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A case of entecavir resistance which is developed after complete viral suppression during entecavir treatment for nucleoside-naive chronic hepatitis B

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ABSTRACT

Entecavir (ETV) is a potent nucleoside analogue against hepatitis B virus (HBV), and the emergence of drug resistance is rare in nucleoside-naive patients because development of ETV resistance (ETVr) requires at least three amino acid substitutions in HBV reverse transcriptase. We observed a case of genotypic ETVr with viral and biochemical breakthrough during ETV treatment of nucleoside-naive patients with chronic hepatitis B (CHB). A 57-years-old HBeAg-positive man received ETV 0.5 mg/day for 145 weeks. HBV DNA was 7.7 log10 copies/ ml at baseline, decreased to below 2 at week 48, declined to a nadir of 0 (negative) at week 72, and rebounded to 2.2 log10 copies/ ml at week 90 and remained this level until 109 weeks and increased to 6.8 log10 copies/ ml at week 145. Alanine aminotransferase (ALT) level increased to 440 IU/ L at week 145. The ETVr-related substitution (rtS202P) and lamivudine resistance-related substitutions (rtL180M + rtM204V) were detected by DNA sequencing analysis at week 145. The patient discontinued ETV therapy at week 145, and then received 245 mg of tenofovir disoproxil fumarate (TDF). Afterwards, HBV DNA level dropped to below 2.6 log10 copies/ml and ALT level was normalized after 19 weeks of TDF dosing. The three substitutions associated with ETV and lamivudine resistance developed after complete viral suppression in a nucleoside-naive CHB patient during ETV treatment. In spite of the extremely rare chance of viral mutation during ETV treatment, nucleoside-naive patients should be carefully monitored for resistance even if complete suppression is present.

Keywords: Entecavir, chronic hepatitis b, drug resistance, nucleoside-naive

Case Report

INTRODUCTION

Approximately 400 million people worldwide have chronic hepatitis B virus (HBV) infections, with a risk for chronic, life-threatening liver disease (1). Antiviral therapy for HBV can provide suppression of viral replication and halt disease progression (2,3). However, therapeutic benefits are diminished with the emergence of drug-resistant virus, which occurs most often with prolonged therapy and incomplete viral suppression (4).

Resistance to nucleoside/nucleotide antivirals arises through substitutions in the HBV polymerase (*pol*) reverse transcriptase domain (RT) that arise spontaneously through low-fidelity replication and are enriched through drug-selective pressure (2,3). Antiviral therapies are characterized by their barrier to resistance, which includes three components: (1) the potency of the antiviral in suppressing viral replication, (2) a "genetic barrier", i.e., the number of genetic changes required to effectively reduce drug susceptibility that results in virologic breakthrough, and (3) the replication fitness of the resistant virus. For lamivudine (LVD), adefovir-dipivoxil (ADV), and telbivudine (LdT), the genetic barrier to resistance in antiviral-naive patients can be as low as a single substitution. So, developments of resistance of these drugs are higher.

Several factors contribute to the high barrier to resistance with entecavir (ETV). ETV is potent, resulting in a higher proportion of patients achieving undetectable HBV DNA than those treated with LVD (5,6) or ADV (7). Marked reductions in ETV susceptibility require substitutions at residues rtT184, rtS202, or rtM250 in LVD resistance (LVDr) HBV with changes at rtM204I/V ± rtL180M

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(8,9). Thus, the *genetic* barrier to ETV resistance (ETVr) involves multiple substitutions. In-vitro studies demonstrated that the highest levels of phenotypic resistance, leading to virologic breakthrough, require both the rtM204V and rtL180M LVDr substitutions with at least one ETVr substitution (8,10). Additionally, ETVr HBV exhibits impaired replication fitness (9). Thus, the finding that ETVr has been rarely observed in nucleoside-naive patients (11,12) is likely due to a combination of a high genetic barrier, potent viral suppression, and reduced fitness of resistant viruses.

So, it has been reported that the development of ETVr in nucleoside-naive patients is very rare, even after 5 years of therapy (13). Recently, however, rare cases of ETVr, which developed in nucleoside-naive patients, have been reported (14-16). We also observed one patient who developed ETVr-associated HBV RT substitutions, followed by virologic and biochemical breakthrough after complete viral suppression in longterm ETV treatment of nucleoside-naive CHB patients. In this study, we report this case in detail.

CASE REPORT

A 57-years-old Turkish man with CHB received a checkup in April 2009, and was found to be seropositive for hepatitis B surface antigen (HBsAg) with normal liver enzymes. He has a history of asthma and use of inhaling corticosteroids for 9 years. He didn't drink and smoke. Hepatitis B e antigen (HBeAg) was positive and serum HBV DNA was 7.7 $\log_{10}$ copies/mL (Cobas AmpliPrep-Taqman HBV Test, Roche Diagnostics, Mannheim, Germany). Other baseline characteristics are shown in Table 1.

He was diagnosed with CHB by percutaneous liver biopsy (mild activity [7] and severe fibrosis [F3], according to Knodell score) at another hospital in May 2009. At this time, treatment with ETV was started at 0.5 mg/day. After the start of ETV, the serum HBV DNA level decreased to below 2 $\log_{10}$ copies/ml at week 48 and declined to a nadir of 0 copies/ ml at week 72 of ETV treatment. Thereafter, HBV DNA level rebounded to 2.2 $\log_{10}$ copies/ml at week 90 and remained this level until 109 weeks and increased to 6.8 $\log_{10}$ copies/ ml at week 145. ALT levels increased from 19 IU/L at week 122 to 440 IU/L at week 145. The patient discontinued ETV therapy at week 145, and then received 245 mg of tenofovir disoproxil fumarate (TDF). Afterwards, HBV DNA level dropped to below 2.6 log10 copies/ml and ALT level was normalized after 19 weeks of TDF dosing (Figure 1).

A pair of primers was designed (forward: 5'-TC-GTGGTG-GACTTCTCTCAATT-3' and reverse: 5'-CGTTGA-CAGACTTTC-CAATCAAT-3') for amplification of the HBV *pol* region for the analysis of HBV drug resistance. PCR conditions were 95°C for 15 min, followed by 45 cycles consisting of 95°C for 45 s, 56°C for 45 s, and 72°C for 45 s. The final primer concentration was 0.3 µM, and the HBV amplicon size was 742 bp. All PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany) and directly sequenced on ABI PRISM 310 Genetic Analyzer equipment using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc., Piscataway, USA). For cycle sequencing, we used the following thermal protocol: 35 cycles consisting of 95°C for 20 s, 50°C for 25 s, and finally 60°C for 2 min. The reverse primer was used as the sequencing primer at a final concentration of 0.5 µM. Electropherogram-obtained sequences were assembled using Vector NTI v5.1 (InforMax, Invitrogen Life Science Software, Frederick, MD, USA).

Table 1. Baseline characteristics of patient

	Normal range	Unit	Case
Age	-	-	57
Gender	-	-	Male
Fasting blood glucose	74-106	mg/dL	104
Blood Urea Nitrogen	6-20	mg/dL	12
Creatine	0-0.9	mg/dL	0.9
Aspartate Aminotransferase	0-31	IU/L	28
ALT	0-33	IU/L	31
Alkaline Phosphatase	0-98	IU/L	78
GGT	0-36	IU/L	103
T. Bil	0-1.1	mg/dL	1.3
Albumin	3.5-5.2	g/dL	4.7
Hb	12.3-15.3	g/dL	15
WBC	4.4-11.3	103/µ	7.5
Plt	136-380	103/µ	145
Prothrombin time	12-15	second	13.8
INR	0.8-1.2	-	1.18
AFP	0-5.8	ng/ml	14
ANA	Negative	-	Negative
AMA	Negative	-	Negative
SMA	Negative	-	Negative
HBsAg	0-0.9	IU/mL	250 (Positive)
HBeAg	0-1	S/CO	18.7 (Positive)
Anti-HBe	0-50	S/CO	1.8 (Negative)
Anti-HBs	0-10	IU/mL	0 (Negative)
Anti-HCV	0-0.9	IU/mL	0 (Negative)
Anti-Delta	Negative	IU/mL	Negative
HBV DNA (PCR)	Negative	Log10 copies/mL	7.7
HBV genotype	-	-	D
HBV subgenotype	-	-	D2
Liver histology	Normal	-	HAI 7, F 3 (Knodell)

INR: International normalized ratio; HbsAg: hepatitis B surface antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; PCR: polymerase chain reaction; ALT: alanine Aminotransferase; GGT: gamma-glutamyl transpeptidase; T. Bil: total bilirubine; Hb: hemoglobine; WBC: white blood cells; AFP: alpha-fetoprotein; ANA: anti-nuclear antibody; AMA: anti-mitochondrial antibody; ASMA: anti-smooth muscle antibody

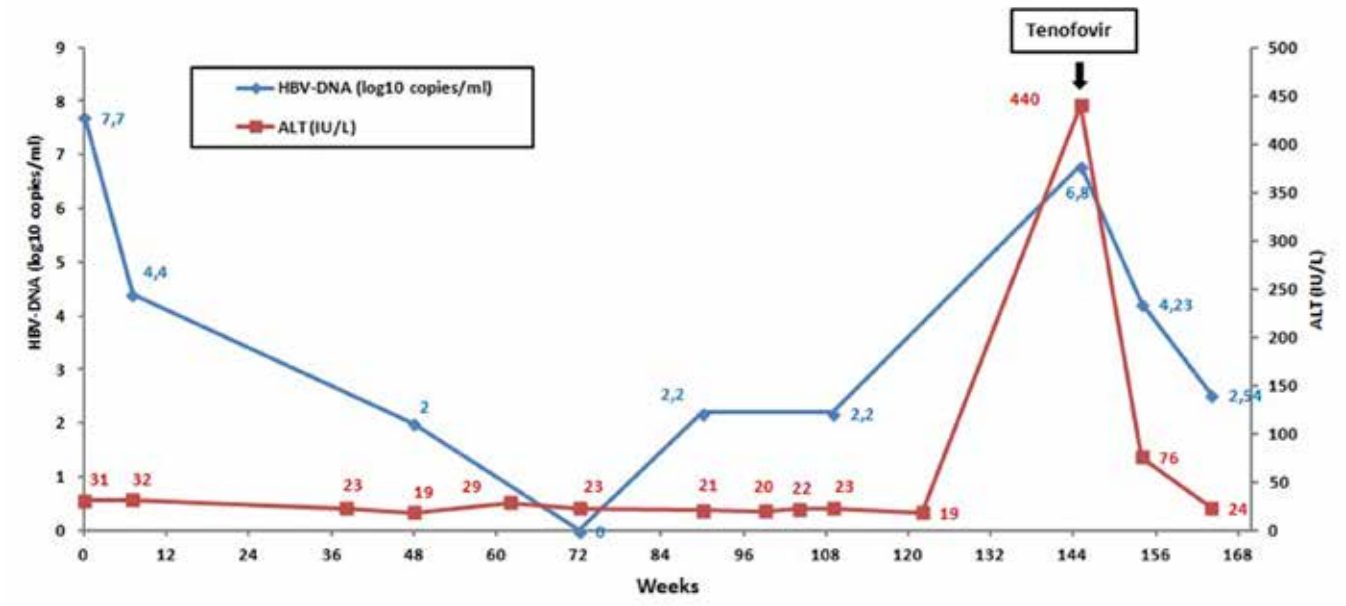

Figure 1. Clinical course of case.

The LVDr-related substitutions rtL180M and rtM204V, as well as ETVr-related substitution rtS202G, were detected at week 145.

DISCUSSION

Treatment of CHB has evolved markedly with the introduction of nucleoside-analogue antivirals, that is, LVD, ADV, ETV, and LdT, to clinical practice. The most important limitation of long-term nucleoside analogue treatment for CHB is the emergence of drug resistant mutations in HBV followed by viral breakthrough and hepatitis flare (3). The most common mutation associated with LVDr involves substitution of methionine in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV DNA *pol* gene RT domain with valine or isoleucine (rtM204V/I), with or without a leucine-to-methionine substitution in an upstream region (rtL180M) (17). It was reported that LVDr was detected at a rate of 14 to 32% after 1 year and 60 to 70% after 5 years of LVD treatment (3). LdT resistance also arises at the YMDD motif and has been reported in the context of virologic breakthrough, at 22% and 9% over 2 years in patients who are positive and negative, respectively, for the hepatitis B e antigen (11,12). The substitutions conferring resistance to ADV are asparagine to threonine (rtN236T) and alanine to valine or threonine (rtA181V/T) (20), and the cumulative probability of ADV resistance with elevation of HBV DNA level has been reported to be 20% at 5 years in HBeAg-negative patients (21) and as high as 42% in HBeAg-positive patients (22).

Entecavir displays several properties for consideration as the first-line nucleoside analogue because of its potent antiviral activity and a lower frequency of drug resistance than LVD, ADV, or LdT (23). In the case of ETV, it has been reported that resistance to the drug requires at least one of three substitutions in HBV RT, that is, rtT184, rtS202, and rtM250, as well as LVDr-related substitutions rtL180M and rtM204V (24).

There is a high genetic barrier to resistance to ETV in nucleoside-naive patients and <1% experience virologic breakthrough with ETVr through 5 years of therapy (13). In that study, ETVr substitutions were detected in only three nucleoside-naive patients (3 of 663). Two of these patients with wild-type virus at baseline developed rtM204V+rtL180M and rtS202G simultaneously. They did not achive undetectable HBV-DNA (<300 copies/mL) on ETV. Only HBeAg-negative one patient with the rtM204I substitution achieved undetectable HBV DNA (<300 copies/mL) in that study. So, it was concluded that, ETVr changes combined with only the rtM204I LVDr substitutions displayed lower levels of phenotypic ETV resistance (25).

The results of a parallel survey of surveillance conducted in Japan in which nucleoside-naive patients received the 0.5 mg dosage of ETV for 3 years yielded only one of 66 patients who developed genotypic resistance (1.7%) (12).

Simultaneous emergence of all three resistance substitutions has been noted in other reports of ETVr.(14). In that report, ETVr developed in a nucleoside-naive patient with genotype H of HBV, which did not achieve undetectable HBV DNA levels. In another report, ETVr have been emerged in two Japanese nucleoside-naive CHB patients after prolonged therapy and incomplete suppression (15). Finally, in a recent report, the three substitutions associated with ETV and LVD resistance has been developed simultaneously without complete suppression in a nucleoside-naive CHB patient after extended therapy (16).

Entecavir resistance has been attributed to high pretreatment viral loads and persistently detectable HBV-DNA by PCR during the treatment course in all reports of ETVr. It is believed that some subpopulations of HBV that proliferate very actively and are not completely suppressed by ETV may have a chance of

being selected for the resistance substitutions required for ETV virologic failure. Phenotypic analyses of samples associated with virologic breakthrough confirmed that ETV susceptibility correlates with the spectrum of these additional substitutions conferring genotypic resistance and the increased level of circulating HBV DNA (25). Thus, all of the ETVr cases had incomplete viral suppression except one HBeAg-negative patient presented in the study of Tenney et al (13). Our patient also had complete viral suppression before the development of ETVr. The three substitutions associated with ETV and LVD resistance developed after complete viral suppression. So, circulating HBV DNA could not a reason for ETVr. We could not perform DNA sequence analysis before the HBV treatment. So we dont know whether baseline rtM204I substitution was present or not as in the study of Tenney et al (13).

CONCLUSION

In this article, we report a case with confirmed genotypic resistance to ETV, virologic and biochemical breakthrough during long-term ETV treatment for nucleoside-naive CHB patients. The present case of ETV resistance is particularly notable for its emergence in a case with complete viral suppression, which is rare. To our knowledge, this is the second report of emerging resistance to ETV in a nucleoside-naive patient after complete viral supression. In spite of the extremely rare chance of viral mutation during ETV treatment, nucleoside-naive patients should be carefully monitored for resistance even if complete suppression is present.

Conflict of Interest: No conflict of interest was declared by the authors.

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