Oral Ganciclovir for Treatment of Lamivudine-Resistant Hepatitis B Virus Infection: A Pilot Study

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Although liver disease seems to be stable in most patients who are infected with lamivudine-resistant mutant hepatitis B virus (HBV) in the short term, it may progress to more-advanced disease in some patients. In our pilot study, we investigated the efficacy of oral ganciclovir for the treatment of lamivudine-resistant HBV infection. Six patients infected with lamivudine-resistant HBV (3 patients had decompensated cirrhosis and 3 had chronic active hepatitis without cirrhosis) were included. Ganciclovir was administered at a dosage of 3 g daily for 6 months. Four of 6 patients completed the 6-month treatment period. Two patients with cirrhosis completed only 2 months of ganciclovir treatment because they died of cirrhosis complications. None of the patients had a >2-log10 reduction of HBV DNA and complete alanine aminotransferase normalization at the end of their treatment regimens. In conclusion, 6 months of ganciclovir treatment is not effective for suppression of lamivudine-resistant HBV infection.

Lamivudine has a major role in the treatment of chronic hepatitis B virus (HBV) infection. It inhibits the reverse transcriptase of HBV and effectively suppresses viral replication without any significant side effects. Viral kinetic and clinical studies have suggested that prolonged therapy is necessary for viral clearance [1–3]. Seroconversion of hepatitis B e antigen (HBeAg) to antibody against hepatitis B e (anti-HBe) can be achieved in 40% of patients at the third year of lamivudine treatment [4]. The major problem with prolonged therapy is the development of mutations in the catalytic domain of the HBV polymerase gene that confer resistance to lamivudine. A methionine-to-valine or -isoleucine change at codon 552 (M552V/I) of the conserved "tyrosine-methionine-aspartate-aspartate" (YMDD) motif and leucine-to-methionine change at codon 528 (L528M), which is located outside of YMDD motif, are common mutations that confer lamivudine resistance [5].

The risk of development of lamivudine-resistant mutations increases with time, at a frequency of 15%–25% within the first year to 67% by the fourth year of treatment [6]. The long-term outcome of mutant infection has not been well established. A relatively benign course, with lower alanine aminotransferase (ALT) levels and lower HBV DNA levels and comparable liver histologic findings, has been reported in short-term mutant infection, compared with prior wild-type YMDD infection [4, 6]. In addition, one-quarter of the patients with mutant infection have been shown to develop HBe seroconversion [4]. However, progressive and severe liver disease with hepatic decompensation has also been reported after the development of mutant infection in both immunocompetent [7, 8] and immunocompromised patients [9–12]. The treatment of these patients is of major importance. Some newer
antiviral agents, such as adefovir and entecavir, appear to be promising, but health care professionals are awaiting the results of the phase III clinical trials for their clinical use [13, 14].

Ganciclovir is an acyclic nucleoside analogue. Its parenteral [15–18] and oral [19] forms have been found to be effective in small series of both liver transplant recipients and immunocompetent patients with hepatitis B. In a recent case report, oral ganciclovir was shown to successfully suppress lamivudine-resistant mutant HBV infection in a patient [20], but this finding needs to be confirmed. The aim of our pilot study was to investigate the efficacy of oral ganciclovir for the treatment of lamivudine-resistant HBV infection.

PATIENTS AND METHODS

Patients. Six patients (5 men and 1 woman; mean age, 33 years) who developed a clinical breakthrough characterized by the reappearance of HBV DNA and ALT elevation while receiving lamivudine treatment (100 mg daily) were included in the study. Genotypic resistance was confirmed by sequencing a PCR-amplified HBV polymerase gene in 5 patients, whereas the remaining patient had a wild-type HBV polymerase sequence (table 1). Two patients (patients 2 and 4) had the M552I mutation, whereas 3 patients (patients 1, 3, and 5) had a co-occurrence of M552V and L528M mutations. The mean (± SD) interval from the commencement of lamivudine treatment to the development of lamivudine resistance was 20 ± 7 months (range, 12–30 months; table 1).

None of the patients were suitable candidates for interferon treatment because of the presence of advanced liver disease, previous interferon nonresponse, or the presence of a concomitant disease that precluded interferon treatment. Three patients (2 of whom were HBeAg positive and 1 of whom was HBeAg negative/anti-HBe positive) had decompensated cirrhosis with actively replicating virus at the time of entry to the study. Two of these patients (patients 1 and 3) already had decompensated liver cirrhosis when they began receiving lamivudine treatment, whereas the remaining patient (patient 2) had compensated cirrhosis when lamivudine treatment was started, but which became decompensated after the development of lamivudine resistance. The remaining 3 patients were HBeAg positive, with chronic active hepatitis but without cirrhosis. Two of these 3 patients previously had interferon nonresponse (patients 4 and 6), and 1 patient (patient 5) had psychosis that precluded interferon treatment. The laboratory parameters for the patients at baseline are shown in table 2.
Ganciclovir treatment (1 g t.i.d.) was commenced at a mean (± SD) of 12 ± 5 months (range, 5–18 months) after the development of lamivudine resistance and was administered for 6 months, with continued use of lamivudine (100 mg q.d.). Although, in a study elsewhere [19], no difference was found between 1.5-g and 3-g doses of ganciclovir with regard to the inhibition of HBV replication and suppression of disease activity, we preferred the 3-g dose, because mutant virus could have had a resistance to ganciclovir that might be overcome by higher doses. The primary response was defined as undetectable HBV DNA, as determined by use of a hybridization assay, or a ≥2-log_{10} reduction of HBV DNA, as determined by use of a real-time PCR assay, as well as complete normalization of ALT levels at the end of the treatment regimen. Patients were observed at monthly intervals, and ALT and HBV DNA levels (determined using a hybridization assay) were measured at each visit. Informed consent was obtained from each patient, and the study was approved by the local ethics committee.

Serologic testing for the detection of hepatitis. Hepatitis B surface antigen (HBsAg), antibody against HBsAg (anti-HBs), HBeAg, antibody against HBe, antibody against hepatitis C virus, and antibody against HIV were determined by use of a microparticle EIA, and antibody against hepatitis D virus was determined by use of EIA (Abbott Laboratories).

Hybridization assay for the detection of HBV DNA. HBV DNA levels were determined by use of a liquid hybridization assay (Digene). The lower and upper detection limits of the assay were 5 pg/mL and 2000 pg/mL, respectively.

Real-time PCR quantification for the detection of HBV DNA. Real-time PCR–based quantification of HBV DNA was performed with serum samples obtained at baseline, at the third month, and at the end of treatment. HBV DNA was extracted from the serum samples of patients by using a spin column kit (Nucleospin blood kit; Macherey-Nagel), in accordance with the manufacturer’s instructions. PCR amplification was performed by use of a primer and dual-labeled probe set deduced from a conserved HBV DNA polymerase region that generated a product of 81 bp. PCR primers and probes were designed by using Primer Express software (Perkin Elmer) and were purchased from Oswel. The primers used for PCR amplification and the Taqman probe were 5′-GTT CAG TGG TTC GTA GGG CTT T-3′ (nucleotides 694–715), 5′-TGT ACA GAC TTG GCC CCC A-3′ (nucleotides 775–757), and 5′-FAM-CAC TGT TTG GCT TTC AGT ATG GAT GAT GTG GTA-TAMRA-3′ (nucleotides 720–755). Amplification was performed by using the Taqman PCR Core Reagent kit (Perkin Elmer) in a 50-µL reaction mixture that contained 50 mM KCl, 10 mM of Tris-HCl (pH, 8.3), 5 mM of magnesium chloride, 200 µM of dATP, 200 µM of dCTP, 200 µM of dGTP, 400 µM of dUTP, 200 nM of forward primer, 200 nM of reverse primer, 100 nM of Taqman probe, 0.5 U of AmpErase UNG, 1.25 µM of AmpliTaq Gold DNA polymerase, and 5 µL of template DNA. Detection and amplification were performed by use of an ABI prism 7000 sequence-detection system (Perkin Elmer). After initial incubation for 2 min at 50°C, which allows uracil N′-glycosylase to inactivate the contaminating ampiclon, incubation for 10 min at 95°C allowed AmpliTaq Gold polymerase to be activated and to inactivate the uracil N′-glycosylase. The PCR cycling program consisted of 40 cycles of 15 s at 95°C and 60 s at 60°C. The external standards were prepared from 10^6 to 10^10 copies/mL by using 10-fold dilutions of a serum sample in which the virus load was adjusted according to reference sera (Accrometrix). The sensitivity of the assay was 5 × 10^2 copies/mL.

Amplification and direct sequencing of the polymerase

Table 3. Hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) levels before commencement of lamivudine treatment, at the time of breakthrough, and before and after ganciclovir treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Beginning of LAM regimen</th>
<th>At time of breakthrough</th>
<th>Beginning of GAN regimen</th>
<th>End of GAN regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT a (IU/L)</td>
<td>HBV DNA c (pg/mL)</td>
<td>ALT a (IU/L)</td>
<td>HBV DNA c (pg/mL)</td>
</tr>
<tr>
<td>1</td>
<td>108</td>
<td>&lt;5</td>
<td>180</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>&lt;5</td>
<td>122</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>&lt;5</td>
<td>67</td>
<td>139</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>&gt;2000</td>
<td>36</td>
<td>9</td>
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<tr>
<td>5</td>
<td>60</td>
<td>&gt;2000</td>
<td>298</td>
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<tr>
<td>6</td>
<td>85</td>
<td>&gt;2000</td>
<td>43</td>
<td>166</td>
</tr>
</tbody>
</table>

NOTE. GAN, ganciclovir; LAM, lamivudine.

aALT and HBV DNA levels at the time of breakthrough, the beginning of the GAN regimen, and the end of the GAN regimen were not statistically different.

bNormal range, 0–37 IU/L.
cLower and upper detection limits, 5 pg/mL and 2000 pg/mL, respectively.
gene. After extraction of HBV DNA by proteinase K digestion, PCR amplification and direct sequencing of the polymerase gene were performed as described elsewhere [21].

Statistical analyses. The Wilcoxon rank sum test was used for comparisons.

RESULTS

Four of the 6 patients completed the 6-month treatment period, whereas the remaining 2 patients with cirrhosis (patients 1 and 2) completed only 2 months of ganciclovir treatment because they died of progressive liver failure (table 1). At baseline, both patients had very high bilirubin levels (24 mg/dL and 8 mg/dL, respectively) and prolonged prothrombin times (41 s and 28 s, respectively; table 2). Neither patient had hepatitis flare, which might be related to ganciclovir toxicity during the 2 months of ganciclovir treatment. There was no clinically significant side effect that necessitated dose reduction for or withdrawal of the drug from the other 4 patients. No change in blood chemistry values, whole blood cell counts, or urinalysis findings attributed to ganciclovir treatment were recorded.

None of the 6 patients lost HBV DNA, as determined by use of a hybridization assay (table 3), and none had a ≥2-log₁₀ reduction in the HBV DNA level at the end of treatment (figure 1). However, 3 subjects (patients 3, 4, and 6) had a 1-log₁₀ decrease in their HBV DNA levels (figure 1). HBV polymerase sequences of these patients revealed M552V/L528M, M552I, and wild-type 552/528 sequences, respectively. We did not observe complete normalization of the ALT level or a >50% reduction in the ALT level in any of the 6 patients (table 3). No ALT flares were recorded in any patient, and none of the 5 HBeAg-positive subjects had seroconversion while receiving treatment. HBV DNA (detected by use of a hybridization assay) and ALT levels remained high after the cessation of ganciclovir therapy in the 4 patients who completed the 6 months of treatment.

DISCUSSION

It has been reported elsewhere that 8 weeks of oral ganciclovir treatment is effective in inhibiting HBV replication and disease activity in patients with chronic HBV infection [19]. Apart from a study of a 6-month combination of oral ganciclovir and interferon treatment [22], no study has addressed the efficacy of longer-duration oral ganciclovir monotherapy in a series of immunocompetent patients with chronic hepatitis B. To our knowledge, our study is the first to involve administration of oral ganciclovir monotherapy for 6 months to patients with chronic HBV infection. Successful treatment of a patient with lamivudine-resistant mutant HBV infection with oral ganciclov-

![Figure 1](https://academic.oup.com/cid/article-abstract/35/8/960/330586)
The results of our study suggest that 6 months of ganciclovir treatment has no effect on lamivudine-resistant HBV infection. No patients showed a ≥2 log₁₀ decrease in HBV DNA levels during or after treatment. In a previous pilot study that involved 15 patients infected with wild-type HBV who were treated for 8 weeks with oral ganciclovir, baseline HBV DNA levels decreased by 99%, or 2 log₁₀. Four of these 15 patients lost their HBV DNA, as detected by use of a monitor assay with a detection limit of 10¹ copies/mL [19]. In contrast, we did not observe such a remarkable decrease in HBV DNA levels during the first 3 months of the treatment, which suggests that lamivudine-resistant mutant viruses are not as susceptible as wild-type virus to ganciclovir. However, the relative resistance of mutant viruses to ganciclovir can be confirmed only by in vitro studies. We also failed to demonstrate a significant inhibition of HBV replication at the end of the 6-month treatment period, which suggests that ganciclovir is unlikely to have a role in the treatment of lamivudine-resistant mutant HBV infection. However, one-half of our patients had advanced liver disease, and this drug may have more effect on less severe hepatic disease. The finding of successful treatment of a patient who had lamivudine-resistant mutant infection with oral ganciclovir is in contrast to our results [20]. All 9 individual clones had an M552I substitution in this patient. Of interest, 4 clones also had a glycine-to-arginine change at codon 145 or a glycine-to-asparagine change at codon 130 in the major antigenic “a” epitope of the corresponding surface sequence. The role of such mutations in response to nucleoside analogue treatment is currently unknown. However, it seems unlikely that these changes would sensitize the virus to ganciclovir.

Some patients showed a reduction in their serum HBV DNA levels, but only to a limited extent (<2 log₁₀). Because we directly sequenced the PCR-amplified products, it is not possible to deduce whether the small reductions of HBV DNA levels represent the inhibition of mutant HBV replication by ganciclovir in these patients. Cloning and sequencing of individual clones before and after treatment can demonstrate relative inhibitions of wild-type and mutant viruses in response to ganciclovir treatment. In addition, it is unlikely that the type of mutation determines the relative susceptibility to ganciclovir, because 3 patients who had a reduction in HBV DNA levels had completely different HBV sequences at positions 552 and 528. One patient had wild-type sequences at positions 528 and 552, despite the development of lamivudine resistance. After the alignment of a sequenced polymerase region spanning codons 455–559, no sequence difference between patients’ HBV sequence and subtype ayw HBV sequence, the most prevalent HBV subtype in Turkey [23], was observed. The HBV DNA polymerase sequence in the serum sample obtained 11 months after the development of clinical breakthrough was also the same. Mutations observed elsewhere that conferred resistance to lamivudine in the other parts of the polymerase region [24] have yet to be determined.

The natural course of YMDD mutant infection is not well established, but progressive liver disease after the development of YMDD mutant infection has been reported [7–12]. This pattern can be best exemplified by the demonstration of progression from compensated cirrhosis to decompensated cirrhosis, as seen in patient 2 in the present study. This patient had compensated cirrhosis in his first presentation, and his liver disease was stable until the development of lamivudine resistance. He developed decompensated cirrhosis after the development of YMDD mutant infection and died after a relatively short period of time. In contrast to this type of unfavorable outcome, some patients may have a better outcome, even at an advanced stage of the disease. Patient 3 had uncompensated cirrhosis before he began to receive lamivudine treatment. Lamivudine resistance occurred 2.5 years after the initiation of lamivudine treatment. His liver disease remained stable for >2 years, and he survived for >3 years after the development of the mutation. The reason for these contrasting patterns of disease course cannot be explained simply by the pathogenicity of the dominant virus population at a given time point. Predictors of a worse outcome of YMDD mutant infection should be documented in further studies with larger numbers of patients. It is also necessary to conduct controlled studies with more-effective drugs to understand which patients will benefit from treatment. However, because the natural history of infection with a lamivudine-resistant hepatitis B mutant virus is currently unknown, it is difficult to ascertain the efficacy of experimental antiviral therapies.

In conclusion, 6 months of ganciclovir treatment is not effective for the inhibition of lamivudine-resistant HBV replication. It does not suppress disease activity and does not change the clinical course of YMDD mutant HBV infection. Thus, ganciclovir shows no promise for the treatment of lamivudine-resistant hepatitis B infection.

References
5. Doo E, Liang TJ. Molecular anatomy and pathophysiologic implica-