In Vitro Evaluation of Hepatitis B Virus Polymerase Mutations Associated With Famciclovir Resistance

XIAOFENG XIONG, HUILING YANG, CHRISTOPHER E. WESTLAND, RUIMING ZOU, AND CRAIG S. GIBBS

Several mutations (V521L, P525L, L528M, T532S, and V555I) in the gene for hepatitis B virus (HBV) polymerase have been identified in HBV isolated from patients that displayed break-through viremia during famciclovir treatment. To determine whether these mutations cause phenotypic resistance to famciclovir, we compared the inhibition constants (K_i) of penciclovir triphosphate (PCVTP, the active metabolite of famciclovir) for recombinant wild-type and mutant HBV polymerases containing these mutations. In *in vitro* enzymatic assays, the V555I mutation displayed the most resistance (with K_i increased by 6.2-fold) to PCVTP. The V521L and L528M mutations showed moderately decreased sensitivity to PCVTP (K_i increased by $>$3-fold). We also analyzed the cross-resistance profiles of these variants for adefovir and lamivudine, two other antiviral agents that also inhibit DNA replication by HBV polymerase. All 5 famciclovir-associated mutations were sensitive to adefovir diphosphate (ADVDP) in *in vitro* enzymatic assays ($<$2.3-fold decreased sensitivity). The V521L, L528M, and T532S mutations were also sensitive to lamivudine triphosphate (LAMTP); however, the P525L and V555I mutations displayed moderately decreased sensitivity to LAMTP in enzymatic assays (3.6-fold decreased sensitivity). The lamivudine-resistant mutations M552I, M552V, and L528M+M552V, which were previously shown to display 8- to 25-fold resistance to LAMTP, were less resistant ($\leq$3.1-fold) to PCVTP. (HEPATOLOGY 2000;31:219-224.)

Hepatitis B viral infection is a major cause of acute and chronic hepatitis, cirrhosis, and hepatocellular carcinoma, and it is one of the 10 most common causes of death worldwide.[1] Current treatment regimens rely primarily on interferon alfa therapy. However, interferon has limited efficacy, is expensive, requires parenteral administration, and is associated with frequent and unpleasant side effects. Nucleoside or nucleotide analogs, which inhibit DNA replication through hepatitis B virus (HBV) polymerase, represent a new class of promising anti-HBV therapeutics. In addition to lamivudine,[2-9] which was approved recently for the treatment of hepatitis B infection in the United States, several nucleoside and nucleotide analogs including famciclovir[5,10,11] and adefovir dipivoxil[12-14] are currently being evaluated in late stage clinical trials. These compounds have been shown to be well tolerated and can produce rapid and marked decreases in serum HBV-DNA levels.

Famciclovir is the oral prodrug of penciclovir, which has to be converted to penciclovir triphosphate by cellular enzymes to become an active inhibitor against HBV polymerase by competing with the natural substrate deoxyguanosine triphosphate (dGTP).[15] Famciclovir was originally approved for the treatment of herpes simplex virus and herpes zoster virus.[16,17] Later, famciclovir was shown to have activity against human and duck hepatitis B viruses in enzymatic and cell culture assays and in animal models of infection.[15,18-21] Clinical studies subsequently showed that famciclovir can reduce serum HBV levels in patients chronically infected with HBV.[5,10,11]

In analogous fashion to the treatment of human immunodeficiency virus (HIV) infection, HBV infection also requires prolonged administration of nucleoside analogs to eliminate the virus from patients' sera, and emergence of drug-resistant HBV during extended monotherapy appears to be a factor that may ultimately limit treatment efficacy. During extended lamivudine therapy, genotypic resistance has been observed in 14% to 39% of patients treated with lamivudine for 12 months.[22,23] The resistance is associated with mutations of methionine 552 to either isoleucine (M552I) or valine (M552V)[23-27] in conserved domain C (YMDD motif) of HBV polymerase.[28] The M552V and M552I mutations are most often observed in conjunction with an additional mutation, L528M, in conserved domain B.[27] The emergence of these mutations is associated with a rebound in serum HBV-DNA levels and clinical deterioration in transplant patients. These mutations have been directly associated with a lamivudine-resistant phenotype in several *in vitro* models of HBV replication.[27,29-32]

Several mutations, also in the subdomains B and C of HBV DNA polymerase, including V521L, P525L, L528M, T532S, and V555I, have been reported to arise in HBV patients during famciclovir treatment (Fig. 1).[33-38] These mutations were associated with an increase in serum HBV-DNA levels, suggesting that they may confer reduced sensitivity to famciclovir. In early reports, famciclovir-associated mutations were found most frequently in the conserved domain B of HBV polymerase. In subsequent reports, famciclovir-associated mutations were revealed to be scattered throughout HBV polymerase/reverse transcriptase domain, and multiple muta-

Abbreviations: HBV, hepatitis B virus; dGTP, deoxyguanosine triphosphate; HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen; dNTP, deoxynucleotide triphosphate; dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dTTP, deoxythymidine triphosphate; K_m, Michaelis constant; K_i, inhibition constant; PCVTP, penciclovir triphosphate; ADVDP, adefovir diphosphate; LAMTP, lamivudine triphosphate.

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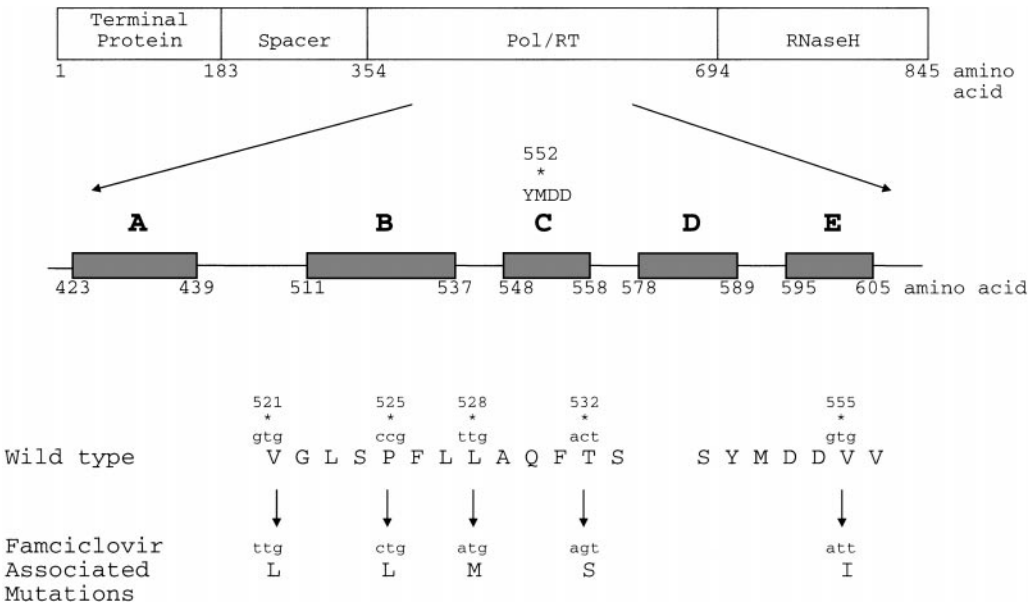

FIG. 1. Structure organization of HBV polymerase and mutations associated with famciclovir resistance.

tions were typically present in individual viral isolates.[39,40] Some of these mutations could be caused by the evolution of HBV during the natural history of the infection. HBV polymerase has a high spontaneous mutation rate of 2×10^{-4} base substitutions/site/year,[41] and individual mutations in the HBV polymerase gene could be fixed in the population through selection by the immune system mediated through the overlapping reading frame with the gene for HBV surface antigen (HBsAg) or through genetic drift caused by decimation of the virus population after antiviral therapy. Therefore, it is important to correlate HBV genotypic changes with phenotypic drug resistance to guide future clinical decisions on anti-HBV therapy.

The aim of this study was to evaluate the phenotypic resistance profiles of the famciclovir-resistance–associated mutations: V521L, P525L, L528M, T532S, and V555I with respect to famciclovir, lamivudine, and adefovir in *in vitro* enzymatic assays.

MATERIALS AND METHODS

Materials. Adefovir diphosphate was prepared by Sierra Bioresearch (Tucson, AR). Lamivudine triphosphate was obtained from Moravek (Brea, CA). Racemic (R/S)-penciclovir triphosphate was kindly provided by Dr. Klaus Esser (SmithKline Beecham, King of Prussia, PA). The baculovirus expression system was purchased from Clontech (Palo Alto, CA). Activated calf thymus DNA was from Pharmacia Biotechnology (Piscataway, NJ). RNasin was from Promega (Madison, WI).

Construction of HBV Polymerase Expression Vector. Human HBV polymerase (adw2 serotype, GenBank X02763) was cloned into a baculovirus transfer vector pBacPAK9 (Clontech) as described by Xiong et al.[30] In this construct (pSX20) a FLAG affinity tag (Met-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) was fused to the N-terminal of the polymerase to facilitate protein purification. The V521L, P525L, T532S, L528M, and V555I mutations were generated by polymerase chain reaction mutagenesis[42]; their codon sequences are shown in Fig. 1. Recombinant baculoviruses were obtained according to the instructions in Clontech's baculovirus expression system.

Immunoaffinity Purification of HBV Polymerase. Sf 21 cells from a 1-L cell culture were harvested by centrifugation 48 hours postinfection with recombinant baculoviruses expressing wild-type or mutant HBV polymerases. HBV polymerases were purified using an affinity column containing the anti-FLAG M2 monoclonal antibody as described.[43] Briefly, the baculovirus-infected Sf21 cell pellet was extracted with 20 mL of phosphate-buffered saline containing 10% glycerol, 0.5% NP-40, protease inhibitors (100 μmol/L leupetin, 1 mmol/L pefabloc, 10 μmol/L aprotinin, 1 μg/mL pepstatin, and 1 mmol/L ethylenediaminetetraacetic acid), 50 U/mL RNasin (Promega). The lysate was cleared by centrifugation at 15,000*g* for 10 minutes. The clarified extract was passed over a 2-mL M2-agarose (Sigma, St. Louis, MO) column twice, and the column was washed sequentially with TNG (0.1 mol/L Tris-HCl [pH 7.5], 30 mmol/L NaCl, 10% glycerol), TNG plus 1 mol/L NaCl, and again with TNG. HBV polymerase was eluted with 0.1 mol/L glycine (pH 3.0) plus 10% glycerol, collected in 1-mL fractions, and immediately neutralized with 67 μL of 0.8 mol/L Tris HCl, 3% Triton X-100, 80 mmol/L DTT, and 50 units of RNasin. The final HBV polymerases were 5% to 20% pure as judged by Coomassie Blue–stained protein gel. The concentration of HBV polymerase preparations were determined by quantitative Western blots using a commercially available Flag-tagged protein (Flag-BAP; Sigma) as the reference standard. The protein standards were electrophoresed along with HBV polymerase preparations of unknown concentration in a sodium dodecyl sulfate polyacrylamide gel (10%). The proteins were subsequently transferred onto a nitrocellulose membrane and probed with the M2 monoclonal antibody (Sigma) followed by [^{35}S]-labeled anti-mouse immunoglobulin G (IgG; Amersham, Piscataway, NJ) as the secondary antibody. After washing, the blot was scanned in a Storm 860 PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The concentration of HBV polymerase in each preparation was determined from a standard curve generated using the Flag-BAP standards. One liter of cell culture typically yielded 25 to 250 μg HBV polymerase.

Inhibition of HBV Polymerase. HBV polymerase activity was monitored by measurement of the incorporation of [α-^{32}P]-labeled deoxynucleotide triphosphate (dNTP) into acid-precipitatible products. Assays were performed in 40 μL of a solution containing 100 mmol/L Tris (pH 7.5), 10 mmol/L $MgCl_2$, 0.6 U/μL RNasin, 5% glycerol, 0.2 μg/μL activated calf thymus DNA, 100 μmol/L unlabeled dNTPs (*e.g.* deoxyadenosine triphosphate [dATP], deoxycytidine triphosphate [dCTP], and deoxythymidine triphosphate [dTTP]), various concentrations of a [α-^{32}P]-labeled dNTP (approximately 500 Ci/mmol) and various concentrations of inhibitors. For the determination of the inhibition constants for penciclovir triphosphate (a dGTP substrate analog), adefovir diphosphate (a dATP substrate analog), and lamivudine triphosphate (a dCTP substrate

analog), [α-^{32}P]-labeled dGTP, [α-^{32}P]-labeled dATP, and [α-^{32}P]-labeled dCTP were used, respectively. Five microliters of HBV polymerase (approximately 0.1 μg) was added to start the reaction. Twelve-microliter aliquots were taken at various time points between 0 to 20 minutes and transferred onto 3MM paper discs. The paper discs were washed 3 times in 5% trichloroacetic acid plus 1% sodium pyrophosphate and once in 95% ethanol. The incorporated radioactivity was measured in a Beckman scintillation counter.

Enzyme Kinetics. Kinetic constants were determined by fitting the initial rates to a Lineweaver-Burk plot based on the algorithms described by Cleland.[44]

RESULTS

A series of recombinant HBV polymerases were characterized enzymologically to evaluate mutations observed to arise in HBV from patients failing famciclovir therapy. In our enzymatic assay we determined that the Michaelis constant (K_m) for dGTP was 0.12 μmol/L for the wild-type HBV polymerase, within the range of K_m values for dGTP previously reported for HBV polymerase activity associated with recombinant (0.012 μmol/L)[45] or plasma-derived HBV core particles (0.09 μmol/L, 0.07 μmol/L, and 0.32 μmol/L).[15,31,46] The 25-fold range of variation between the values reported in the literature illustrates the difficulty in comparing the absolute values of kinetic constants between different studies in which different serotypes/genotypes of HBV and different assay conditions were used. In our study it is the relative difference in inhibition constant (K_i) values between the wild-type and mutant enzymes that is important; however, these could also be affected by the background genotype of the particular viral isolate selected, the assay conditions used, and the presence or absence of the HBV core protein.

Penciclovir triphosphate (PCVTP), the active metabolite of famciclovir, is an analog of the HBV polymerase substrate dGTP. Lineweaver-Burk plots indicated that PCVTP was a competitive inhibitor of HBV polymerase with respect to dGTP (Fig. 2). The K_i for PCVTP was calculated to be 4.8 μmol/L (Table 1), 40-fold higher than the K_m for dGTP (0.12 μmol/L) suggesting that PCVTP will be at a considerable competitive disadvantage with respect to the natural substrate dGTP *in vivo*. Comparison of the K_i values for adefovir diphosphate (ADVDP) (K_i = 0.1 μmol/L) and lamivudine triphosphate (LAMTP) (K_i = 0.25 μmol/L) with that of PCVTP showed that the potencies of these 3 compounds were in the order of ADVDP > LAMTP >> PCVTP in *in vitro* HBV polymerase assays (Table 1).

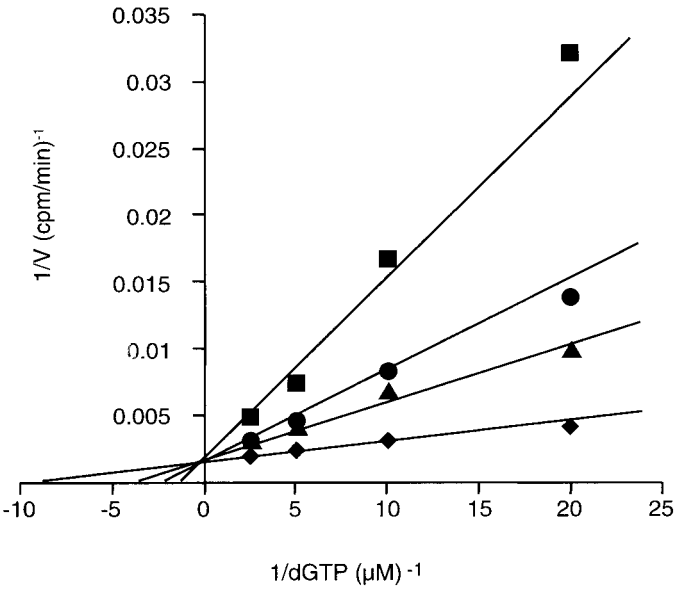

FIG. 2. Competitive inhibition of wild-type HBV polymerase by PCVTP. (■), 40 μmol/L PCVTP; (●), 20 μmol/L PCVTP; (▲), 10 μmol/L PCVTP; (◆), 0 μmol/L PCVTP.

TABLE 1. Inhibition of HBV Polymerases Expressing Famciclovir-Associated Mutations

	Famciclovir (PCVTP)		Adefovir (ADVDP)		Lamivudine (LAMTP)	
Enzyme	K_i (μmol/L)*	Fold Increase†	K_i (μmol/L)*	Fold Increase†	K_i (μmol/L)*	Fold Increase†
Wild type	4.8 ± 0.1	1	0.10 ± 0.01	1	0.25 ± 0.03	1
V521L	15.2 ± 1.8	3.2	0.15 ± 0.04	1.5	0.31 ± 0.06	1.2
P525L	11.4 ± 1.4	2.4	0.23 ± 0.06	2.3	0.89 ± 0.06	3.6
L528M	15.1 ± 3.4	3.1	0.23 ± 0.04	2.3	0.64 ± 0.04	2.6
T532S	6.3 ± 1.2	1.3	0.18 ± 0.01	1.8	0.54 ± 0.08	2.2
V555I	29.7 ± 5.8	6.2	0.19 ± 0.01	1.9	0.87 ± 0.13	3.6

NOTE. All inhibition constants (K_i) are the average of 3 separate experiments.

*K_i values are means ± SD.

†Fold increase = (mutant K_i)/(wild-type K_i).

In this study, the differences between K_i values determined for mutant and wild-type enzymes were used to evaluate the effect of specific HBV polymerase mutations on sensitivity to anti-HBV agents *in vitro*. For the purposes of discussion, the mutants were classified according to subjective criteria where a less than 3-fold increase in K_i was scored as sensitive, a greater than 5-fold increase in K_i was scored as resistant, and a 3- to 5-fold increase was scored as low-level resistance or moderately decreased sensitivity. As debated in the Discussion, the relationship between these *in vitro* resistance phenotypes and actual clinical resistance may vary for different agents depending on the therapeutic threshold or potency of the particular agent *in vivo*.

To determine whether the V521L, P525L, L528M, T532S, and V555I mutations cause phenotypic resistance to famciclovir, we compared the inhibition constants of PCVTP for wild-type and mutant HBV polymerases containing these mutations. In *in vitro* enzymatic assays, the V555I mutation caused the most resistance to PCVTP with the K_i increased by 6.2-fold (Table 1). The V521L and L528M mutations caused moderately decreased sensitivity to PCVTP (Table 1, K_i values increased by >3-fold). The P525L and T532S mutant polymerases remained sensitive to PCVTP with K_i values increased by less than 3-fold (Table 1).

We also studied the cross-resistance profiles of these mutations with respect to two other anti-HBV compounds, adefovir and lamivudine. Adefovir is an analog of deoxyadenosine monophosphate, which is converted *in vivo*, by the addition of two phosphate groups, to ADVDP, which is a competitive inhibitor of HBV polymerase with respect to the substrate dATP (K_i = 0.1 μmol/L). All 5 famciclovir-associated mutations remained sensitive to ADVDP in *in vitro* enzymatic assays as indicated by a less than 2.3-fold increase in the K_i (Table 1). Lamivudine is a nucleoside analog that requires the addition of three phosphate groups *in vivo* for conversion to the active form. LAMTP is a competitive inhibitor of HBV polymerase with respect to the substrate dCTP (K_i = 0.25 μmol/L). The V521L, L528M, and T532S

mutations were sensitive to LAMTP, whereas the V555I and P525L mutations displayed moderately decreased sensitivity to LAMTP in the enzymatic assays (3.6-fold decreased sensitivity, Table 1).

We previously reported that the lamivudine-resistant mutations M552I, M552V, L528M+M552I, and L528M+M552V mutant HBV polymerases displayed resistance to LAMTP with K_i values increased by 8- to 25-fold compared with that of wild-type HBV polymerase and that all these mutants remained sensitive to ADVDP.[30] In this study, we found that the M552I and L528M+M552V mutants remained sensitive to PCVTP with K_i values increased by 1.1- and 2.5-fold, respectively. However, the M552V mutant displayed moderately decreased sensitivity to PCVTP (3.1-fold) (Table 2).

DISCUSSION

Potency of anti-HBV nucleoside analogs is typically accessed at three different levels in preclinical studies: (1) inhibition of HBV polymerase in enzymatic assays, (2) inhibition of HBV replication in cell culture, and (3) inhibition of duck HBV or woodchuck hepatitis virus replication in animal models. We determined that PCVTP has a K_i value of 4.8 μmol/L, indicating that PCVTP is approximately 20- and 50-fold less potent than LAMTP (0.25 μmol/L) and ADVDP (0.1 μmol/L), respectively, in inhibiting HBV polymerase in *in vitro* enzymatic assays. Two different enantiomers of PCVTP could potentially be generated after the intracellular phosphorylation of penciclovir, and it is not known whether either or both enantiomers are present *in vivo*. Previous studies have shown that both the (R)- and (S)-enantiomers can inhibit HBV polymerase; however, the (R)-enantiomer was over 4-fold more potent than the (S)-enantiomer.[15,19] In our studies we used racemic PCVTP, which contains an equal proportion of both enantiomers.

Our estimate of 4.8 μmol/L for the K_i for inhibition of recombinant HBV polymerase by PCVTP is consistent with two previous reports in which the IC_{50} for the inhibition of duck HBV polymerase produced by *in vitro* translation was determined to be 20 μmol/L[19] and the IC_{50} for HBV polymerase in core particles isolated from transfected HepG2 cells was determined to be 5 μmol/L.[31] For competitive inhibition, when the concentration of the competing substrate (dGTP) is present at a concentration close to or below the K_m, the IC_{50} provides a reasonable approximation of the K_i as related by the equation $K_i = IC_{50}/(1 + [S]/K_m)$, in which [S] is the substrate concentration. However, in a third report, the K_i for racemic PCVTP versus HBV polymerase in core particles was reported to be at 0.04 μmol/L, 120-fold lower than our estimate.[15]

Similarly there are some discrepancies in the literature regarding the potency of PCV in inhibiting HBV replication in cell culture assays, where early reports showed PCVTP was a potent inhibitor of HBV replication with IC_{50} values of 0.2 μmol/L and 0.7 μmol/L.[18,20] However, several recent reports have indicated that penciclovir has high IC_{50} values in the range of 82 μmol/L to 100 μmol/L.[38,47,48]

The lower potency of penciclovir, compared with adefovir and lamivudine, for the inhibition of HBV polymerase and HBV replication *in vitro* is more consistent with the apparent lower efficacy of famciclovir compared with adefovir and lamivudine in recently published clinical studies. Patients treated with 30 mg adefovir daily for 12 weeks displayed ≥99.99% decreases in median serum HBV-DNA levels from baseline.[13,14,49] A recent open-label comparative study of lamivudine and famciclovir for the treatment of chronic HBV infection showed that patients treated with 100 mg lamivudine daily for 12 weeks demonstrated ≥99% decreases in median serum HBV-DNA levels from baseline, whereas patients treated with 500 mg famciclovir daily for 12 weeks had 68% to 90% decreases in median serum HBV-DNA levels from baseline.[5]

Of the 5 mutations evaluated in the present study, V555I was the most resistant mutation, showing a 6.2-fold decreased sensitivity to PCVTP. The V521L and L528M mutations only displayed 3.1-fold decreased sensitivity compared with 8- to 25-fold increases in K_i values for LAMTP for mutations associated with lamivudine resistance in the same assay.[30] However, the 3-fold decreased sensitivity could be enough to cause clinical resistance to famciclovir.[33] Because suppression of HBV replication by famciclovir in chronically infected patients appears to be suboptimal, famciclovir has a low threshold to become clinically ineffective. Any mutation that causes low-level resistance to famciclovir could result in clinical failure. However, mutations that cause a 3-fold decreased sensitivity to lamivudine and adefovir in our *in vitro* evaluation assays probably will not result in clinical failure because the enhanced efficacy of these two compounds increases the therapeutic threshold. A low-level of resistance (3- to 5-fold) may be sufficient escape suppression by famciclovir whereas a higher level of resistance is required to circumvent suppression by lamivudine. The frequency of resistance mutations may inversely relate to the level of resistance. This may explain why famciclovir-associated mutations are more diverse and scattered throughout the polymerase gene, whereas lamivudine-associated mutations are concentrated in the YMDD motif. The higher therapeutic threshold of lamivudine limits the number and type of mutations that are sufficient to allow the virus to escape suppression, whereas the lower potency of famciclovir permits more possibilities.

Another possible explanation for the lower levels of resistance to PCVTP observed for these mutants compared with lamivudine-resistant mutations may be that a combination of several of these mutations may be required to cause higher levels of resistance to PCVTP because multiple mutations have been observed in HBV isolates from single famciclovir-treated patients.[33-40] However, because these were aggregate sequences of polymerase chain reaction products it is unclear whether multiple mutations were present in individual viral clones. A third possible explanation is that these

TABLE 2. Inhibition of HBV Polymerases Expressing Lamivudine-Associated Mutations by PCVTP

	Famciclovir (PCVTP)	
Enzyme	K_i (μmol/L)*	Fold Increase†
Wild type	4.8 ± 0.1	1
M552I	5.5 ± 1.2	1.1
M552V	14.7 ± 3.8	3.1
L528M	15.1 ± 3.4	3.1
L528M/M552V	11.9 ± 1.7	2.5

NOTE. All inhibition constants (K_i) are the average of 3 separate experiments.

*K_i values are means ± SD.

†Fold increase = (mutant K_i)/(wild-type K_i).

mutations are natural HBV variants that have nothing to do with famciclovir resistance; however, all 5 of these mutations have been reported in famciclovir-treated patients on at least 2 separate occasions and none of them were present in a survey of 49 isolates of wild-type HBV taken from Genbank.

Our *in vitro* enzymatic assays indicated that the V555I mutation in HBV polymerase was clearly associated with a phenotypic resistance to famciclovir. This mutation has also recently been shown to be resistant to penciclovir in a cell culture model of HBV replication by Pichoud et al.[38] The valine to isoleucine mutation at position 555 may directly interfere with the binding of PCVTP to HBV polymerase or indirectly interfere by inducing a conformational change in the PCVTP binding site. The exact mode of PCVTP binding to HBV polymerase is unknown because of the lack of a crystal structure for the complex HBV polymerase. Sequence alignments of RNA-dependent DNA polymerases including HBV polymerase and HIV reverse transcriptase revealed 5 conserved domains A-E.[28] Based on the crystal structure of HIV reverse transcriptase, domains A, C, and D may participate directly in nucleotide triphosphate binding and catalysis. Domain B is involved in template binding and in the discrimination of the sugar ring of the nucleotide. Domain E is involved in the positioning of primer.[50] Valine 555 is located in the C-domain of HBV polymerase and therefore may be directly involved in the binding to PCVTP. Mutations in the C-domain of HBV polymerase have been found to be associated with strong phenotypic resistance to another nucleoside analog, whereas the lamivudine-resistant M552I and M552V mutations are also located in the conserved C-domain of HBV polymerase. The V555I mutation has been reported in both famciclovir- and lamivudine-treated HBV patients[26,38] and interestingly, 2 different sets of codons were used for the isoleucine mutation at 555. In the famciclovir-treated patient, adenosine-uridine-adenosine was used to encode isoleucine, which leads to a stop codon in the overlapping reading frame for HBsAg at amino acid 199, and this was shown to be defective in HBsAg production in cell culture.[38] Presumably, this variant is dependent on coinfection with a second virus producing normal HBsAg to produce infectious HBV particles. In the lamivudine-treated patient adenosine-uridine-cytidine was used to encode isoleucine, which causes a tryptophan to serine change in HBsAg at amino acid position 199.

As with all direct anti-HBV agents, prolonged administration of famciclovir is likely to be required to control chronic HBV infection. Unfortunately, famciclovir-resistant HBV has emerged during prolonged famciclovir therapy. Because of a favorable safety profile, famciclovir remains a potentially useful drug for the treatment of HBV infection. However, it may be best used in combination with other anti-HBV agents to prevent the emergence of resistant HBV. Our *in vitro* evaluation showed that both adefovir and lamivudine were active against famciclovir-resistant HBV polymerases, suggesting that in addition to their potential as first-line therapies for chronic HBV, they are also candidates for the treatment of HBV patients who have failed famciclovir therapy because of the emergence of resistance, or for coadministration with famciclovir to prevent the emergence of resistant virus.

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