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Analysis of CYP3A4-HIV-1 protease drugs interactions by computational methods for Highly Active Antiretroviral Therapy in HIV/AIDS

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ABSTRACT

HIV infected patients often take at least three anti-HIV drugs together in Highly Active Antiretroviral Therapy (HAART) and/or Ritonavir-Boosted Protease Inhibitor Therapy (PI/r) to suppress the viral replications. The potential drug–drug interactions affect efficacy of anti-HIV treatment and major source of such interaction is competition for the drug metabolizing enzyme, cytochrome P450 (CYP). CYP3A4 isoform is the enzyme responsible for metabolism of currently available HIV-1 protease drugs. Hence administration of these drugs in HARRT or PI/r leads to increased toxicity and reduced efficacy in HIV treatment. We used computational molecular docking method to predict such interactions by which to compare experimentally measured metabolism of each HIV-1 protease drug. AutoDock 4.0 was used to carry out molecular docking of 10 HIV-protease drugs into CYP3A4 to explore sites of reaction and interaction energies (i.e., binding affinity) of the complexes. Arg105, Arg106, Ser119, Arg212, Ala370, Arg372, and Glu374 are identified as major drug binding residues, and consistent with previous data of site-directed mutagenesis, crystallography structure, modeling, and docking studies. In addition, our docking results suggested that phenylalanine clusters and heme are also participated in the binding to mediate drug oxidative metabolism. We have shown that HIV-1 protease drugs such as tipranavir, nelfinavir, lopinavir, and atazanavir differ in their binding modes on each other for metabolic clearance in CYP3A4, whereas ritonavir, amprenavir, indinavir, saquinavir, fosamprenavir, and darunavir share the same binding mode.

1. Introduction

Human immunodeficiency virus-1 (HIV-1) is one of the important targets for clinical research causes AIDS in human. This RNA virus replicates inside the host cell by integrating its genetic material with the host cell genome [1]. Several researches are happening worldwide to neutralize the activity of this virus for curing HIV-1 infected patients. There are no effective drugs yet to treat the patients because this virus is highly resistant to the drugs [2]. Hence a single HIV-drug administration is not advisable for AIDS treatment. Highly Active Antiretroviral Therapy (HAART) and/or Ritonavir-Boosted Protease Inhibitor Therapy (PI/r) and salvage therapy are widely in practice with the co-administration of three or more HIV drugs simultaneously to treat AIDS patients [3–5]. Researchers are mainly targeting the HIV-1 reverse transcriptase, HIV-1 protease and HIV-1 integrase to block the virus multiplication inside the host cell. HIV-1 protease enzyme is essential for the life-cycle of this virus [6,7]. The virus synthesizes its proteins as polyprotein precursor form, and should be cleaved to transform into mature, fully functional proteins to infect the cells [8]. HIV-1 protease is required for proteolytic cleavage of the viral polyprotein precursors to transform into the individual functional proteins [9,10]. Inactivation of HIV-1 protease by specific chemical compounds should render the virus noninfectious [11]. The compounds such as ritonavir (RTV) [12], amprenavir (APV) [13], tipranavir (TPV) [14], indinavir (IDV) [15], saquinavir (SQV) [15], nelfinavir (NFV) [15], lopinavir (LPV) [16], fosamprenavir (FOS-APV) [17], darunavir [18] and atazanavir (ATV) [19] are the US-FDA approved drugs available for HIV-1 protease inhibition (http://www.fda.gov/oashi/aids/virals.html).

In general, drugs are to be metabolized by enzymes to avoid toxic effects of drug accumulation. Cytochrome P450 (CYPs) is superfamily of heme-thiolate containing enzymes that are responsible for the oxidative metabolism of xenobiotic compounds [20]. Human CYP450 proteins CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are the major drug metabolizing isoforms, which play an important role in the oxidative metabolism of 90% drugs in the

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current clinical use [21,22]. Of these isoforms, CYP3A4 is involved in the metabolism of more than 50% drugs and other xenobiotics [23]. In the present form, there are number of crystal structures for human CYP3A4 (PDB ID: 1TQN, 1WOE, 1WOF, 1WOG, 2JOD, and 2VOM) available in the protein structure database (http://www.rcsb.org/pdb). The most interesting features of the CYP3A4 structures are the shape and size of the active site cavity, which is adjacent to the heme iron. The two research groups Yano et al. [24] and Williams et al. [25] reported two different volumes of active site cavity 1386 and 520 Å respectively. This result indicates that CYP3A4 active site cavity can metabolize several bulky substrates [26] and also supporting binding of multiple compounds simultaneously for oxidative metabolism [27–29]. The methods of X-ray crystallography, site-directed mutagenesis, modeling and docking studies have revealed an important catalytic active site residues Val101, Asn104, Arg105, Met114, Ser119, Leu211, Arg212, Asp214, Asp217, Pro218, Glu374, Ile301, Ala305, Thr309, Ile369, Ala370, Leu373, Glu374, Ser478, Leu479, and 'Phe-cluster' (Phe108, Phe215, Phe219, Phe220, Phe241, Phe304 and Phe215) for interaction with substrates and inhibitors of CYP3A4 [24,25,30–32].

All currently available HIV-1 protease drugs of FDA approved (U.S. Food and Drug Administration) are metabolized by CYP3A4 [12–19]. As the HIV-1 treatment employs HAART practice, the co-administration of protease drugs leads to CYP3A4-related drug–drug interactions occur for metabolic clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events. The simple reason for this is to one drug modulates the metabolism of other (drug–drug interactions) by simple competition for the same active site, and/or by binding in an allosteric region of the same enzyme [33]. Hence for the effective treatment, it is important to have the co-administration of protease drugs having diverse binding modes and affinity into the CYP3A4 active site for metabolic clearance. In this present investigation, we used molecular docking method to predict such binding modes and affinity (i.e., interaction energies) of currently available 10 HIV-1 protease drugs into CYP3A4. In addition, understanding of how this complex CYP3A4 enzyme recognizes multiple, diverse chemical structures was investigated to rational design of potential drug candidates.

2. Methods

The atomic coordinates of ligand-free human microsomal cytochrome P450 3A4 (CYP3A4) was retrieved from Protein Data Bank [PDB ID: 1TQN] [34]. This structure was determined by X-ray crystallography to 2.05 Å resolutions [24]. A set of 10 drugs approved by FDA for HIV-1 protease were used for this docking study. These drugs are ritonavir [DrugBank: DB00503], amprenavir [DrugBank: DB00701], tipranavir [DrugBank: DB00932], indinavir [DrugBank: DB00224], saquinavir [DrugBank: DB01232], nelfinavir [DrugBank: DB00220], lopinavir [DrugBank: DB01601], fosamprenavir [DrugBank: DB01319], darunavir [DrugBank: DB01264] and atazanavir [DrugBank: DB01072] was retrieved from DrugBank database [35]. The two-dimensional structures of all these drugs are shown in Figs. 1 and 2. The geometry of drug molecules and CYP3A4 structure were optimized via Smart Minimizer protocol (Steepest Descent method followed by Conjugate Gradient method with 1000 steps) using Accelrys Discovery Studio (Version 1.7, Accelrys Software Inc.), the most comprehensive suite of modeling and simulation solutions for drug discovery available. Each of the minimization was carried out with CHARMm force field.

Docking studies for these drugs were carried out as per the protocol described by our previous work [36,37]. In brief, AutoDock 4.0 software package [38] was used for conducting docking studies on CYP3A4. The graphical user interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. The Kollman charges, solvation parameters and polar hydrogen were added into the water free CYP3A4 structure for the preparation of protein in docking simulation. The Gasteiger charge was assigned into ligands (i.e., HIV-1 protease inhibitors) and then non-polar hydrogen was merged. The rigid roots of each ligand were defined automatically instead of picking manually and amide bonds were made non-rotatable. AutoTors program was used to assign all rotatable dihedrals in the ligand. The number of rotatable bonds varied based on size of the ligand in the range 12–23 and were allowed to rotate freely. AutoDock requires pre-calculated three dimensional grid maps, one for each type of atom present in the ligand and its stores the interaction energy based on a macromolecular target using the AMBER force field. This grid must surround the region of interest in the macromolecule. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to generate grid maps for the ligands. The grid box was fixed in the catalytic active region between heme moiety and SER119 of human CYP3A4. The box size in *x*-, *y*- and *z*-axis was normally set at 60 Å × 60 Å × 60 Å, though it was changed depending on the size of the ligands. The spacing between grid points was 0.375 Å. The GA-LS search algorithm (Lamarckian Genetic Algorithm) was chosen to search for the best conformers with 10 runs of each ligand. During the searching process, the enzyme was regarded as rigid, while ligands (i.e., drugs) were regarded as being flexible, to explore in any of the six degrees of freedom. The population size was set to 150 and the individuals were initialized randomly. The maximum number of energy evaluation was set to 250,000; maximum number of generations was to 1000. The maximum number of top individual that automatically survived was set to one. The rates of gene mutation and crossover were set at 0.02 and 0.80 respectively. All the AutoDock docking runs were performed in Intel Pentium PD-925 CPU at 3.0 GHz of HCL infosystem origin, with 2 GB DDR RAM. AutoDock 4.0 was compiled and run under Microsoft Windows XP operating system.

3. Results

The three dimensional structures of both CYP3A4 and HIV-1 protease drugs were optimized to have minimal potential energy using Smart Minimizer and values are shown in Table 1. After minimization, the drug molecules have the potential to change the initial states of it into a system of binding state in target CYP3A4 molecule when the energy is released. Docking simulation of 10 runs of GA-LA was performed for a set of 10 protease drugs into a catalytic active site of CYP3A4. The best docked conformation of each drug into CYP3A4 binding site was determined as the one which having the lowest interaction energies of both docking energy and binding free energy among the 10 different poses generated. The binding free energy, docking energy and binding site residues of CYP3A4 which involved in hydrogen bond and vdW interaction are given in Table 2. We were further analyzed the docked conformation for finding the binding mode of each protease drug into CYP3A4. We were compared our docking results with the experimentally measured metabolism of each HIV-1 protease drug to validate the drug orientation and position obtained likely to represent reasonable binding modes or conformations.

4. Discussion

4.1. Docking ritonavir into CYP3A4

Ritonavir, a potent HIV-1 protease inhibitor, is extensively metabolized by CYP3A4. The majority of currently available protease drugs are co-administered with low doses of RTV for