Antiviral Research 80 (2008) 339–353

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Understanding the molecular basis of HBV drug resistance by molecular modeling

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ARTICLE INFO

Article history:
Received 16 May 2008
Received in revised form 25 July 2008
Accepted 29 July 2008

Keywords:
Hepatitis B virus
Drug resistance
Nucleoside analog
Polymerase inhibitor

ABSTRACT

Despite the significant successes in the area of anti-HBV agents, resistance and cross-resistance against available therapeutics are the major hurdles in drug discovery. The present investigation is to understand the molecular basis of drug resistance conferred by the B and C domain mutations of HBV-polymerase on the binding affinity of five anti-HBV agents [lamivudine (3TC, **1**), adefovir (ADV, **2**), entecavir (ETV, **3**), telbivudine (LdT, **4**) and clevudine (L-FMAU, **5**)]. In this regard, homology modeled structure of HBV-polymerase was used for minimization, conformational search and induced fit docking followed by binding energy calculation on wild-type as well as on mutant HBV-polymerases (L180M, M204V, M204I, L180M + M204V, L180M – M204I). Our studies suggest a significant correlation between the fold resistances and the binding affinity of anti-HBV nucleosides. The binding mode studies reveals that the domain C residue M204 is closely associated with sugar/pseudosugar ring positioning in the active site. The positioning of oxathiolane ring of 3TC (**1**) is plausible due the induced fit orientation of the M204 residue in wild-type, and further mutation of M204 to V204 or I204 reduces the final binding affinity which leads to the drug resistance. The domain B residue L180 is not directly close (~6 Å) to the nucleoside/nucleoside analogs, but indirectly associated with other active-site hydrophobic residues such as A87, F88, P177 and M204. These five hydrophobic residues can directly affect on the incoming nucleoside analogs in terms of its association and interaction that can alter the final binding affinity. There was no sugar ring shifting observed in the case of adefovir (**2**) and entecavir (**3**), and the position of sugar ring of **2** and **3** is found similar to the sugar position of natural substrate dATP and dGTP, respectively. The exocyclic double bond of entecavir (**3**) occupied in the backside hydrophobic pocket (made by residues A87, F88, P177, L180 and M204), which enhances the overall binding affinity. The active site binding of LdT (**4**) and L-FMAU (**5**) showed backward shifting along with upward movement without enforcing M204 residue and this significant different binding mode makes these molecules as polymerase inhibitors, without being incorporated into the growing HBV-DNA chain. Structural results conferred by these L- and D-nucleosides, explored the molecular basis of drug resistance which can be utilized for future anti-HBV drug discovery.

Published by Elsevier B.V.

1. Introduction

More than 350 million people are chronically infected with hepatitis B virus (HBV), resulting about 1 million death per year (Lai et al., 2003b). HBV, a member of the hepadnavirus family, is an enveloped virus that contains a partially double stranded DNA genome (3 kbp). In addition to regular transcription and translation processes, it has a reverse transcription process similar to HIV. This is mediated by a single enzyme, catalyzing RNA- and DNA-dependent DNA polymerase, RNase H and protein priming activities (Seeger and Mason, 2000). Analogous to HIV, the HBV-polymerase is also a good target for inhibiting the viral replication. Several nucleoside-analogs such as lamivudine (**1**, 3TC, a cytosine L-nucleoside analog) (Dienstag et al., 1995), adefovir (**2**, ADV, a adenosine analog), entecavir (**3**, ETV, a carbocyclic guanosine analog) (de Man et al., 2001) and telbivudine (**4**, LdT, a thymidine L-nucleoside analog) (Lai et al., 2004) have been approved by the US-FDA for the treatment of chronic HBV infection (Fig. 1).

Previously, we have synthesized a number of L-nucleosides. Among which clevudine (**5**, L-FMAU, a thymidine L-nucleoside analog) has been discovered as a potent anti-HBV agent (Chu et al., 1995; Marcellin et al., 2004; Yoo et al., 2007a) and more recently it was approved for the treatment of chronic hepatitis B virus infection in South Korea in 13 November 2006. Currently, it is undergoing Phase III clinical trials in US and Europe (Marcellin et al., 2004).

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0166-3542/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.antiviral.2008.07.010

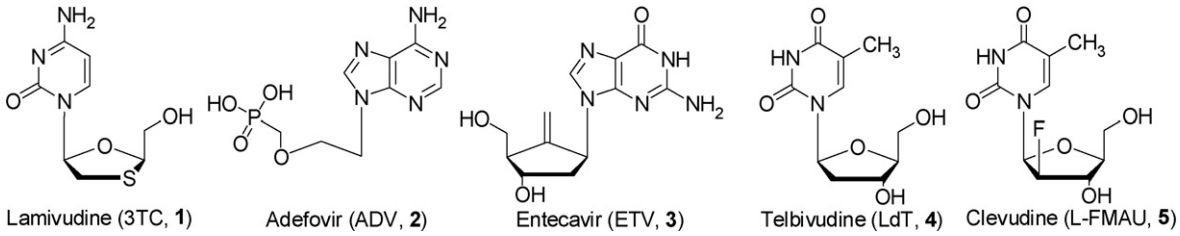

Fig. 1. The chemical structure of potential HBV-polymerase inhibitors.

Table 1
Antiviral activity (IC_{50} in μM) and fold resistance (FR)[a] of anti-HBV agents (**1**–**5**) against wild-type and 3TC-resistant HBV-polymerases (Chin et al., 2001; Locarnini, 2003; Brunelle et al., 2005)

HBV-strain	3TC (**1**)		ADV (**2**)		ETV (**3**)		LdT (**4**)		L-FMAU (**5**)	
	IC_{50}	FR	IC_{50}	FR	IC_{50}	FR	IC_{50}	FR	IC_{50}	FR
Wild-type	0.6	1.0	3.9	1	0.8	1	0.17	1	0.1	1
L180M	0.80	1.7	2.0	0.5	na	1[b]	1.96	>10	>100	>100
M204V	8.5	18	2.8	0.7	na	10[b]	na	na	1.5	15
M204I	>50	>100	2.7	0.7	na	na	39.5	>100	>100	>100
L180M – M204V[c]	>50	>100	0.6	0.2	5.0	6	22.2	>100	>100	>100

[a] Fold resistance = (mutant IC_{50})/(wt IC_{50}).
[b] Separate study on ETV, shows that M204V mutation had 10-fold reduction on the potency of ETV, while L180M had no significant effect.(Levine et al., 2002).
[c] Dual mutant 3TC-resistant strain (L180M – M204V and L180M – M204I) having similar pattern of resistance profile against most of the potential compounds (**1**–**5**) (Das et al., 2001; Langley et al., 2007).

Despite the above significant successes in the discovery of an anti-HBV agent (Ferir et al., 2008; Palumbo, 2008), the critical issue is the development of drug resistance and cross-resistance against available therapeutics. Recent studies reveal that adefovir resistance increases to 29% after 5 years of use (Locarnini et al., 2005). Therefore, increasing use of adefovir against lamivudine-resistant HBV infection can increase a risk of multidrug-resistant HBV. The development of drug resistance is not unexpected if viral replication continues during the current monotherapy. The prevention of resistance requires the adoption of strategies that can more effectively control the virus replication, including the combination therapy (Sasadeusz et al., 2007; Hui et al., 2008). For the past several years, our group has been involved in understanding the HBV drug resistance issue at molecular level by molecular modeling (Chong and Chu, 2002; Yadav and Chu, 2004).

Recently, several publications related to HBV drug resistance issue appeared on different classes of anti-HBV agents (Ayres et al., 2004; Bartholomeusz et al., 2004; Chong and Chu, 2004; Tenney et al., 2004; Jacquard et al., 2006; Locarnini and Mason, 2006). HBV-polymerase has several domains that are homologous to other RNA-dependent DNA polymerases (Poch et al., 1989; Lesburg et al., 1999). A revised numbering system for HBV-polymerase in comparison to HIV RT proposed by Stuyver et al. (2001) has been generally accepted and it is being used in this article.

A current anti-HBV agent (**1**–**5**) causes a specific primary mutation during the treatment and the published biological data (Table 1) (Chin et al., 2001; Delaney et al., 2001a,b; Levine et al., 2002; Ono-Nita et al., 2002; Angus et al., 2003; Locarnini, 2003; Angus and Locarnini, 2004; Locarnini and Mason, 2006) provides an opportunity to understand the structural and functional insight of the active-site of HBV-polymerase.

Several groups have developed the structural models of HBV RT based on HIV RT (Allen et al., 1998; Bartholomeusz et al., 1998; Das et al., 2001), although homology models may not be accurate due to low sequence homology between HIV and HBV. Fortunately, the active site residues of HBV-polymerase are highly conserved in with respect to HIV RT (Bartholomeusz et al., 2004). Therefore HBV-polymerase-modeled structure can be utilized to draw some useful conclusions. A similar right-hand structure (fingers, palm, and thumb subdomains) was assigned for the modeled structure of HBV-polymerase with respect to HIV RT (Fig. 2a). The catalytic site residues are highly conserved (Bartholomeusz et al., 2004) and is responsible for polymerase residue-recognition with the template, primer or an incoming nucleotide analog.

In our molecular modeling studies, we focused our attention on highly conserved regions surrounding the binding site of natural substrate thymidine triphosphate (TTP). The initial modeling of TTP highlighted its binding mode in the active site of wild-type HBV-polymerase (Fig. 2b). Some of the important non-covalent interactions (Table 2) that stabilize the complex (HBV-poly-DNA-TTP) and required for any molecule to fit into the active-site to behave as a perfect mimic of incoming natural nucleoside (TTP) are shown in Fig. 2b. The strong hydrogen-bonding network between the triphosphate moiety with HBV-polymerase residues (S85, A86, A87) is conserved for all nucleoside reverse transcriptase inhibitor-triphosphates (NRTI-TPs) and natural nucleosides.

Fig. 2b also focuses on the two-metal-ion (Mg^{2+}) mechanism (Steitz, 1998) for facilitating the 3′O^- attack on the α-phosphate with help of the conserved catalytic residues D83 and D205 for the addition of TTP to the primer (+1) for DNA chain elonga-

Table 2
Molecular recognition element (non-covalent interaction) required for NRTIs to mimic dNTP in HBV-polymerase based on Fig. 2b (dTTP-binding)

NRTIs	Interaction detail with HBV-polymerase
Base pairing	Thymine base is forming two strong H-bond with Adenine of DNA–template
Base stacking	Thymine base is showing stacking interaction (centroid distance ~4 Å) with the guanine base of DNA–primer (+1) nucleotide
Two-metal-ion mechanism	Conserved catalytic residue D83 and D205 with two Mg^{2+} is responsible for dNTP addition to the DNA–primer (+1) for chain elongation
Triphosphate positioning	Triphosphate positioning is mediated by H-bonding with residues S85, A86 and A87