ISSN(Online): 2320-9801
ISSN (Print): 2320-9798

International Journal of Innovative Research in Computer and Communication Engineering

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 12, December 2015

Molecular Docking Studies of CYP Enzymes Polymorphism on Drug Binding

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ABSTRACT *:* Among various enzymes of vital importance in human biological system, Cytochrome P450 enzymes (P450) is essential for the metabolism of many medications. Cytochrome 2B6 (CYP2B6) an important enzyme of Cytochrome enzyme super family plays a major role in the metabolism of clinically important drugs. Owing to the existence Single Nucleotide Polymorphism (SNP) of this enzyme, the drugs metabolized by CYP2B6 show significant inter-patient variability in their response. The present work embodies the impact of SNP of CYP2B6 enzymes on drug binding of anticancer drug Cyclophosphamide in terms of binding energies by molecular docking. Binding energies reflect the binding affinity of ligands and higher the binding energy lower the binding affinity and vice versa. The outcome of the present work may find its application in pharmacogenomics which holds a promise in personalized medicine that drugs might one day be tailor-made for individuals and adapted to each person's own genetic makeup.

KEYWORDS: receptor,ligand,docking, polymorphism, drug binding, binding energy, binding affinity

I. INTRODUCTION

Medicinal activities are triggered by interaction of drug with the receptors in the human body. Most of the receptors are proteins in the body. When a drug binds with a protein, it results in the change in structure of three dimensional structure of protein leading to change in functions of the receptor, thus showing the response of the body to the drug. As there have been found variations in genes of the receptors among individuals, there are inter-individual differences in therapeutic response to the same drug. Study of the effect of variations in the gene of target protein among individuals on drug binding and response by both experimental and computational methodologies is an area of intense research . Hence an understanding of the binding interactions between drug like molecule (ligand) and receptor and the effect of variations in genes of the receptor on binding interactions in terms of binding energies will be helpful in successful design and discovery of more efficient drugs. As the computational structures of both the receptors and ligand are available, Bioinformatics or Computational Biology speeds up the process of understanding the binding interactions by using computational methods called molecular docking .

Molecular docking is a computational method of placing a computer generated representation of a small molecule (ligand) into the active site of the computer generated target structure in a variety of positions, conformations and orientations . Each such position, orientation and conformation of the ligand in the active site of the protein is called as a 'pose' [1]. As each of these poses induces a total binding energetic cost of the system, the system's total binding energy is calculated after every move. The main objective of molecular docking is to identify a pose which has the lowest binding energy as binding energy is one of the important criteria in measuring binding affinity of a ligand. The affinity is a measure of how tightly a drug binds to the receptor. If the drug does not bind well, then the action of the drug will be shorter. Molecular docking methods help reduce the cost and time of drug discovery and development process [2].

II. LITERATURE REVIEW

The use of molecular docking , one of the *in silico* methods in virtual screening of drug discovery process has been the subject of discussion in terms of its importance and vital role in cutting cost and time of drug discovery cycle [2] . Many

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enzymes are present in the human body. Among them, there is a family of enzymes called P450 or CYP enzymes which are essential for the metabolism of many medications [3,4,5]. Hence these enzymes are found to be very important to pharmaceutical industry for discovering new, safe and effective drugs. Poly and morph is a term used in genetics to describe the multiple forms of a single gene existing in an individual or among a group of individuals. Genesequencing studies of the Human Genome Project have revealed that, at the nucleotide level, the gene encoding a specific protein can have a number of differences in sequence [6]. These differences may or may not alter the overall product significantly enough to produce a different protein, but may have an effect on drug-receptor binding interactions [7] in terms of binding energies. One form of genetic variation called as Single Nucleotide Polymorphism (SNP) is very important to pharmaceutical industry [8]. SNPs are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genomic sequence is altered in a population at a frequency greater than 1%. When a single base change occurs at less than 1% it is considered to be a mutation. For example, SNP might change the DNA sequence AAGGCTAA to ATGGCTAA. CYP enzymes are known for its highly polymorphic nature [9]. It is reported that Polymorphisms of this enzyme can influence how humans metabolize drugs [10,11].

Based on the amino acid sequence identity, CYP enzymes are classified into various families and subfamilies. Among the known families, the CYP1A, CYP1B, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E and CYP3A are known to be involved in drug metabolism. It has been reported that CYP2B6 is the only active member of the CYP2B subfamily in homosapians [12] and is also known to be involved in the metabolism of many clinically important drugs and a less understood enzyme too. Also CYPP2B6 is highly polymorphic in nature.

The alkylating agent, Cyclophosphamide, is a common therapeutic agent used in the treatment of many cancers. Importantly, the efficacy and toxicity of Cyclophosphamide varies greatly in patient populations. CYP2B6 is involved in the activation and metabolism of Cyclophosphamide and a potential explanation for the observed therapeutic discrepancies is believed to be the highly polymorphic nature of CYP2B6 [13,14].

III. METHODS

It is evident from the literature review that very little research has been done in investigating the effect of Single Nucleotide Polymorphism of CYP2B6 enzymes on binding of anticancer drugs Cyclophosphamide by molecular docking. Hence in the current work CYP2B6 and some of its SNPs are chosen as receptors and anticancer drug Cyclophosphamide is chosen as ligand. The structure of ligand and receptor is elementary to molecular docking studies. 2D structure of Cyclophosphamide which is aquired from PubChem database of NCBI (National Centre For Biotechnology Information) is as displayed below.

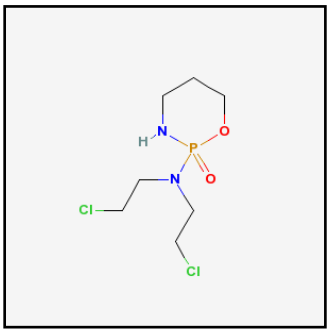

Figure 1 : 2D Structure of Cyclophosphamide

Since 3D mol file of Cyclophosphamide is required for docking studies, the 2D structure is converted into 3D mol file by Chimera Software. 3D structure of the receptor CYP2B6 WildType(without polymorphism) is procured from the NCBI structure database and is as displayed below.

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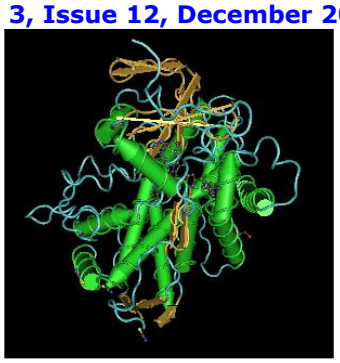

Figure 2: 3D structure of CYP2B6 WildType

Various SNPs of CYP2B6 are reported in the dbSNP database of NCBI [15]. Though there are many SNPs of CYPP2B6 reported [16], in the present study only five SNPs are chosen. The SNPs of CYP2B6 selected for investigation are listed in the following table .

	Amino Acid Position	Amino acid Replacement
1	99	G with E
2	259	S with R
3	336	R with C
4	423	T with N
5	487	R with C

Table 1: SNPs selected for study in the present work

Proteins are complex, organic compounds composed of many amino acids . Each amino acid substitution potentially affects the protein function. Therefore only SNPs of CYP2B6 where the amino acids are replaced are chosen as receptors for the present study. In the present work above listed SNPs are referred to as receptors with the name CYP2B6 SNP 99, CYP2B6 SNP 259, CYP2B6 SNP 336, CYP2B6 SNP 423, CYP2B6 SNP 487. To understand the influence of SNPs on drug binding , 3D structures of SNPs of CYP2B6 are also essential. The 3D structures of SNPs of CYP2B6 are not available in the PDB database of NCBI. Hence they are constructed by the researcher using Homology Modeling [17] by giving protein sequence of each SNP as input. But the protein sequences of SNPs of CYP2B6 are not available in any database. Therefore researcher constructed the protein sequences of SNPs of CYP2B6 using the protein sequence of CYP2B6 WildType which is available in the protein database of NCBI. The protein sequence of each SNP is fed as input to the Swiss Model and 3D structure is obtained. Once the required structures are ready, molecular docking is perormed using "AutoDock" molecular docking tool [18] and binding energy differences between the molecular dockings performed on the ligand Cyclophosphamide with CYP2B6 WildType and each of its SNP is calculated. Appropriate conclusions are drawn on the effect of SNP on drug binding

IV. RESULTS AND DISCUSSIONS

Results of molecular docking studies are displayed graphically. It is evident from the graph that each of the SNPs of CYP2B6 have higher binding energy than the CYP2B6 WildType receptor

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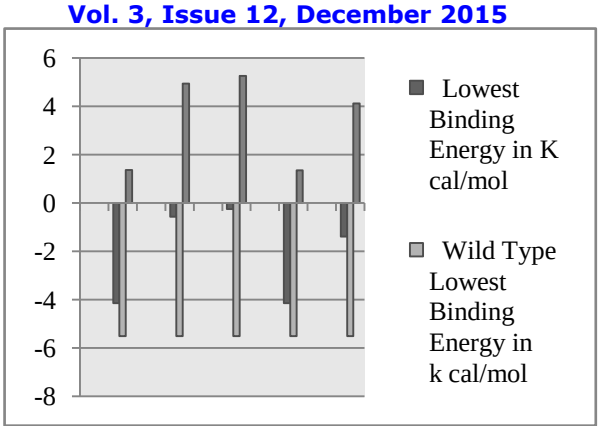

Graph 1: Lowest Binding Energies of CYP2B6 and its SNPs

Difference in binding energies arising out of SNPs can cause conformational changes in the receptor that affect the binding affinity of a ligand. It is well known that binding energies reflects the binding affinity. Thus increase in binding energy due to SNP of CYP2B6 may decrease the binding affinity of Cyclophosphamide. However, along with binding energies, several other physical effects like electrostatics, van der waals forces, hydrogen bonding, and hydrophobic and entropic effects influence the binding affinity. These are also needed to be evaluated elsewhere for calculating binding affinity of ligands or drug candidates.

V. CONCLUSIONS

In the present study, influence of SNP of CYP2B6 on drug binding of Cyclophosphamide is understood in terms of binding energies by molecular docking. On docking Cyclophosphamide to CYP2B6 which has SNP, binding energies obtained were found to be significantly higher than binding energies obtained while docking Cyclophosphamide to CYP2B6 without SNP. Best binding or interaction is indicated by lowest binding energy. Lower the binding energy, more stable the complex is. Also lower binding energy implies higher binding affinity. Thus increase in binding energy due to SNP of CYP2B6 may lower the binding affinity of Cyclophosphamide. Binding affinity is a pointer to drug efficacy. Lower binding affinity because of SNPs of CYP2B6 points to altering the dosage for patients with SNPs to overcome inter-patient variability. From the study, inter patient variabilty which was observed in the treatment of cancer using Cyclophosphamide can be attributed to the existence of SNPs of CYP2B6 among individuals. This implies that medical treatments have to be tailored according to the gene make up of an individual. Hence the present study has made a contribution to the emerging personalized medicine field.

ACKNOWLEDGMENT

Our sincere thanks are due to Dr. P.K.Butey, Dr. Satish Sharma for all valuable suggestions , support and encouragements made throughout the course of this research work. We also thank Dr. Kavita Rattan for all her valuable inputs during the course of this work.

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