obtained from the simulation showed the stability of binding of the drug to the enzyme. Also, RMSD results showed the stability of glucocorticoid and dexamethasone complex during molecular dynamics simulation. It reached relative stability at 0.8 nm after 80,000 ps until the end of the simulation.

***Keywords*:** In silico, Dexamethasone, Molecular docking, Bacterial and viral diseases

Introduction

Biotransformation is the process by which organisms such as bacteria, fungi, and enzymes transform organic compounds from one form to anothe (Baillie et al 2015). The purpose of biotransformation is to facilitate the excretion of these compounds from the body. Cytochrome P450 genes belong to a multigene group that encodes the functions of monooxygenated mixtures, which are responsible for the oxidative metabolism of a wide range of substrates (Crake et al 2021). The concentration of the cytochrome P450 enzyme depends on physiological mechanisms that can be used as adjustment exogenous inducers. In addition, some non-physiological conditions (pathophysiological changes, toxic agents, viruses, and tissue damage affect Cytochrome P450 enzymes (Pascussi et al 2001).CYP3A4 is an isozyme of Cytochrome P450, comprising approximately 30% of the total liver P450 content (Zerilli et al 1998). This enzyme oxidizes small foreign xenobiotic molecules such as toxins and drugs. Many compounds can inhibit or induce this enzyme, including dexamethasone.

Dexamethasone is a steroidal anti-inflammatory drug used in the treatment of inflammatory diseases and chronic autoimmunity. In addition, it acts effectively as an anti-emetic agent in cancer chemotherapy. In human liver cell culture, dexamethasone is a potent inducer of CYP3A4 (Villikka et al 1998). Glucocorticoids induce CYP3A subtype genes in humans and many other species (Al Rihan et al 2020). A glucocorticoid

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receptor (GR) is a receptor to which cortisol and other glucocorticoids bind. When glucocorticoids bind to GR, its main mechanism of action is the regulation of gene transcription (Kino et al., 2009). The activated GR complex regulates the expression of anti-inflammatory proteins in the nucleus or suppresses the expression of inflammatory proteins in the cytosol by preventing the transfer of other transcription factors from the cytosol to the nucleus (Lu NZJPR 2006). In molecular biology, the PXR 1 receptor is a nuclear steroid receptor or a subset of the nuclear receptor family. PXR is a nuclear receptor whose main task is to monitor the presence of foreign toxins. PXR is one of the transcriptional regulators for the cytochrome P450 3A4 gene. It is activated by dexamethasone and rifampicin that stimulate P450 3A4 (Goodwin et al 2002). Molecular docking, a sub-technique of molecular modeling in bioinformatics, has become increasingly popular due to the advancement of science and technology. The use of interdisciplinary sciences, including bioinformatics, offers the possibility of saving time and money in research. Various docking software such as HADDOCK, Autodock, and Molegro Virtual Docker can be used to investigate the interaction between protein-ligand, protein-protein, and protein-DNA (Prieto-Martínez and Arciniega 2018). With this in mind, the current study investigated the interaction between dexamethasone and cytochrome P450 3A4 enzyme by in silico analysis.

Materials and Methods

In this project, dexamethason was selected as the enzyme inducer (Pascussi et al 2001) The structure of the drug with the chemical formula C22H29FO5 was extracted from the Drug Bank database (code: DB01234). The desired ligand and the protein structure of the enzyme were extracted from the Protein data bank database (code: 1TQN).

Investigation of protein-ligand interaction

Molegro Virtual Docker software was used to investigate the interaction between cytochrome P450 3A4 protein and dexamethasone ligand. Also, the interaction between glucocorticoid proteins with dexamethasone ligand was assayed. This software was used to simulate molecular dynamics and protein-ligand docking. GROMACS software was used to perform Molecular dynamics simulation.The results were observed and analyzed using Pymol and VMD software. In addition, the root-mean-square deviation (RMSD) and radius of gyration of C-alpha atoms were calculated and analyzed using the GRACE software. Also, the PDB files of the two proteins were aligned using the Pymol software (Sadus 2002).

Investigation of protein-Protein interaction

To explore the interaction between glucocorticoid and cytochrome P450 proteins, a completely flexible protein-protein docking approach was used with the ClusPro software. (Nola and Roccatano 1994, Kozakov et al 2017). ln this software, biochemical and biophysical information obtained from laboratory methods were used to predict the manner of interaction. Glucocorticoid receptor because dexamethasone activates this receptor and acts by binding to the promoter regions of the target gene, including cytochrome P450.

Molecular dynamics simulation

GROMACS 2019.6 software was used to simulate molecular dynamics. Input structures were prepared with the ff99SB force field. Chlorine ions were added to neutralize the surface charge of the structure. Gmx solvate tool was used to place the complex in a layer of 9 A_o-thick TIP3P water molecules inside an octahedron box. The energy of the structures was reduced using the steepest descent method with 50,000 steps to minimize hydrogen bonds and van der Waals interactions between the complex of glucocorticoid and cytochrome P450 proteins and water molecules. Next, the system's temperature was gradually increased from 0 to 310 K for 100 ns (nanoseconds), then it was balanced at constant pressure for 100 ns.

MD simulations were performed at 37°C for 100 ns. The SHAKE method surrounded hydrogen atoms involved in bonding to increase computing performance. RMSD and RG was used to analyse all changes over time. The RMSD which is a suitable and common standard analyses was calculated during the MD simulation in the time distance, to ensure the stability of the protein structure. Therefore, RMSD changes related to alpha carbon atoms of the protein were calculated and extracted relative to the original structure during the simulation time (100 ns). RG analysis was used to study protein size changes during MD simulation.

Results

Molecular modeling of glucocorticoid and cytochrome P450

Given the availability of the three-dimensional

structure of the proteins in the PDB database, this structural information was utilized in this study. Pymol software can also display the protein's three-dimensional structure based on the PDB file. To examine the interaction between glucocorticoid and cytochrome P450, a fully flexible protein-protein docking analysis was conducted using the ClusPro software. Results from molecular docking of glucocorticoid and cytochrome P450 show that the best docking cluster with the lowest bond energy was -1125.5 (Figure 1). Molecular dynamics simulation results were observed and analyzed using Pymol and VMD software. As shown in Figure 2, the structural stability and molecular diffusion per unit of time were evaluated after performing the molecular dynamics simulation. At 50,000 ps, the RMSD decreased slightly. At 60,000 ps, it reached 1.1 nanometers and remained constant until the end of the simulation, indicating the stability of the protein structure at the end of the simulation.

The results of the radius of gyration indicated that glucocorticoid and cytochrome P450 have the minimum compactness of their structures. At 80,000 ps, the value of the protein's radius was about 2.18 nm (nanometers), after which it remained constant until the end of the simulation, informing the stability of the protein (Figure 3).

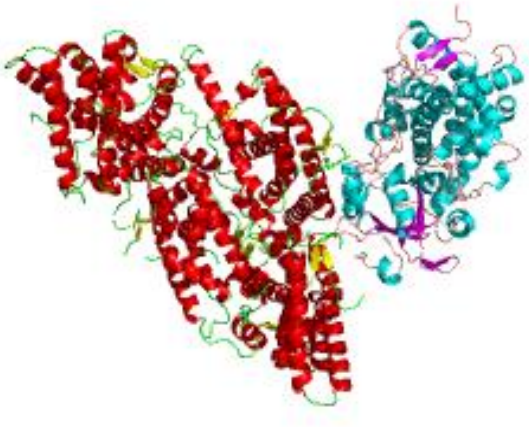

Figure 1. The ClusPro software was employed to generate the three-dimensional structure of the docked protein-protein; glucocorticoid protein is red, and cytochrome P450 protein is blue.

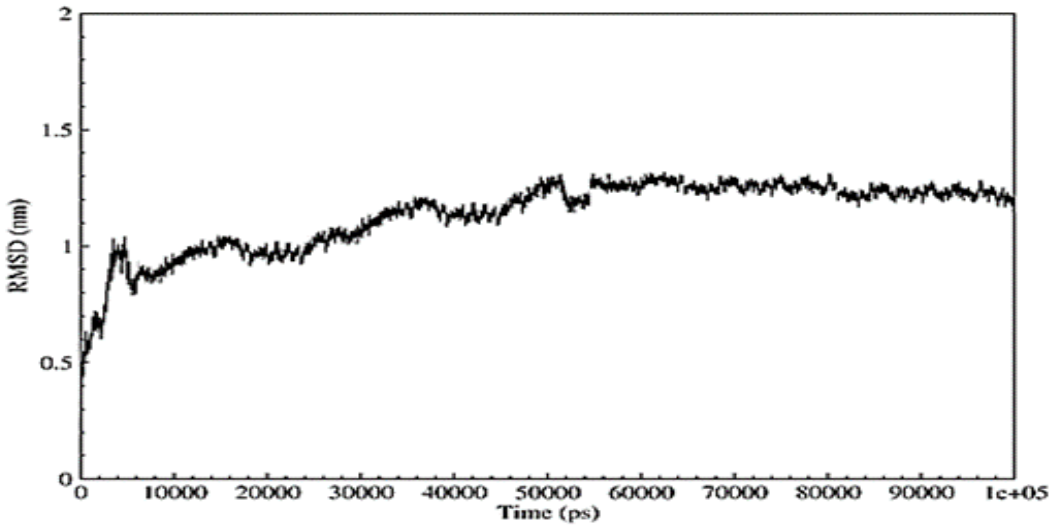

Figure 2. The stability of the complex of glucocorticoid and cytochrome P450protein structure at the end of a 100 ns. MD simulation at 310 K is demonstrated by the plot of root-Mean-Square Deviation (RMSD) of recombinant proteins throughout the simulation.

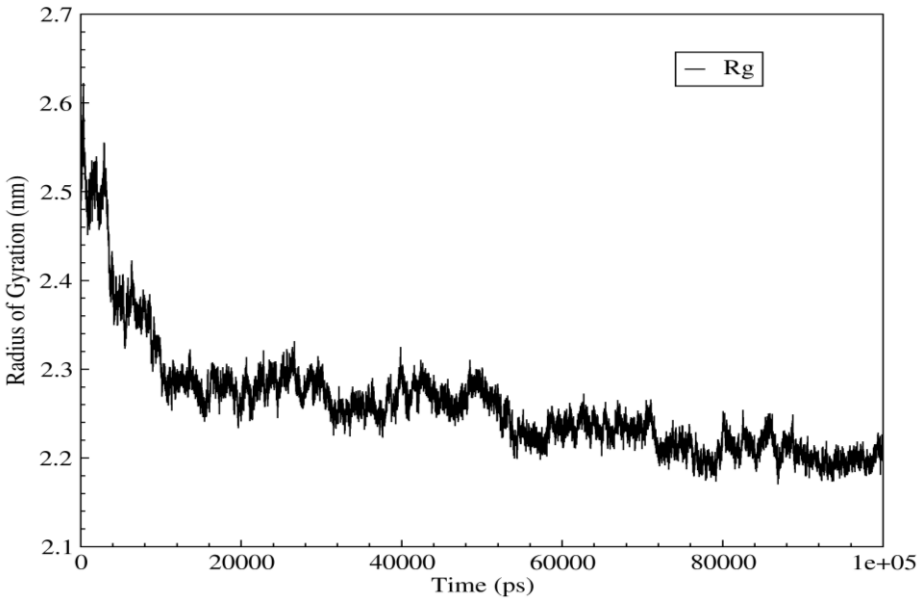

Figure 3. Radius of gyration glucocorticoid and cytochrome P450 during 100 ns molecular dynamics simulation. At 80,000 ps, the value of the protein's radius was about 2.18 nm (nanometers), after which it remained constant until the end of the simulation, indicating the stability of the protein.

Molecular docking of glucocorticoid protein with dexamethasone ligand

The Molegro Virtual Docker software was used to investigate the interaction of GR protein with dexamethasone ligand (Figure 4). To limit the volume of docking calculations, residues directly involved in the connection were identified. The docking results were ranked based on the intermolecular interaction energy after molecular docking. This energy is the sum of electrostatic energies.

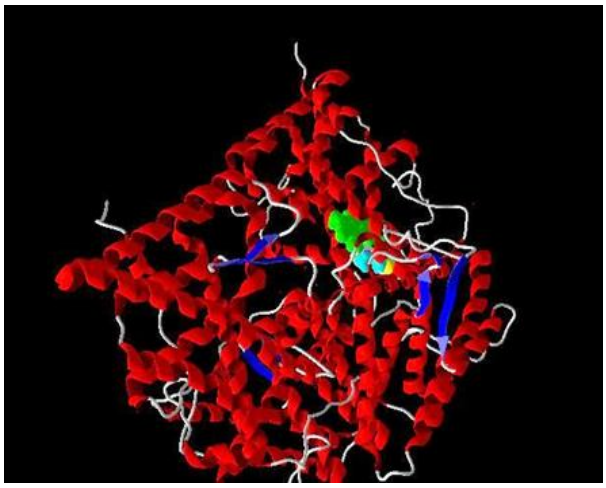

Figure 4. The three-dimensional structure of the glucocorticoid protein docked with the dexamethasone ligand using Molegro Virtual Docker software. The glucocorticoid protein is represented in red, and the dexamethasone ligand is shown in green, which denotes its optimal docking position with the glucocorticoid protein.

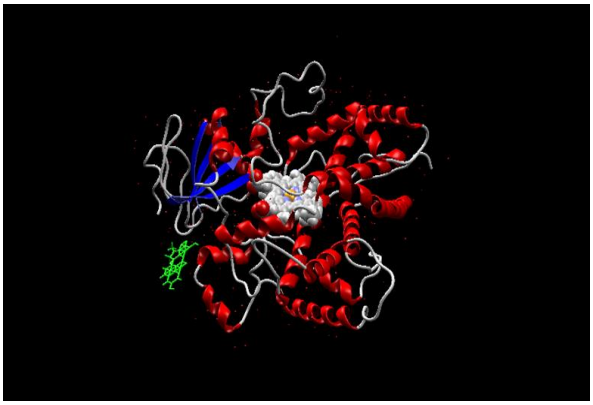

Figure 5. The molecular docking complex between cytochrome P450 and dexamethasone, created using Molegro Virtual Docker software. The cytochrome P450 protein is shown in red, while the dexamethasone ligand is represented in green and is positioned next to the cytochrome P450 protein. The best pose is represented by white.

Molecular docking of cytochrome P450 protein with dexamethasone ligand

Molecular docking was performed to investigate the interaction of cytochrome P450 protein with dexamethasone ligand. After docking, the results will show a list of ligands with their binding energy or binding affinity. A general statement usually states that for "binding affinity," the more negative the energy is, the better the ligand (Figure 5).

Molecular dynamics simulation of glucocorticoid and dexamethasone complex

After performing molecular docking, the best-selected complex was used as input to simulate molecular dynamics. The protocol mentioned in GR and cytochrome P450 was used to place the complex inside the membrane and perform molecular dynamics simulations. The only difference was the number of phospholipids, water molecules, and sodium and chlorine ions used. There were 120 POPC phospholipid molecules, 1340 water molecules, 34 sodium atoms, and 79 chlorine atoms. The simulation time was 100 ns. RMSD results showed the stability of glucocorticoid and dexamethasone complex during molecular dynamics simulation. It reached relative stability at 0.8 nm after 80,000 ps until the end of the simulation (Figure 6).

Molecular dynamics simulation of cytochrome P450 and dexamethasone complex

After molecular docking, the best complex obtained from molecular docking was utilized as input for molecular dynamics simulation. The only variation was the number of phospholipids, water molecules, and sodium and chloride ions used compared to the previous two sections. There were 83 POPC phospholipids molecules, 22 sodium atoms, and 49 chlorine atoms. The simulation time was 100 ns.
The RMSD parameter was used to study the stabilit y of the cytochrome P450 on dexamethasone ligand during molecular dynamics simulation. It reached relative stability at 0.7 nm after 80,000 pc until the end of the simulation (Figure 7).

Discussion

Eun Chae Gong et al., 2018 investigated the activity of cytochromes D192, 2B6, 2E1, and 3A4 using molecular docking. This study found that cytochrome P450 isozyme has the highest negative energy in the active site region, resulting in better binding with ligands (Gong et al 2018). These findings were consistent with our results. Therefore, according to our results, dexamethasone is placed in the best binding position to enzyme CYP3A4 and has the lowest binding energy. The molecular docking of cytochrome P450 with inhibitory drugs was investigated. As a result, the drug celecoxib was identified as an inhibitor of the CYP 4A11 isoenzyme, which is used to treat pain, swelling, and dryness caused by arthrosis or joint wear, sprain or muscle strain, etc. (Gujjula et al 2018). Based on studies conducted Goodwin et al,. 1998 CYP3A4 the main hepatic P450 protein in the human liver, can be regulated by several hormones at physiologically relevant concentrations (LIDDLE et al 1998). Experiments in rodents have shown that most of the constitutive liver P450 enzymes are under hormonal regulation (Zerilli et al 1998). Cytochrome induction can lead to severe drug-food interactions, especially if the drug plasma level is critical because it can reduce therapeutic effects and cause side effects (Koe et al 2014).

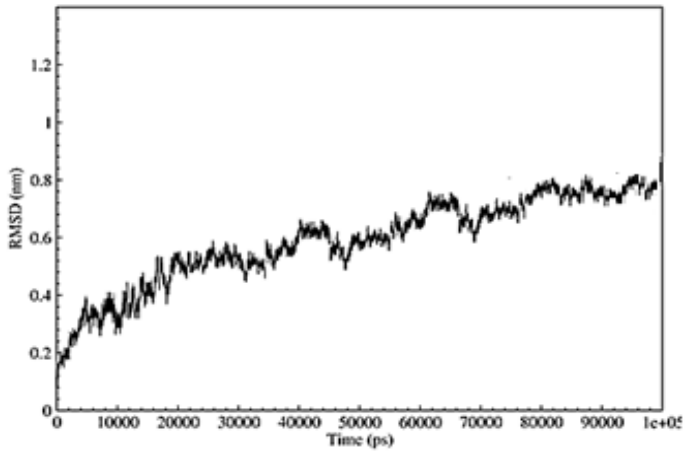

Figure 6. Changes in the RMSD diagram of the glucocorticoid and dexamethasone complexes during molecular dynamics simulations.

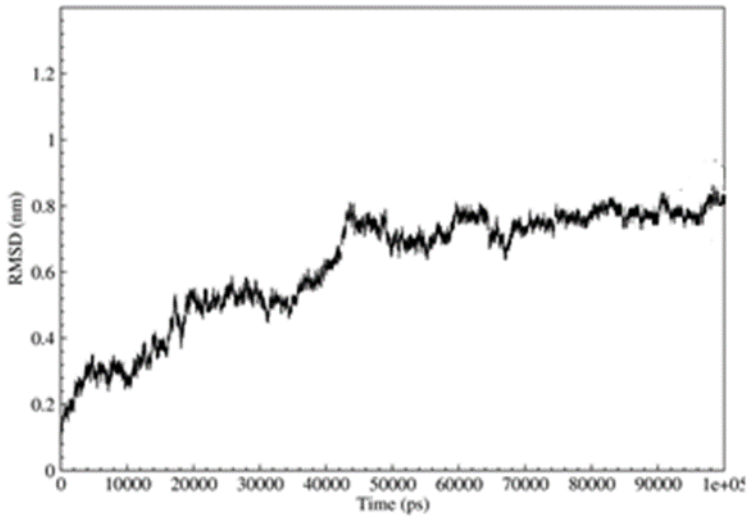

Figure 7. Changes in the RMSD diagram of the cytochrome P450 and dexamethasone complexes during molecular dynamics simulations, it reached relative stability at 0.7 nm after 80,000 ps until the end of the simulation.

For the current study, the PDB database was utilized to examine the three-dimensional structure of proteins. These proteins were then modeled using the SWISS-MODEL online server, and the Molegro Virtual Docker program was employed for molecular docking. The findings of this experiment emphasize that dexamethasone can be placed in the best pose of the enzyme P450 3A4 and form a stable complex to induce the enzyme. This study aimed to examine the induction of the cytochrome P450 3A4 enzyme, which activates the GR receptor following dexamethasone, a glucocorticoid drug. Furthermore, this study sought to evaluate the impact of dexamethasone on the expression of PXR and CAR (Goodwin er al 1999). If GR has a drug-binding site, it will induce the enzyme. However, since GR does not have a region that can bind directly to the P450 3A4 promoter, GR must first activate the PXR and CAR genes. These genes are linked to dexamethasone and activate other enzyme compounds. By activating the enzyme cytochrome P450 3A4, it can play its role in metabolizing xenobiotics and toxic substances produced by bacterial infections in the liver, causing them to leave the body. So, in this research, we investigated the effect of dexamethasone on the CYP3A4 enzyme using molecular docking. So, docking results showed that dexamethasone is placed in the best position and has an inducer effect on the CYP3A4 enzyme.

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