Virologic breakthrough in a patient with chronic hepatitis B by combination treatment with tenofovir disoproxil fumarate and entecavir

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Abstract: Tenofovir disoproxil fumarate (TDF) is widely used to treat hepatitis B virus (HBV) patients in the USA and Europe. No confirmed report of resistance selection during treatment with TDF in treatment-naïve and nucleoside/nucleotide analog-treated chronic hepatitis B patients has yet been reported. Here, we report for the first time a patient with chronic hepatitis B and cirrhosis who emerged with virologic breakthrough during combination therapy with TDF and entecavir (ETV), against ETV-resistant virus. A 51-year-old Japanese woman with hepatitis B e-antigen (HBeAg), whose genotype was C, received ETV monotherapy continuously followed by TDF and ETV combination therapy, because her HBV DNA levels had been >3.5 log copies/mL. At the start of combination therapy, amino acid substitutions of the reverse transcriptase (rt) gene, rtL180M, rtT184I/M, and rtM204V, were detected. After this, serum HBV DNA decreased to less than 2.1 log copies/mL and remained at this level until 31 months of combination therapy, when it again began to increase. Amino acid substitutions of rtL180M, rtS202G, and rtM204V emerged and were associated with an increase in serum HBV DNA at virologic breakthrough. Long-term therapy with TDF against the ETV-resistant virus has the potential to induce virologic breakthrough and resistance, and careful follow-up should be carried out.

Keywords: hepatitis B virus, resistant

Introduction

Hepatitis B virus (HBV) infection is a common disease that can induce a chronic carrier state and is associated with the risk of progressive disease and hepatocellular carcinoma.¹ Interferon (IFN) and several nucleoside/nucleotide analogs (NAs), such as lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), and tenofovir disoproxil fumarate (TDF), are currently approved for the treatment of chronic hepatitis B (CHB) in most countries.^{2–5} Because NA analogs inhibit reverse transcription of the HBV polymerase but do not directly interfere with the formation of covalently closed circular DNA (cccDNA), they require long-term administration, which is usually accompanied by the emergence and selection of drug-resistant mutations in the viral polymerase.⁶

TDF is widely used to treat HBV patients in the USA and Europe. This agent is equally effective against multiple HBV genotypes (A–H) as well as against LAM-resistant isolates.⁷ No confirmed report of resistance selection during treatment with TDF in treatment-naïve CHB patients has yet been reported.^{8–10} In a recent study, long-term TDF monotherapy provided durable antiviral efficacy for 240 or up to 288 weeks (6 years) of treatment, and comprehensive genotypic and phenotypic analyses detected no evidence of TDF resistance.^{11,12} Additionally, longer treatment duration

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did not increase the incidence of virologic breakthrough.¹² Moreover, TDF monotherapy has demonstrated the long-term (median 23 months) efficacy of this agent in NA-experienced patients with treatment failure, and virologic breakthrough was not observed in any patient during the entire observation period.¹³

Here, we report for the first time a patient with CHB and cirrhosis who emerged with virologic breakthrough during TDF and ETV combination therapy against ETV-resistant virus.

Case report

A 51-year-old Japanese woman with CHB underwent a checkup in February 1999 and was found to be seropositive for hepatitis B surface antigen (HBsAg), with mild alanine aminotransferase (ALT) elevation. Hepatitis B e-antigen (HBeAg) was positive, and serum HBV DNA was >7.6 log copies/mL (Amplicor HBV Monitor assay; F Hoffman-La Roche Ltd, Basel, Switzerland). The HBV genotype was C, and human immunodeficiency virus (HIV) status was negative. She was diagnosed with cirrhosis by peritoneoscopy and liver biopsy (moderate hepatitis [A2] and severe fibrosis [F4]) in February 2000. She received LAM (100 mg/day) monotherapy from September 2006. The nadir of HBV DNA was 2.5 log copies/mL in January 2007. HBV DNA levels gradually increased, and LAM-resistant virus emerged (reverse transcriptase [rt] M204I). Treatment was switched

from LAM to ETV (0.5 mg/day) in October 2007 (HBV DNA 3.9 log copies/mL) following the emergence of ETV-resistant virus (rtL180M, rtS202G, and rtM204V) and higher elevation in HBV DNA. However, she discontinued therapy of her own volition from February 2009 to May 2010. She returned to our hospital in May 2010 because of general fatigue and ascites, at which time serum HBV DNA was >7.6 log copies/mL, ALT was 687 IU/L, and bilirubin was 3.8 mg/dL. Treatment with ETV (0.5 mg/day) was restarted immediately, and ALT and serum HBV DNA levels gradually decreased. However, because HBV DNA levels remained at >3.5 log copies/mL until September 2010, she was started on TDF (300 mg/day) and ETV combination therapy (HBV DNA 3.9 log copies/mL). Serum HBV DNA then decreased to less than 2.1 log copies/mL (COBAS® TaqMan® HBV Test, v2.0; F Hoffman-La Roche Ltd) at November 2011 (month 14 of TDF and ETV treatment) and remained at this level until April 2013 (month 31 of TDF and ETV treatment), when it again began to increase (HBV DNA 3.9 log copies/mL). Moreover, ALT was elevated in September 2013 (Figure 1). Compliance with TDF and ETV was good throughout the course of treatment.

During treatment, nucleotide sequences of the polymerase gene were determined by polymerase chain reaction (PCR) direct sequencing, as previously described.¹⁴ The viral polymerase reverse transcriptase (rt) gene at the baseline of LAM treatment (September 2006) showed the wild type sequence

Figure 1 Clinical course of lamivudine or entecavir and tenofovir disoproxil fumarate therapy.

Abbreviations: ALT, alanine aminotransferase; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate.

course of treatment. Virologic breakthrough in compliant patients is generally related to viral resistance.⁶ Amino acid substitutions of rtL180M, rtS202G, and rtM204V have emerged in cases in which serum HBV DNA increased during TDF and ETV therapy. Moreover, these amino acid substitutions changed from rtL180M, rtT184M, and rtM204V to rtL180M, rtS202G, and rtM204V. This clinical course suggests that these amino acid substitutions are resistant to TDF and ETV therapy, although in vitro confirmation is necessary. Kim et al reported that among 18 patients who failed multiple NA treatments, including LAM, ADV, and ETV, 17 patients achieved virologic response and one patient showed a viral reduction of 3.9 log IU/mL, nearly reaching virologic response within 24 months.²⁰ These findings indicate that genotypic resistance to ETV does not affect the probability of an initial virologic response to TDF therapy.²⁰ Petersen et al reported that four patients harboring ETV-resistant virus achieved a virologic response within 9 months.¹⁹ Recently, Seto et al reported 142 Asian CHB patients with at least 6 months exposure to other NAs (including ETV) who received TDF with or without LAM. With a median 2.25 years of follow-up, 45 patients had detectable viremia in at least one time point.²¹ For these 45 patients, which included ten with virologic breakthrough, both line probe assay and direct sequencing revealed no new amino acid substitutions, including substitutions that could be associated with reduced TDF susceptibility (rtA181V/T, rtN236T, or rtA194T). Moreover, Karatayli et al reported that HBV DNA, in seven of eight patients with ETV resistance mutations (T184F/A/L/I, S202G, and M250V), became undetectable with TDF and LAM after 6 months of treatment.²² In vitro drug susceptibility showed that TDF displayed one- to twofold resistance to ETV-resistant viral strains (N123D + H124Y + L180M + S202G + M204V + Y257H, I163V + L164M + L180M + S202G + M204V + C256S, and H124Y + L180M + S202G + M204V + Y257H). However, in other cases, the treatment period was relatively shorter. In our case, virologic breakthrough occurred at month 31 of TDF and ETV therapy, and the ETV-resistant strain (L180M + S202G + M204V) of our case was not identical with that in the in vitro drug susceptibility study above. Clarification of virologic breakthrough and resistance of TDF against patients with NA-resistant virus, especially ETV, will likely require further studies with a longer time frame.

In conclusion, this study shows that long-term treatment of ETV-resistant virus with TDF has the potential to induce virologic breakthrough and resistance, and careful follow-up should be done.

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Disclosure

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