The mechanisms of action of antivirals against hepatitis B virus infection

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Although vaccination programmes among neonates in intermediate to high endemic countries have been successful in preventing vertical transmission of hepatitis B virus (HBV), an estimated 350 million persons are still chronically infected with HBV. Although HBV is transmitted in a fashion similar to that of HIV, HBV infection is more prevalent in certain groups, even in developed countries. Interferon-α is licensed to treat chronic HBV infection but it is effective in only a minority of patients.1 Thus, the therapeutic challenge to effectively treat chronic HBV infection continues, and the past decade has seen the introduction of new therapeutic strategies to HBV infection.

HBV is a small, partially double-stranded DNA virus and a prototype member of the hepadnavirus family. Its 3.2 kb genome possesses four overlapping open reading frames encoding the envelope (pre-S/S), core (precore/core), polymerase and X proteins. Among these hepadnavirus proteins, a single protein contains the enzyme catalysing RNA- and DNA-dependent DNA polymerase, RNase H and protein priming activities. Hepadnavirus polymerase plays a critical role in the replication of the hepadnavirus genome (Figure 1).2 Analogous to HIV, the viral reverse transcriptase is a good target for inhibiting hepadnavirus replication. Indeed, some nucleoside reverse transcriptase inhibitors (NRTIs) are also suggested to have an anti-HBV activity in vitro.

Lamivudine (β-L-2′,3′-dideoxy-3′-thiauridine), one of the dideoxycytidine (ddC) analogue NRTIs, is the first nucleoside analogue approved to treat chronic HBV infection and has been shown to benefit various categories of patients. These include HBe-positive and -negative patients, nonresponders to interferon-α therapy, and patients with decompensated cirrhosis.3 Lamivudine’s D-isomer is less active against HBV replication and more cytotoxic than the L-isomer. Both the D- and L-isomers of 2′,3′-dideoxy-3′-thiauridine were found to be inactive against HBV replication, and the susceptibility of the thiauridine stereoisomers to cytidine and/or deoxycytidine deaminase was suspected to be the reason. In fact, the racemic mixture and the D-isomer of 2′,3′-dideoxy-3′-thiauridine were deaminated with partially purified human deaminase and were found to be processed to the D-isomer of the thiauridine, while lamivudine and ddC were not.4 Despite the intracellular stability of its cytosine base, ddC was less effective than lamivudine in a cell culture system. Until recently, it was thought that the potency of lamivudine reflected the higher incorporation efficiency into newly synthesized DNA by the viral DNA polymerase. Lamivudine and ddC are phosphorylated to their triphosphates and then the triphosphates act as chain terminators during viral and cellular DNA synthesis.

Pyrophosphorolysis and resistance

Pyrophosphorolysis, another catalytic function of the reverse transcriptase, has recently been investigated. It may play a role in determining susceptibility to drugs and should be discussed. Although the genotype of HIV for 3′-azido-3′-deoxythymidine (AZT) resistance had been well characterized, the phenotypic mechanism of AZT resistance remained unclear for a long time. It was reported that AZT-resistant reverse transcriptase showed an enhanced rate of pyrophosphorolysis compared with that of wild-type HIV under physiological concentrations of inorganic pyrophosphate (PPi),5 and subsequently shown that nucleotide-dependent phosphorolysis was also enhanced in AZT resistance.6 Furthermore, the development of phosphonoformic acid (PFA, a pyrophosphate analogue) resistance derived from AZT-resistant variants of HIV restored susceptibility to AZT.7 These results suggest that the pyrophosphorolytic...
removal of a chain terminator from elongating DNA may be one of the important mechanisms for establishing drug resistance. In hepadnaviruses, replication of a viral genome mainly occurs within the core particles, which have a pore size 2 nm in diameter, which restricts access of larger molecules.8 Using purified duck HBV (DHBV) core particles, pyrophosphorolysis was found to be catalysed by the viral polymerase.9 This study showed that the addition of exogenous PPi succeeded in stimulating pyrophosphorolysis catalysed by DHBV polymerase, but when PPi was substituted with ribonucleoside triphosphates or PFA, they failed. More interestingly, the efficiency of PPi-dependent pyrophosphorolysis on chain-terminated DNA was not affected by the presence of a 3′ hydroxyl group or a kind of nucleoside base on the chain terminator. Furthermore, the excretion of lamivudine monophosphate from lamivudine-monophosphate-terminated viral DNA was at least 60-fold less efficient than the excretion of ddCMP or dCMP. These results indicate that pyrophosphorolysis catalysed by the viral polymerase may contribute to both NRTI resistance and selectivity against HIV or HBV replication in vivo. This indicates that the use of PPi-analogue inhibitors in combination with NRTIs should be further investigated.

Figure 1. Life cycle of hepadnavirus. The steps that are catalysed by hepadnavirus polymerase are indicated by filled arrows. The details of the replication cycle are discussed in Seeger & Mason.2 cccDNA, covalently closed circular DNA.

Figure 2. Schematic domains of HBV polymerase. The reverse transcriptase domain is located in the middle. Since amino acid positions of conserved motifs vary among the genotypes of HBV, a standardized amino acid numbering system of the reverse transcriptase domain was proposed.10 The amino acid substitutions of the drug-resistant strains of HBV discussed in the text are also indicated.

Mutation studies

Clinical studies of lamivudine in treating chronic HBV infection show that short-term therapy is not sufficient to clear the virus, but long-term therapy is associated with increased emergence of lamivudine-resistant strains of HBV.3 Most of the lamivudine-resistant strains of HBV have methionine to valine or isoleucine substitutions (M204V/I, formerly M552V/I) in the tyrosine-methionine-aspartate-aspartate (YMDD) motif. This YMDD motif is highly conserved in other virus polymerases and in the C domain of the HBV polymerase (Figure 2). Another substitution of methionine for leucine in the B domain (L180M, formerly L528M) frequently accompanies the M204V/I mutation, and HBV carrying these two mutations shows cross-resistance to lamivudine and famciclovir. It was known that the single substitution of a valine or isoleucine for methionine in the YMDD motif resulted in lamivudine resistance. In a study of the contribution of Met-184 in HIV reverse transcriptase to the binding of nucleoside 5′-triphosphates, the $K_i$ values of L-nucleoside triphosphates for M184V mutant HIV reverse transcriptase were 50- to 300-fold higher than $K_i$ values of D-enantiomers of the same inhibitors, including lamivudine (3TC), emtricitabine (FTC), ddC and ddT.11 Furthermore, the crystal structures of the M184I mutant HIV reverse transcriptase in both the presence (3.5 Å resolution) and absence (2.85 Å resolution) of 3′ DNA template primer were determined.12 Comparing the crystal structure of the M184I mutant reverse transcriptase with that of the wild-type reverse transcriptase, the template primer position was substantially shifted at the polymerase active site; however, the effect of DNA binding to residue 184 was

Figure 2.
similar for the wild-type and mutant reverse transcriptases. Modelling analysis of the interaction of dCTP or lamivudine triphosphate with the amino acid residues at the active site of HIV reverse transcriptase indicated that the ribose ring of L-entantiomer projected 1.5–2.0 Å further towards residue 184 than that of the D-entantiomer, and the close contact between the oxathiolane ring of lamivudine and the methyl group of the β-branched Ile-184 side chain may result in steric hindrance.

Another resistance mechanism of the M184I/V mutants—effecting catalytic efficiency—has also been proposed. In this model, lamivudine triphosphate would bind to the M184I/V mutant; however, the distance between the 3'-hydroxyl group of the terminal of the primer strand and the α-phosphate group of lamivudine triphosphate was increased to a suboptimal configuration for efficient catalysis. In HBV, modelling of the active site of HBV polymerase was carried out based on the sequence and structure analysis with the retrovirus reverse transcriptase. The study indicated that the oxathiolane ring of lamivudine or emtricitabine interfered with the β-branched side chain of Ile-204 or Val-204 at the active site of HBV polymerase. Contrary to M204I/V, the methionine substituted for Leu-180 introduced a longer side chain that might interact with docked L-nucleotides, but the side chain of Met-180 was more flexible than the β-branched side chains of Ile/Val-204 and might not interfere sterically with the nucleotides. Since the M204I/V mutant HBV polymerase seemed to regain its enzymatic activity of deoxyribonucleoside triphosphate incorporation when associated polymerase seemed to regain its enzymatic activity of deoxyribonucleoside triphosphate incorporation when associated with the nucleotides. Since the M204I/V mutant HBV polymerase seemed to regain its enzymatic activity of deoxyribonucleoside triphosphate incorporation when associated with the nucleotides.

Leading article

HBV infection. Adefovir dipivoxil is a prodrug of adefovir, which has an acyclic and monophosphonic modification of the sugar ring of adenosine and is active against HBV and HIV replication. Preliminary clinical studies have indicated that adefovir dipivoxil could suppress the replication of the lamivudine-resistant HBV mutants. Some other acyclic and cyclobutyl nucleoside analogues that have a broad antiviral spectrum including anti-herpesvirus activity (e.g. ganciclovir, famciclovir and lobucavir) were also evaluated in clinical trials, but were discontinued for treating HBV due to insufficient efficacy and/or unacceptable toxicity.

Protein priming

Besides the structural similarity between HIV and HBV reverse transcriptase, protein priming activity, which is a distinctive feature of hepadnavirus polymerase, may also be important for determining active compounds against hepadnaviruses. During the first step of hepadnavirus genome replication (Figure 1), the polymerase binds to an RNA stem-loop structure at the 5' end of positive strand pre-genome RNA, named epsilon, and the polymerase itself acts as protein primer for DNA synthesis. A bulge sequence on the epsilon of pre-genome RNA is a template for a 3mer or 4mer of DNA that usually starts with deoxyguanosine and is covalently linked to the N-terminal domain of hepadnavirus polymerase. A potent inhibitor of hepadnavirus primer synthesis may possess a selective or specific antiviral profile against HBV rather than HIV. Since entecavir was shown to have a potent anti-HBV activity but no anti-HIV activity in a cell culture, the mechanisms of action of this compound could become useful for determining specificity to hepadnavirus polymerase. Inhibitory activities of triphosphates of lamivudine and six guanosine analogues, including entecavir, were evaluated in each of the three different steps of hepadnavirus genome replication, i.e. RNA- and DNA-dependent DNA polymerase and protein priming activities. The study revealed that only triphosphates of entecavir and lobucavir (cyclopentyl and cyclobutyl deoxyguanosine derivatives having a hydroxyl group, which is equivalent to the 3'-hydroxyl group of guanosine) were potent inhibitors of the synthesis of the DNA primer product. However, triphosphates of lamivudine and the other guanosine analogues, such as ddG, aciclovir and ganciclovir, were much weaker or inactive against the synthesis of the primer oligonucleotides, even though they could inhibit the RNA- and DNA-dependent DNA polymerase activities. Carbocyclic 2'-deoxyguanosine (2'-CDG), which is structurally related to entecavir, was also reported to be a potent inhibitor of hepadnavirus priming. These three carbocyclic deoxyguanosine analogues were synthesized as novel anti-herpesvirus drugs but it was later thought that they might be better inhibitors of hepadnavirus than of HIV. It was reported that some truncated hepadnavirus
polymerases could catalyse the initial attachment of the first nucleotide (i.e., dGMP) to polymerase, but could not catalyse the subsequent primer DNA polymerization. This different requirement of hepadnavirus polymerase sequence might reflect the different catalytic mechanisms of hepadnavirus polymerase between the first deoxyguanosine incorporation and the subsequent deoxynucleotide polymerization. These observations indicate that guanosine analogues with a 3′-hydroxyl group may be favourable to confer specificity against hepadnavirus replication. However, it is possible that these carbocyclic analogues could induce toxicity by internal incorporation into newly synthesized mitochondrial and/or cellular DNA. While it is generally agreed that hepadnavirus polymerase requires conformational maturation by host factors before it binds to pre-genome RNA, the requirements and mechanisms of the maturation are not fully understood. Further studies to reveal the initial conformational states of hepadnavirus polymerase will lead to better HBV-specific drug candidates.

Novel drug candidates

Recently, some new HBV-specific drug candidates have been found. Phenylpropenamide derivatives, AT-61 and AT-130, were active against wild-type and lamivudine-resistant strains of HBV, but had no effect on the replication of other hepadnaviruses, including DHBV and woodchuck hepatitis virus (WHV). However, it seemed that their IC₅₀ₐ₈ against the wild-type HBV might not be satisfactory (21.2 ± 9.5 and 2.40 ± 0.92 μM, respectively), when compared with lamivudine (0.064 ± 0.020 μM). We have reported that MCC-478, a novel phosphonomethoxyethyl purine derivative, was more potent than lamivudine and had HBV-specific antiviral activity. There is clearly no structural similarity between MCC-478 and the carbocyclic guanosine analogues discussed above. Since MCC-478 was also effective against DHBV and WHV replications, its activity spectrum seems to be different from that of the phenylpropenamide derivatives, and the mechanisms of action of MCC-478 are clearly of interest. Studies that elucidate the mechanisms of action of novel anti-HBV compounds, together with the rapid progress of hepadnavirus virology, will provide the basis for the design of more effective antiviral therapies, which are required to treat the enormous number of patients with chronic HBV infections.

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References


