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Computational study on the drug resistance mechanism of HCV NS5B RNA-dependent RNA polymerase mutants V494I, V494A, M426A, and M423T to Filibuvir

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ABSTRACT

Filibuvir, a potent non-nucleoside inhibitor of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase (RdRp), has shown great promise in phase IIb clinical trial. However, drug resistant mutations towards Filibuvir have been identified. In the present study, the drug resistance mechanism of wild-type (WT) and mutant NS5B polymerases (including V494I, V494A, M426A, and M423T) toward Filibuvir was investigated by molecular modeling methods. The predicted binding free energy of these five complexes is highly consistent with the experimental EC_{50} values of Filibuvir to the wild-type and mutant NS5B RdRps, V494I < WT < V494A < M426A < M423T. Analysis of the individual energy terms indicates that the loss of binding affinity is mainly attributed to the decrease in the van der Waals interaction contribution. Through detailed analysis of the interaction between FBV and $RdRp^{V494I}$, $RdRp^{WT}$, $RdRp^{V494A}$, $RdRp^{M426A}$, and $RdRp^{M423T}$, several conclusions are made. Firstly, the smaller size of residue 494 side chain results in the smaller binding affinity between FBV and RdRp. Secondly, the poor inhibition capacity of Filibuvir toward $RdRp^{M426A}$ is mainly due to the decrease in the van der Walls interaction between Filibuvir and residue Leu-497^{M426A} caused by the spatial structure change of Ala-426^{M426A}. Thirdly, the decrease in the binding affinity in mutation M423T is attributed to the smaller binding cave and the cyclopentyl group of Filibuvir exposing outside the cave. Our computational results will provide valuable information for developing more potent and selective inhibitors toward HCV NS5B polymerase.

1. Introduction

According to a data released from the World Health Organization, about three percent of the world's population has been infected with the hepatitis C virus (HCV) (World Health Organization, 2012), and many of these infected individuals are likely to develop serious HCV-related liver diseases, such as liver cirrhosis, hepatocellular carcinoma (Leone and Rizzetto, 2005). Therefore, HCV has become a great threat to human health.

HCV, a member of the Flaviviridae family, is a single-stranded, positive-sense RNA virus. Its genome includes approximately 9600 nucleosides, and encodes a polyprotein precursor which is made up of the core protein, envelope glycoproteins and the non-structural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Tang and Grise, 2009). Among these proteins, NS5B polymerase is an important component of the viral replication machinery as it encodes the viral RNA-dependent RNA polymerase (RdRp) which is the key enzyme for HCV RNA synthesis (Behrens et al., 1996; Moradpour et al., 2004). Structurally, like other RdRps, NS5B RdRp contains canonical thumb, finger, and palm domains resembling the human's right hand. It has an encircled enzyme active site, and its fingers and thumb subdomains interact with RNA (Lesburg et al., 1999). As one of the viral RdRp, HCV NS5B RdRp has its own particular architecture that the fingers and thumb domains are connected. This particularity makes it distinct from related mammalian DNA and RNA polymerases, hence using NS5B RdRp as a drug target will greatly reduce the damage on human cells. Therefore, HCV NS5B RdRp has represented an attractive drug target for the development of specific anti-HCV drugs and vaccines (Beaulieu and Llinas-Brunet, 2002). Currently, a certain number of NS5B RdRp inhibitors have been reported and entered into clinical trials. Based on their mode of action (Wang et al., 2012; Powdrill et al., 2010), these inhibitors can be broadly categorized into nucleoside or nucleotide inhibitors (NIs) (Cole et al., 2009; Cretton-Scott et al., 2008), non-nucleosides inhibitors (NNIs) (Bedard et al., 2009; Lazerwith et al., 2013) and pyrophosphate (PPi) analogues (Koch et al., 2006; Summa et al., 2004). The NNIs

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bind at allosteric pockets of NS5B and prevent a conformational change needed for initiation of RNA synthesis (Tomei et al., 2004, 2003; Gu et al., 2003).

Filibuvir (FBV, Fig. 1) (Li et al., 2009) is a potent non-nucleoside inhibitor (NNI) developed by Pfizer in 2009 and shows significant promise in phase IIb clinical trial (Love et al., 2003; Shi et al., 2009). It binds to the "Thumb II" allosteric pocket of NS5B RdRp and affects the formation of a productive replicase complex. In the study by Shi et al. (2009), they described that FBV shows no cytotoxic effect in several human cell lines, up to the highest concentration evaluated (320 μmol/L). These properties of FBV support its use as a potential antiviral agent to HCV-infected patients. Unfortunately, company Pfizer had discontinued the development of FBV after a strategic review of its pipeline in March 11, 2013. But they also declared that this decision was not related to any safety issue of FBV. Actually, the research of FBV is still ongoing (Jiao et al., 2014).

However, NS5B mutations that mediate resistance to FBV have been selected both in cell culture (replicons) and in therapy studies. Experimental studies in cell culture with replicons (Troke et al., 2012) demonstrated that the EC_{50} values of FBV to the WT, V494I, V494A, M426A, and M423T NS5B RdRps are 18, 13, 120, 172, and >11,000 nmol/L, respectively. Thus, the treatment of chronic HCV infections with FBV still faces a great challenge. So, it is necessary to identify the relationship between residue mutations of NS5B RdRp and the inhibition capacity of FBV. At the present time, molecular modeling methods have been proved to be the effective techniques for investigating the drug resistance mechanism of inhibitors. Several typical works aimed at the drug resistance mechanism studies related to the HCV NS3/4A protease and NS5B polymerase have been reported in the past few years (Davis and Thorpe, 2013; Guo et al., 2006; Klibanov et al., 2012; Welsch et al., 2008, 2012; Xue et al., 2012, 2013, 2014; Guan et al., 2014; Jiao et al., 2014; Yi et al., 2012; Wang et al., 2014). It is worth mentioning that in Davis and Thorpe's study, they first demonstrated the evidence for a mechanistic basis of allosteric inhibition in NS5B (Davis and Thorpe, 2013). And in the study by Xue et al. (2014), they focused on the three representative mutations (M423T/V/I) in NS5B RdRp to explore the drug resistance mechanism of HCV to FBV. Their results indicated that the threonine, isoleucine, and valine with a larger side chain than methionine is the main reason leading to the decrease in the binding affinity between FBV and NS5B RdRp.

In this work, to elucidate the drug resistance mechanism of FBV, we systemically investigate the interaction mechanisms between FBV and the wild-type and mutant (V494I, V494A, M426A, and M423T) NS5B RdRps (genotype 1) by using MD simulations, molecular mechanics/Poisson–Boltzmann surface area (MM/PBSA) free energy calculations, and molecular mechanics/generalized born surface area (MM/GBSA) free energy decomposition analysis (Wang and Kollman, 2000, 2001; Lee et al., 2000; Kuhn and Kollman, 2000; Hou et al., 2002, 2003, 2006a,b, 2008, 2010, 2011, 2012; Kollman et al., 2000; Wang et al., 2001; Lepšík et al., 2004; Weis et al., 2006; Hou and Yu, 2007; Luo et al., 2002;Gohlke and Case, 2004; Fang et al., 2008; Xu et al., 2013; Sun et al., 2014). Based on the calculations, we will disclose the drug resistance mechanism of FBV toward the wild-type and mutant NS5B RdRps.

2. Materials and methods

2.1. Structure of NS5B RdRp–FBV (RdRp/FBV) complex

The wild-type model of NS5B RdRp/FBV ($RdRp^{WT}$/FBV) complex was derived from the crystal structure of the complex of FBV with the wild-type NS5B RdRp (genotype 1) which was from the Research Collaboratory for Structural Bioinformatics Brookhaven Protein Data Bank (PDB ID: 3FRZ) (Li et al., 2009). The models of NS5B $RdRp^{V494I}$/FBV, $RdRp^{V494A}$/FBV, $RdRp^{M426A}$/FBV, and $RdRp^{M423T}$/FBV complexes were obtained by substituting the residues Val-494, Val-494, Met-426, and Met-423 of the wild-type complex with residues Ile, Ala, Ala, and Thr, respectively. Before the MD simulations were started, the missing hydrogen atoms of FBV were added using SYBYL7.1 while the missing atoms of the wild-type NS5B RdRp were added using the *tleap* program in AMBER11.0 (Case et al., 2005). FBV was minimized using the Hartree–Fock (HF)/3-21G optimization calculations in Gaussian03 (Frisch et al., 2008), and the atom partial charges were generated by fitting the electrostatic potentials derived by Gaussian via the restrained electrostatic potential (RESP) fitting technique in

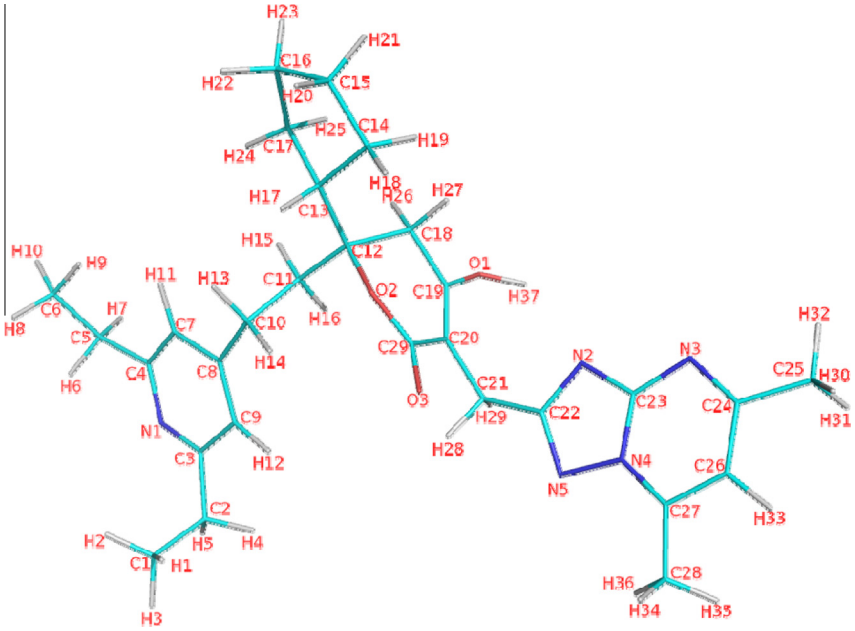

Fig. 1. Chemical structure of Filibuvir with atom notations.