

Case Report

Early emergence of entecavir-resistant hepatitis B virus in a patient with hepatitis B virus/human immunodeficiency virus coinfection

Aimi Kanada,¹ Tetsuo Takehara,¹ Kazuyoshi Ohkawa,^{1,2} Michio Kato,³ Tomohide Tatsumi,¹ Takuya Miyagi,¹ Ryotaro Sakamori,¹ Shinjiro Yamaguchi,¹ Akio Uemura,¹ Keisuke Kohga,¹ Akira Sasakawa,¹ Hayato Hikita,¹ Kiyomi Kawamura,⁴ Tatsuya Kanto,^{1,2} Naoki Hiramatsu¹ and Norio Hayashi¹

¹Department of Gastroenterology and Hepatology, ²Department of Dendritic Cellular Research and Clinical Application, Osaka University Graduate School of Medicine, Yamadaoka, Suita and Departments of

³Gastroenterology and ⁴Immunology Infectious Disease, National Hospital Organization Osaka National Hospital, Hoenzaka, Chuo-ku, Osaka, Japan

The efficacy of entecavir for patients with hepatitis B virus/human immunodeficiency virus coinfection has not been fully elucidated. Here we examined a patient coinfecting with both viruses in whom entecavir-resistant hepatitis B virus appeared. The 60-year-old Japanese male with the coinfection received antiretroviral therapy including lamivudine. The therapy initially suppressed replication of both viruses, followed by reactivation of the hepatitis B virus alone by 2 years of therapy. He subsequently received entecavir therapy in addition to the antiretroviral regimen. After entecavir administration, the hepatitis B virus DNA level was slightly reduced, but then increased after 6 months of entecavir therapy. In the sequencing analysis of hepatitis B virus, no drug resistance-associated amino acid substitutions were observed in the reverse transcriptase (rt) domain before antiretroviral therapy. The lamivudine-resistant amino acid substitutions at rt173, rt180 and rt204 were detected before entecavir administration, and further the entecavir-resistant rt202 substitu-

tion was observed after 6 months of entecavir therapy. The full-length hepatitis B sequences showed that the viral strain derived from the patient belonged to genotype H. In summary, this report describes a patient with hepatitis B virus/human immunodeficiency virus coinfection who received entecavir therapy in addition to an antiretroviral regimen and showed the early emergence of entecavir-resistant hepatitis B virus. In entecavir therapy for patients infected with both viruses, great care should be taken with respect to the emergence of entecavir-resistant hepatitis B virus, especially in patients with pre-existing lamivudine-resistant virus.

Key words: coinfection, drug-resistant hepatitis B virus, entecavir, hepatitis B virus, human immunodeficiency virus, lamivudine

INTRODUCTION

CHRONIC CARRIERS OF hepatitis B virus (HBV) number more than 350 million worldwide.¹ Chronic HBV infection is seen in approximately 10% of human immunodeficiency virus (HIV)-infected

patients,² and coinfection with HBV and HIV is a serious health problem due to the shared mode of transmission. Since the prognosis of HIV-infected patients can be dramatically improved by highly active antiretroviral therapy (HAART), one of the major causes of mortality in HIV-infected patients is chronic liver disease due to HBV infection.³

Lamivudine (LAM, also abbreviated to 3TC), one of the antiretroviral drugs, has also been used for the reduction of HBV replication and improvement of HBV-related liver diseases.^{4,5} However, the anti-HBV effect of LAM is hampered by the emergence of LAM-resistant mutant virus in cases of HBV mono-infection and HBV/

Correspondence: Professor Norio Hayashi, Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Suita 565-0871, Japan. Email: hayashin@gh.med.osaka-u.ac.jp
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HIV coinfection.^{6,7} The LAM-resistant HBV strain is based on point mutation occurring within the reverse transcriptase (rt) domain of the polymerase gene. A methionine-to-valine/isoleucine amino acid substitution at rt204 (rtM204V/I) is known to confer LAM resistance.^{8,9} A leucine-to-methionine substitution at rt180 (rtL180M) and a valine-to-leucine substitution at rt173 (rtV173L) have also been shown to appear in association with LAM resistance.^{8,10,11} The emergence rate of LAM-resistant virus in patients coinfecting with HBV and HIV has been reported to be approximately 50% after 2 years of therapy.⁹

Recently, entecavir (ETV) has been reported to be superior to LAM for the suppression of viral replication and disease activity in patients with HBV mono-infection who had not received previous treatment with other anti-HBV drugs (naïve patients).^{12,13} ETV has also been shown to be effective in HBV-infected patients who had been treated with LAM and showed LAM resistance.¹⁴ It has been demonstrated that ETV resistance occurs based with amino acid substitution(s) at rt184, rt202 and/or rt250, together with the LAM-resistant rtM204V/I and rtL180M substitutions.¹⁵ The emergence rate of ETV-resistant virus after 3 years of therapy has been reported to be less than 1% in naïve patients and 15% in LAM-resistant patients with chronic HBV mono-infection.¹⁶ However, the anti-HBV efficacy of ETV for HBV/HIV coinfection has not been fully clarified.

In this study, we examined a patient with concomitant HBV/HIV infection who underwent HAART including LAM, and showed the appearance of LAM-resistant HBV. Subsequent ETV administration did not lead to an adequate reduction of the HBV replicative level, followed by the early emergence of the ETV-resistant virus. We investigated the serial change in the drug resistance-associated mutation status within the rt domain of the HBV polymerase gene, as well as full-length nucleotide sequences of the ETV-resistant HBV strain derived from the patient.

CASE REPORT

Patient and serum sampling

A 60-YEAR-OLD JAPANESE heterosexual male first visited to the National Hospital Organization Osaka National Hospital in December 2001 due to a positive result from an HIV antibody (anti-HIV) test in voluntary HIV screening. From his anamnestic record, he had been admitted with type B acute hepatitis to another hospital 3 years earlier. Anti-HIV had been

negative at that time. On his first visit, the anti-HIV positivity was confirmed by Western blot analysis. Antibodies to HIV-1 proteins, gp160, gp110/120, p68, p52, gp41, p40 and p34 were positive. As for antibodies to HIV-2 proteins, only an antibody to p68 was positive. According to these, he was judged to be infected with HIV-1. The HIV-RNA level was $10^{4.3}$ copies/mL and the CD4+ T cell counts were 275/mm³ (normal range, >300/mm³). He tested positive for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), and negative for antibody to HBsAg (anti-HBs) and antibody to HBeAg (anti-HBe). The HBV-DNA level was $>10^{7.6}$ copies/mL, and the alanine aminotransferase (ALT) level was 106 IU/L. The patient was free of HIV-related symptoms and had no opportunistic infectious diseases. HAART with LAM (300 mg/day), zidovudine (AZT) (600 mg/day) and efavirenz (EFV) (600 mg/day) was started in April 2002. AZT and EFV were then substituted for didanosine (ddI) (60 mg/day) and avacavir (ABC) (600 mg/day) in July 2002 because of anemia and dizziness. By July 2002, HIV-RNA decreased to below the detection limit ($<10^{1.7}$ copies/mL), whereas the CD4+ T cell counts tended to rise up to >500 /mm³. In August 2006, fosamprenavir (FPV) (2400 mg/day) was commenced in place of ddI due to peripheral nerve palsy. Suppression of HIV-RNA below the detection limit continued at the end of follow-up, irrespective of repeated alterations in the therapeutic regimen of HAART. As for HBV status, HBV-DNA declined to $10^{3.9}$ copies/mL in April 2003 but increased again to $>10^{7.6}$ copies/mL in May 2005. To control HBV replication, ETV (0.5 mg/day) was added in October 2006. After the ETV administration, HBV-DNA slightly decreased from $>10^{7.6}$ to $10^{6.2}$ copies/mL in January 2007 but rose to $10^{7.2}$ copies/mL 3 months later. ALT remained abnormal and HBeAg continued to be positive throughout the follow-up period. The clinical course of the patient is summarized in Figure 1a.

For the nucleotide sequencing of HBV-DNA, the serum samples were obtained in December 2001 (before HAART), August 2006 (before ETV administration), and April 2007 (after 6 months of ETV therapy). These serum sampling points were designated as P1, P2 and P3 (see Fig. 1a). Serum samples were stored at -80°C until use. Informed consent was obtained from the patient.

Virus markers and nucleotide sequencing

HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HIV were tested by chemiluminescent immunoassay. A

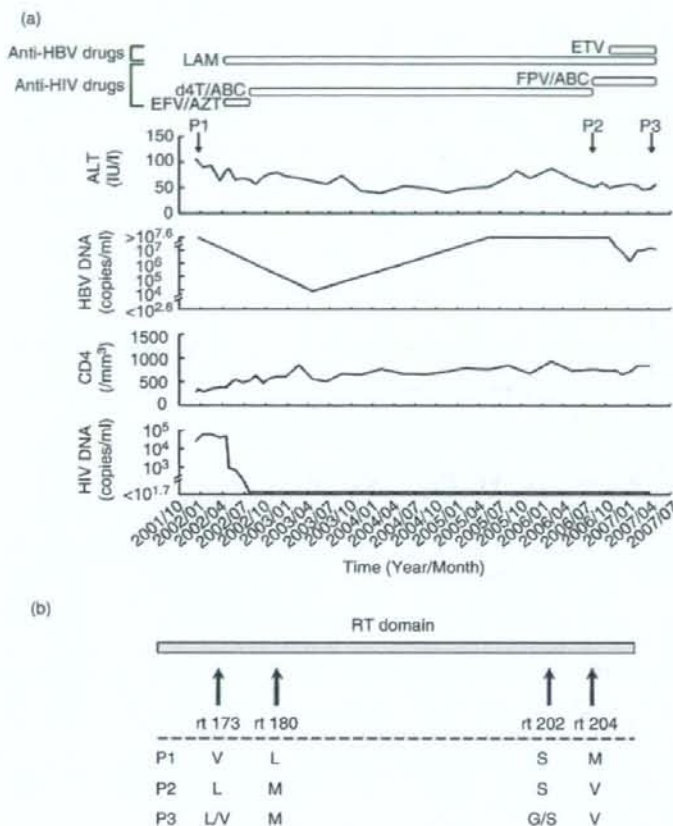


Figure 1 (a) Patient clinical course and serum sampling points. P1, P2 and P3 are the points at which serum samples were obtained. P1 was taken in December 2001 (before HAART), P2 in August 2006 (before ETV administration) and P3 in April 2007 (after 6 months of ETV therapy). ABC, avacavir; ALT, alanine aminotransferase; AZT, zidovudine; d4T, didanosine; EFV, efavirenz; ETV, entecavir; FPV, fosamprenavir; HBV, hepatitis B virus; HIV, human immunodeficiency virus; LAM, lamivudine. (b) Serial change in the status of drug resistance-associated amino acid substitutions.

confirmatory anti-HIV-1/2 testing was carried out by Western blot analysis. Serum HBV-DNA was detected by means of a PCR assay (Amplicor HB monitor; Roche Diagnostics, Basel, Switzerland) with a lower detection limit of $10^{2.6}$ (=400) copies/mL. Plasma HIV-RNA was quantified by a PCR assay (Amplicor HIV-1 monitor; Roche) whose lower detection limit was $10^{1.7}$ (=50) copies/mL.

The nucleotide sequences of HBV-DNA were determined by a method based on nested PCR and direct sequencing, as described elsewhere.¹⁷ In this study, primers BF5-2 (5'-TCC TCA GGC CAT GCA GTG GA-3', nt 3201-20) and BR8 (5'-TTG CGT CAG CAA ACA CIT GG-3', nt 1195-76) were also used. Nucleotide sequences of the entire rt domain in the polymerase gene were examined in HBV strains derived from the P1

and P2 serum samples (GenBank accession nos. AB353765 and AB353766), whereas the full-length HBV-DNA was determined in the strain derived from the P3 serum sample (GenBank accession no. AB353764). The full-length HBV strain obtained in this study (designated as HBDI03), the seven representative HBV strains of genotypes A-G and the eight previously isolated HBV strains of genotype H were aligned, and the phylogenetic tree was constructed. These analyses were done at the homepage of the DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp>).

Results of sequencing analysis of HBV

The serial change in the nucleotide sequences in the rt domain of the HBV polymerase gene was first examined

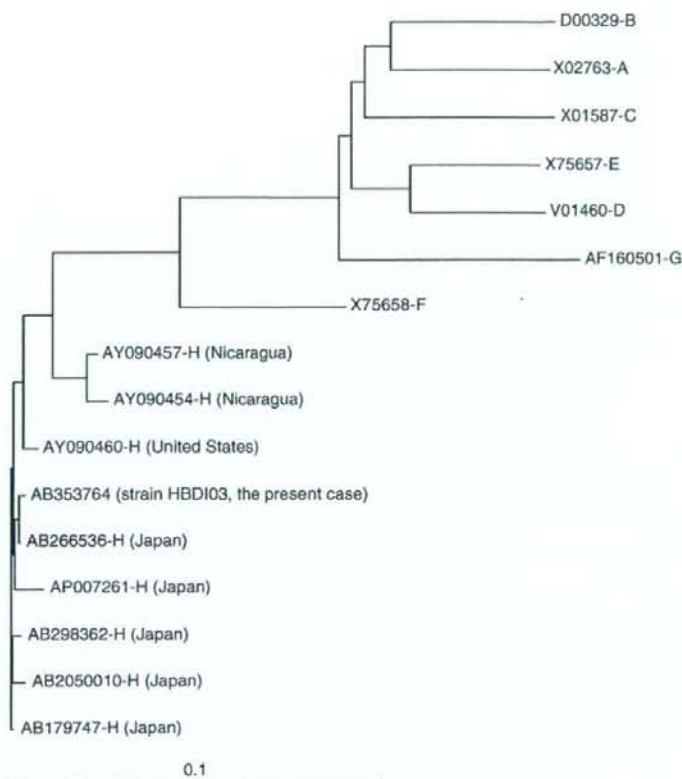


Figure 2 Phylogenetic tree analysis including the HBV strain HBDI03 obtained in this study, the seven representative HBV strains of genotypes A-G, and the eight previously isolated HBV strains of genotype H.

using serum samples obtained at P1-P3 (Fig. 1b). At point P1, no drug resistance-associated mutations were found in the *rt* domain, but three LAM resistance-associated substitutions, *rtM204V*, *rtL180M* and *rtV173L*, emerged at point P2. A serine-to-glycine substitution at *rt202* (*rtS202G*), which has been shown to be one of the ETV resistance-associated substitutions,¹⁵ was further observed at point P3, although *rtS202G* and *rtV173L* substitutions occurred incompletely. No other amino acid substitutions were seen in the *rt* domain of the HBV polymerase gene from point P1 to P3. Thus, in the patient with HBV/HIV coinfection, the emergence of the drug resistance-associated amino acid substitutions revealed a close relationship with the poor anti-HBV efficacy of LAM and ETV.

Next, the full-length nucleotide sequences of HBV were determined from the P3 serum sample of the patient with HBV/HIV coinfection showing ETV resis-

tance. The full-length HBV strain HBDI03 comprised a total of 3215 nucleotide lengths. The phylogenetic tree was depicted using the HBV strain HBDI03, the seven representative HBV strains of genotypes A-G and the eight previously identified genotype H HBV strains. As shown in Figure 2, the HBV strain HBDI03 obtained in this study was classified as genotype H. When the nucleotide sequences of the strain HBDI03 were compared with the eight reported genotype H HBV strains, the strain HBDI03 showed a 97.2-99.8% identity with these strains. The unique amino acid substitutions in the strain HBDI03 were further investigated in comparison with these eight genotype H HBV strains. As shown in Table 1, four drug resistance-associated substitutions within the *rt* domain were observed, as described above. The two amino acid substitutions in the S gene were also caused by the same mutations of the drug resistance-associated *rtV173L* and *rtM204V*

Table 1 The unique amino acid substitutions in strain HBD103 in comparison with eight previously isolated genotype H hepatitis B virus strains

Amino acid position	Consensus residue of genotype H	Residue unique to strain HBD103
Polymerase		
519 (rt173)	V	L/V
526 (rt180)	L	M
548 (rt202)	S	G/S
550 (rt204)	M	V
Surface		
164	E	D/E
195	I	M
X		
32	W	G

Consensus residues of genotype H were from the eight reported hepatitis B virus (HBV) strains (GenBank accession nos. AY090454, AY090457, AY090460, AP007261, AB179747, AB205010, AB266536 and AB298362).

changes. As for the remaining one amino acid substitution in the X gene, the substituted glycine residue observed in the HBD103 strain was a common one in the representative HBV strains of genotypes A-G at the corresponding codon position. Taken together, the HBD103 strain did not appear to have any distinctive features other than the presence of the drug-associated amino acid substitutions.

DISCUSSION

RECENTLY, ETV HAS been widely accepted as an effective drug for the treatment of HBV mono-infection because of its stronger inhibitory effect on HBV replication and lower emergence rate of drug-resistant mutant virus compared to LAM.¹²⁻¹⁴ ETV-resistant HBV has been demonstrated to be established by amino acid substitution(s) at rt184, rt202 and/or rt250, in addition to the LAM-resistant rtM204V/I and rtL180M substitutions.¹⁵ The emergence rate of ETV-resistant virus has been reported to be higher in LAM-resistant patients than in naive patients.¹⁶ There has so far been little evidence concerning the anti-HBV efficacy of ETV for patients with HBV/HIV coinfection. In particular, LAM-resistant HBV has been shown to emerge frequently in patients with HBV/HIV coinfection who received LAM therapy as a component of HAART.⁷ The therapeutic efficacy of ETV on LAM-resistant HBV should be assessed in patients with HBV/HIV coinfection. In this study, we examined a patient with HBV/

HIV coinfection who had LAM-resistant HBV induced by HAART including LAM, and underwent subsequent ETV therapy. The patient showed a rather weak suppressive effect of ETV on HBV replication, followed by the emergence of ETV-resistant HBV in the early phase of therapy.

In the sequence analysis of the HBV genome, no drug-resistant HBV mutations were detected before HAART, but continuous LAM administration induced the LAM-resistant mutant HBV with rtM204V, rtL180M and rtV173L amino acid substitutions. Subsequent ETV therapy resulted in the emergence of an ETV-resistant virus possessing the rtS202G substitution in addition to the three LAM resistance-associated substitutions after no more than 6 months of ETV therapy, although the rtS202G and rtV173L substitutions were incomplete. In LAM-resistant patients with HBV mono-infection, the emergence rate of the ETV-resistant mutation has been reported to be merely 15% after 3 years of therapy.¹⁶ In comparison with this, ETV-resistant HBV appeared in an extremely early phase of therapy in our patient with HBV/HIV coinfection. According to this, ETV resistance is speculated to be established earlier in patients with HBV/HIV coinfection than in those with HBV mono-infection, although concomitant HIV infection has not thus far been suggested to result in a higher incidence of the drug-resistant HBV strain in the treatment with other anti-HBV drugs in chronic HBV infection. The latent immune deficiency caused by HIV infection might prevent HBV eradication through a host immune response, resulting in poor anti-HBV efficacy of ETV. Alternatively, simultaneous usage of multiple antiretroviral drugs might in some way contribute to the emergence of ETV-resistant HBV.

Very recently, it has been shown that ETV possesses modest anti-HIV activity both *in vitro* and *in vivo* and can induce the drug-resistant mutant HIV strain in patients with HBV/HIV coinfection.¹⁸ This suggests that ETV may not be appropriate for the treatment of patients with HBV/HIV coinfection in whom HAART is not needed. On the other hand, ETV is considered to be beneficial for patients with HBV/HIV coinfection undergoing a stable continuation of HAART. In particular, the therapeutic efficacy of ETV may be more promising in patients without LAM-resistant HBV than in those with it. Although the present case of the patient under discussion, who already displayed LAM-resistant HBV due to the preceding HAART, did not support the usefulness of ETV therapy because of the early emergence of ETV-resistant HBV, further studies with a large number of

patients should be completed to assess the antiviral efficacy and deliberate clinical application of ETV therapy for HBV/HIV coinfection.

Both adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF) have recently been shown to effectively inhibit HBV replication in patients with HBV/HIV coinfection, irrespective of LAM resistance.^{19,20} ADV exerts only anti-HBV activity and is available for patients with HBV/HIV coinfection who have no need for HAART or who are receiving a stable HAART regimen. In contrast, TDF can be used as a component of HAART because of its valuable antiviral activity against both HBV and HIV. Accordingly, ADV and TDF are currently useful drugs for patients with HBV/HIV coinfection and may be subsequent therapeutic options for the patient reported in this study.

Our patient was found to be infected with HBV of genotype H, a globally rare genotype. To date, the full-length sequences of eight genotype H HBV strains have been reported from the USA, Nicaragua and Japan (see Fig. 2). Of them, one strain has been obtained from a Japanese patient with chronic HBV mono-infection who underwent ETV therapy as a naïve patient and showed ETV resistance later.²¹ The relevance of the genotype frequency to the therapeutic efficacy of ETV should be studied extensively in HBV-infected patients treated with ETV.

In Japan, genotypes B and C are prevalent in chronic HBV carriers who acquire the infection mainly through the mother-to-child transmission route. In contrast, the foreign HBV strains other than genotypes B and C have been shown to be involved in a considerable proportion of patients with acute HBV infection.²² Infection of such foreign types of HBV possibly occurs through sexual contacts in Japan. In our patient with HBV/HIV coinfection who had genotype H HBV of foreign origin, it is speculated that acute HBV infection occurring 3 years before his first visit led to the transition to chronicity. The time of HIV infection cannot be defined due to the lack of HIV-RNA testing during the period of acute HBV infection. The possibility of simultaneous infection with HBV and HIV cannot be excluded, despite the negative result of anti-HIV at that time, because the test may have taken place during the immunological window period of HIV infection.

In summary, we have introduced a patient with HBV/HIV coinfection who underwent ETV therapy in addition to the HAART regimen and showed ETV resistance in the early phase of therapy. Our finding suggests that, in ETV therapy for patients with HBV/HIV infection, great care should be taken against the emergence of

ETV-resistant HBV, especially in patients with pre-existing LAM-resistant HBV.

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