CASE REPORT

Dynamics of HBV DNA Levels, HBV Mutations and Biochemical Parameters during Antiviral Therapy in a Patient with HBeAg-Negative Chronic Hepatitis B

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SUMMARY   Chronic hepatitis B virus (HBV) infection leads to long-term sequelae such as cirrhosis and hepatocellular carcinoma. Antiviral therapy aims at controlling the viral replication and thus, decreasing the likelihood of such complications. In this study, we evaluated the dynamics of biochemical and virological parameters over 10 years of antiviral therapy in a Thai patient with chronic HBeAg-negative HBV infection, who had relapsed after two courses of interferon alfa treatment. Lamivudine administration initially led to a significant reduction in alanine aminotransferase (ALT) and HBV DNA levels, but a subsequent emergence of YIDD mutants caused an ALT flare and a virus breakthrough. A 4-log HBV DNA decrease and normalization of the ALT level were achieved within 3 months of adefovir monotherapy without any relapse during follow-up exceeding 20 months. Thus, careful monitoring during treatment and knowledge of cross-resistance to antiviral salvage therapy are crucial for the management of patients with chronic hepatitis B.

Treatment of chronic hepatitis B by antiviral therapy is aimed at driving viral replication to the lowest possible level, and thereby halting the progression of liver disease and preventing the onset of complications. The currently approved agents for treatment of chronic hepatitis B are standard or pegylated interferon alfa and nucleos(t)ide analogues such as lamivudine, adefovir and entecavir.[1] Compared with interferon alfa, the nucleos(t)ide analogues, which inhibit viral replication by targeting the HBV reverse transcriptase (RT) activity, have excellent safety and tolerability profiles that allow more patients to be treated for prolonged periods of time. These oral agents consistently result in improved liver histology, including reduced fibrosis, enhanced hepatitis B e antigen (HBeAg) seroconversion, normalization of alanine aminotransferase (ALT) levels, and clinical improvement in patients with advanced liver disease.[2] These agents, however, are also associated with the emergence of point mutations in the HBV

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polymerase gene (genotypic resistance) which can restore partial replication fitness to the mutant strains of HBV and lead to the redetection of previously suppressed HBV DNA and to a biochemical relapse (phenotypic resistance).[3]

Prolonged treatment with lamivudine results in the emergence of resistant viruses in 24% of patients following 1 year of therapy and 70% of patients following 4 years of therapy.[4] Mutations conferring resistance to lamivudine have been mapped in the conserved tyrosine, methionine, aspartate, aspartate (YMDD) motif within the C domain of the viral RT (rtM204I/V).[5,6] They are frequently associated with compensatory mutations in the conserved B domain (rtV173L, rtL180M) that partially restore the replicative capacity of YMDD mutant strains in vitro, and are associated with a 1000-fold reduction in the susceptibility to the drug.[6] Switch or add-on of other antiviral drugs such as adefovir is adequate to suppress lamivudine-resistant HBV and improves liver function in patients with chronic HBV infection.[6] Unlike lamivudine therapy, adefovir therapy is associated with delayed and infrequent selection of drug resistant viruses.[4]

In this report, we describe a patient who failed interferon alfa therapy and subsequently failed lamivudine therapy. The patient was successfully treated with adefovir. The aim of the study was to retrospectively investigate the dynamics of biochemical and virological parameters throughout the oral antiviral therapies administered to this patient.

CASE REPORT

The patient was a 24-year-old Thai man who had been diagnosed with HBeAg-negative chronic hepatitis B without hepatitis C virus (HCV) or human immunodeficiency virus (HIV) co-infections. In 1996, at the age of 13 years, the first diagnosis of chronic hepatitis B was documented because he developed jaundice and had persistently elevated ALT levels. Liver biopsy was not performed because of the patient's refusal. In October 1997 and November 1998, two 6-month courses of interferon-alfa (5 and 3 million units three times per week for the first and second course, respectively) were administered, with a relapse after an initial response. In January 2001, the patient was started on

lamivudine (100 mg per day). In June 2005, after 52 months of uninterrupted lamivudine therapy, the treatment was switched to adefovir (10 mg/day) because of biochemical and virological relapse. Adefovir was then maintained for more than 20 months of follow-up (February, 2007) with a good response.

A total of 52 consecutive serum samples were obtained at various time points before and during oral antiviral therapies to monitor the biochemical and virological response, as well as HBV DNA dynamics. Serum samples were stored at -70°C and retrospectively examined as to genome sequence, amino acid substitutions at the YMDD position of DNA polymerase and HBV DNA viral load.

Prior to the onset of treatment and on each sample taken during anti-viral therapy, the serum ALT level, hepatitis B surface antigen (HBsAg), and hepatitis B e antigen (HBeAg) were determined by automated ELISA, in the routine laboratory, King Chulalongkorn Memorial Hospital, Bangkok, Thailand.

A 100-µl serum sample was incubated with proteinase K in lysis buffer. Following phenol-chloroform - isoamyl alcohol extraction and ethanol precipitation, the resulting DNA pellet was suspended in 30 µl of sterile water and stored at -20°C. HBV DNA levels and YMDD mutants were detected by real-time PCR, as previously described.[7,5] For YMDD detection in samples with a low viral load (< 100 copies/µl), these samples were first amplified using sense and anti-sense primers (primer sequences on request).

The dynamics of biochemical and virological parameters, including HBV DNA levels, are summarized in Fig. 1. Interferon therapy evoked an initial response, which was followed by a relapse once treatment was discontinued. Lamivudine administration induced a sharp decline in HBV DNA with a significant decrease in ALT levels. Yet, after 14 months of stably reduced HBV DNA viral loads, the evolution of a YIDD mutation led to an ALT flare and virological breakthrough characterized by a significant increase in HBV DNA levels exceeding 1.0 log above the nadir. A reduction of HBV DNA
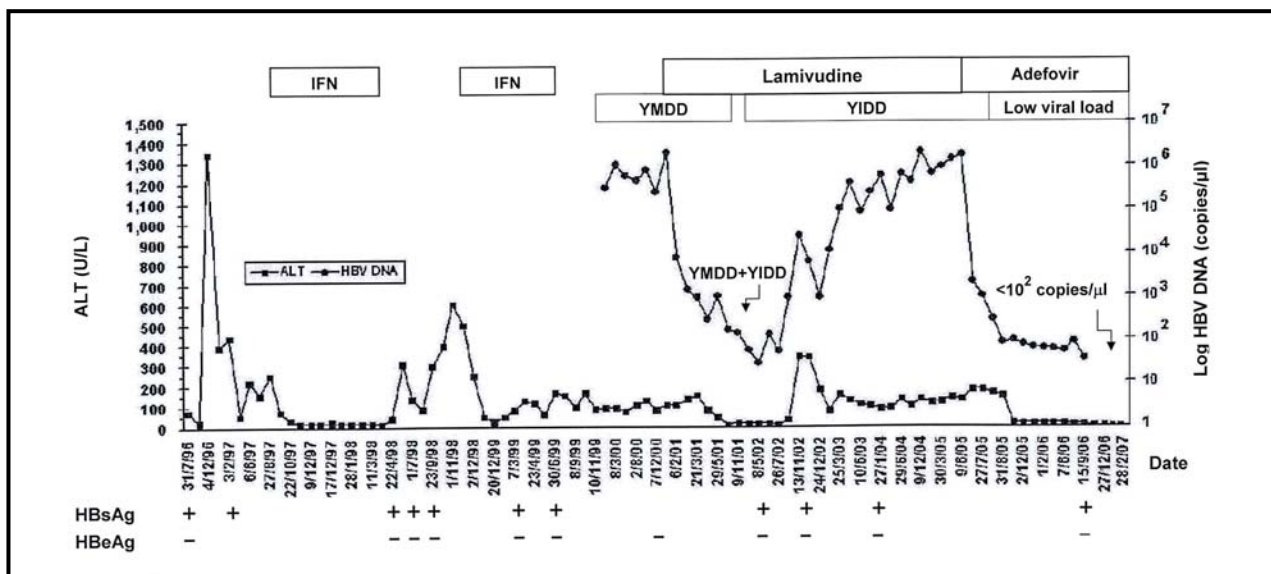
Fig. 1 Association between HBV DNA concentrations, HBV mutants and alanine aminotransferase (ALT) levels during and after anti-viral drug treatment

Table 1 HBV mutations during the course of treatment

Date (accession number)	Position of mutations									N.B.
	Polymerase gene					Core promoter		Precore		
	Adefovir resistance		Lamivudine resistance	Other mutation				Start codon	Stop codon	
	rtA181V/T	rtN236T	rtM204I/V	rtL82M	rtV84E	A1762T	G1764A	ATG	G1896A	
7/12/2000 (EF384200, EF384201)	A	N	M	L	V	T	A	ATG	G	2 years after IFN treatment
13/11/2002 (EF384206, EF394328)	A	N	I	M	E	T	A	ATG	G	2 years after Lamivudine treatment
9/2/2005 (EF384202, EF384203)	A	N	I	M	E	T	A	ATG	G	4 years after Lamivudine treatment
27/7/2005 (EF384204, EF384205)	A	N	I	M	E	T	A	ATG	G	1 month after Adefovir treatment

by 4.0 log IU/ml and ALT normalization was achieved within 3 months of adefovir monotherapy and repeatedly confirmed during the 20- month follow-up period.

To pinpoint the change in the YMDD motif, we first amplified the samples with low viral load by conventional PCR and subsequently detected the YIDD substitution (Fig. 1). We found the typical variant in the virus genome at about 9 months after the onset of lamivudine therapy and later. However, the YMDD substitution was not detectable in samples after treatment with adefovir as conventional PCR failed to amplify those samples due to their very low viral load.

Real-time PCR products representing four specific time points in the course of the antiviral therapy were subjected to nucleotide sequencing. The time points selected comprised 2 months before starting lamivudine therapy (7/12/2000), 2 years of lamivudine (13/11/2002), 4 years of lamivudine (9/2/2005) and 1 month of adefovir (27/7/2005) treatment (Fig. 1). This selection was based on potential resistance to the respective drug as a consequence of mutations in the polymerase gene motif, the core promoter and the precore gene. The details of the sequence method have been reported elsewhere.[5]

Direct sequencing showed the patient to be infected with HBV genotype C with core promoter mutations at positions 1762 (A to T) and 1764 (G to A), point mutations that may cause the absence of HBeAg. However, the precore region did not show any significant nucleotide change. Moreover, we found a mutation of the PreS2 start codon (G to A). Direct sequencing of the HBV genome also showed a G to A substitution at position 741 of the YMDD motif after 9 months of Lamivudine treatment resulting in an amino acid change from M to I (YIDD). The study conducted on this patient confirmed the real-time PCR result and the emergence of YMDD mutant viruses during long-term lamivudine treatment (Table 1). In addition to the above point mutation, we found C to A substitutions at positions 373 (rtL82M) and 400 (rtV84E).

As for adefovir resistance, we could not establish any adefovir-resistant mutation (rtA181V/T or rtN236T) in samples taken after starting adefovir treatment (data not shown) because of an undetectable viral load. All the nucleotide sequences in this study were submitted to Genbank under accession numbers EF384200-EF384206 and EF394328.

DISCUSSION

In this report, we have described the case of a patient chronically infected with HBV genotype C and serologically negative for HBeAg. The patient initially responded to standard interferon alfa therapy, but relapsed after cessation of treatment. Subsequent lamivudine therapy was also insufficient in controlling HBV replication as evidenced by a classical course of initial virological response followed by a viral breakthrough. After the emergence of lamivudine resistance, the patient was successfully treated with adefovir monotherapy.

Long-term administration of lamivudine results in the emergence of drug-resistant strains of HBV through the selection of mutants in the YMDD motif of the HBV polymerase. This results from a spontaneous error rate of the viral polymerase and thus, the accumulation of viral genome mutations during the natural history of infection. Consequently, the viral quasispecies may undergo significant changes under the selective pressure of antiviral therapy with subsequent selection of escape mutants.[3] Resistance should be suspected in patients with virological breakthrough accompanied by an increase in serum ALT levels, despite continuation of therapy. Therefore, as our data suggest, in the course of 14 months, HBV encoding the YIDD mutation had acquired superior replication fitness to the wild-type virus and the patient eventually progressed to virological resistance with ALT elevations. Treatment options for patients with lamivudine-resistant HBV have been limited to continuation or cessation of lamivudine therapy. Continuation of lamivudine therapy in patients with lamivudine-resistant HBV has been associated with deteriorating liver histology in some patients.[8] Cessation of therapy results in re-emergence of wild-type HBV, and patients remain at risk of developing progressive liver disease. Furthermore, re-introduction of lamivudine therapy leads to a more rapid reappearance of the mutant virus than in treatment-naive patients.[9]

Several studies have demonstrated the efficacy of adefovir in the treatment of lamivudine-resistant HBV. In one randomized study conducted on 59 patients with compensated lamivudine-resistant HBV infection, adefovir alone was as effective as adefovir/lamivudine combination therapy in suppressing HBV DNA and improving serum ALT after 1 year.[10] Another trial performed on 135 patients including 30% with decompensated disease showed that after 12 months on average, adefovir combined with lamivudine was superior to lamivudine alone in patients with lamivudine-resistant HBV.[11] Another study executed on 128 pre- and 196 post-liver transplantation patients with

lamivudine-resistant HBV demonstrated that adefovir salvage therapy improved Child-Pugh scores in more than 90% of patients in both groups after a median treatment of 19 and 56 months, respectively. Upon further follow-up, 52% of the 226 patients awaiting transplantation were removed from the waiting list because of improvement in liver function with long-term adefovir therapy. [12] In this study, the switch to adefovir accomplished rapid rescue from lamivudine resistance and thus confirms previously published data.

Whereas prolonged adefovir therapy is not associated with YMDD mutants, unique mutations in the RT region have recently been described. Adefovir resistance is associated with the selection of the rtN236T mutation within the D domain of the viral enzyme or with an rtA181V amino acid change in the B domain of the RT. The rtN236T mutation induces a 3- to 6-fold in vitro reduction in the susceptibility to adefovir compared to wild-type HBV, but retains some level of susceptibility to lamivudine.[13,14] More recent studies have also demonstrated that adefovir monotherapy continuously administered for one year may be associated with a high rate of adefovir resistance in patients with preexisting lamivudine-resistant HBV. For example, 18% of Korean patients with lamivudine-resistant HBV infection developed adefovir resistance after 1 year.[15] In another study carried out on HBeAg-negative lamivudine-resistant Italian patients, adefovir was added to lamivudine either at the time of phenotypic or genotypic resistance.[16] After 2 years of continuous combination therapy, no adefovir resistance was detected in either group. Yet, patients in whom adefovir was initiated at the time of genotypic resistance experienced more rapid viral suppression and ALT normalization than those in whom treatment was delayed. These studies suggest that adefovir should be added to lamivudine rather than substituted for it and that therapy should be started as soon as genotypic resistance to lamivudine is detected.[16] In addition, mutant strains of HBV resistant to both lamivudine and adefovir have been reported. A recent *in vitro* study performed on a viral construct containing both lamivudine- and adefovir-resistant point mutations (ie, rtL180M plus rtM204V plus rtN236T) demonstrated resistance to both

lamivudine and adefovir but persistent susceptibility to entecavir, tenofovir, and interferon alfa.[14]

In conclusion, based on the results of this study as well as other reports, the switch to adefovir accomplished rescue from lamivudine-resistant HBV. However, drug resistance is eventually an expected consequence of long-term exposure to any oral antiviral agent because of the high rate of spontaneous HBV mutations and persistence of the cccDNA. Hence, careful monitoring during therapy and knowledge of cross-resistance to antiviral salvage therapy are essential to optimize the clinical outcomes of patients with chronic hepatitis B.

ACKNOWLEDGEMENTS

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