Hepatitis B e Antigen and Hepatitis B Surface Antigen Seroclearance with the Emergence of Lamivudine-associated and Core Mutations Following CD4 Elevation in a Patient with Hepatitis B and HIV

Takashi Honda¹, Masatoshi Ishigami¹, Fangqiong Luo¹, Yoji Ishizu¹, Teiji Kuzuya¹, Kazuhiko Hayashi¹, Akihiro Itoh¹, Yoshiki Hirooka¹, Tetsuya Ishikawa¹, Isao Nakano¹, Yoshiaki Katano² and Hidemi Goto¹

Abstract

Obtaining a better understanding of the mechanisms associated with hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) loss in patients with hepatitis B virus (HBV) is important for treating patients with chronic hepatitis B. We herein describe the case of a patient with HBV and human immunodeficiency virus whose chronic hepatitis was stabilized due to HBe and HBs seroconversion with the emergence of lamivudine-associated and core mutations after CD4 elevation. A full-length HBV DNA analysis indicated that HBsAg had been lost after the development of the rtS143T mutation, which corresponded to the emergence of the sF134L and core mutations. The details of this case shed some light on the mechanisms associated with HBsAg and HBeAg clearance.

Key words: hepatitis B virus, human immunodeficiency virus, lamivudine, mutation, nucleotide analogue

(Intern Med 54: 585-590, 2015)
(DOI: 10.2169/internalmedicine.54.2038)

Introduction

There are several nucleotide analogues available for treating hepatitis B. However, the administration of long-term antiviral therapy directed towards the hepatitis B virus (HBV) reverse transcriptase (RT) can lead to the selection of drug resistant mutations (1-3), and the efficacy of lamivudine in cases of chronic hepatitis B is limited by the emergence of drug-resistant mutants (1). Mutations conferring resistance to lamivudine are primarily located in the YMDD motif, i.e., M204V or M204I, in the C-terminal domain of RT and may be associated with compensatory mutations in the C-terminal domain, i.e., V173L or L180M (2). Entecavir and tenofovir are highly effective against hepatitis B and less frequently associated with the development of resistant mutants than lamivudine; however, in patients coinfected with HIV, the use of entecavir monotherapy has been reported to result in the accumulation of HIV-1 variants with the lamivudine-resistant mutation M184V (3), and tenofovir monotherapy may induce the accumulation of HIV-1 variants. The introduction of tenofovir and emtricitabine into the HBV armamentarium for HBV/HIV-coinfected patients provides another treatment option in such cases (4). However, the challenge is to increase the number of antiviral drugs available for controlling the emergence of additional mutations.

Patients coinfected with HBV and HIV exhibit more rapid progression of liver fibrosis and a higher rate of decompensated cirrhosis (5). Among these cases, hepatitis B e antigen (HBeAg) and/or hepatitis B surface antigen (HBsAg) seroclearance has been observed in a minority of patients starting antiretroviral therapy (ART) comprising an antiviral drug for HBV (6) and correlates with a sustained HIV response

¹Department of Gastroenterology and Hepatology, Nagoya University Graduate School of Medicine, Japan and ²Department of Gastroenterology, Banbuntane Hotokukai Hospital, Fujita Health University, School of Medicine, Japan

Received for publication November 5, 2013; Accepted for publication July 30, 2014
Correspondence to Dr. Masatoshi Ishigami, masaishi@med.nagoya-u.ac.jp
to antiretroviral therapy consisting mostly of ART regimens that include lamivudine (7).

Selection for both HBV polymerase gene mutations and S gene mutations has been observed during antiviral therapy for HBV in HBV/HIV-coinfected patients (8). These mutations sometimes alter the antigenicity of HBsAg to such an extent that the antigens can no longer be recognized by many monoclonal antibodies (9). As a result, these mutations are very similar to vaccine-escape mutations selected for by HBV vaccination. Moreover, some mutations associated with lamivudine resistance may cause premature stop codons in the S gene, resulting in the impaired secretion of HBsAg, according to a previous report (10).

Obtaining a better understanding of the mechanisms associated with HBsAg and HBeAg loss is important for treating patients with chronic hepatitis B. The following report describes the case of a HBV/HIV-coinfected patient with HBeAg and HBsAg seroclearance and undetectable HBV DNA following the emergence of lamivudine-associated core mutations, without the addition of any new antiviral drugs.

### Case Report

A 35-year-old man with a past history of acute hepatitis B consequently advanced to chronic hepatitis B and was followed up at another hospital. He was subsequently admitted to Nagoya University Hospital due to an elevated level of alanine transaminase (ALT). He had been found to be infected with HIV, and an ART regimen [azidothymidine (AZT) + didanosine (ddI) + indinavir (IDV)] had been initiated in mid-July 2002 (Table). At that time, the laboratory data were as follows: HBsAg= positive, HBsAb= negative, HBeAg= positive, AST= 66 IU/L and ALT= 62 IU/L. However, during follow-up, the viral load did not decrease to an undetectable level; therefore, the medication regimen was changed to stavudine (d4T) + lamivudine (3TC) + efavirenz (EFV) at the end of May in 2003. The HIV RNA viral load transiently became undetectable, although viral mutations conferring resistance to all antiretroviral drugs, including lamivudine, emerged. The previous antiviral drugs were replaced with another regimen [lopinavir (LPV) + ritonavir (RTV) + ddI]. After stopping the dose of lamivudine, the patient’s ALT level increased to 1,161 IU/L then spontaneously decreased to approximately 140 IU/L without any elevation in the total bilirubin level (Fig. 1A). The HIV viral load subsequently became undetectable, and the CD4 cell count recovered to 750 cells/mm³. He was then admitted due to pneumonia and, during hospitalization, was referred to our group at the beginning of April in 2006. At that time, the laboratory data included the following findings: AST=74 IU/L, ALT=91 IU/L, platelet count=369,000/μL, HBeAb= negative, HBV DNA load=8.7 log copies/mL. A liver biopsy was performed, which showed a moderate activity and portal fibrosis without septa (A2/F1), according to the Metavir scoring system (11). After the patient recovered from the pneumonia, he was followed up as an outpatient. During follow-up, the ALT level increased to 502 IU/L and the HBV DNA load increased to 9.1 log copies/mL; therefore, he was admitted to our hospital. The laboratory data on admission were as follows: ALT=613 IU/L, platelet count=299,000/μL, HBeAb= positive. A sequence analysis revealed a wild-type HBV polymerase pattern and genotype A. At the beginning of July 2006, antiviral therapy with lamivudine was restarted, as it was unlikely that the high HBV viral load could be controlled with interferon and, at that time, adefovir and tenofovir were not available under the health insurance system in Japan. The patient’s HBV viral load subsequently decreased to 3.8 log copies/mL, and HBeAg seroconversion began. However, the HBV viral load rebounded to 5.4 log copies/mL and an rt M204V mutation was detected. Since March 2008, the HBeAb titer has demonstrated approximately 100% inhibition, with complete seroconversion. The ALT level then normalized, and the HBV viral load gradually decreased to below 5 log copies/mL. Since July 2010, the HBV viral load has remained below 4.0 log copies/mL and the ALT level has remained below 35 IU/mL. Finally, the HBV DNA and HBsAg titers became negative in April 2012, continuing to July 2013. The HBeAb titer also gradually decreased to approximately 3.0 logU/mL in correlation with the decline in the HBV DNA titer (Fig. 1B). The HBV DNA titer was not measured and no stored serum was available before referral to our liver unit at the beginning of April 2006.

Serum samples exhibiting a peak in the fluctuating HBV viral load were collected at five time points (Fig. 1A). The levels of HBsAg (Architect HBsAg QT, Abbott Laboratories, Park, USA), HBeAg (Architect HBeAg, Abbott Laboratories), antibodies to HBeAg (anti-HBe) (Architect HBeAb,

### Table. HIV Treatment

| Regimen       | Date  | HIV RNA copies/mL | CD4 cell count | CD4/CD8 |
|---------------|-------|-------------------|----------------|---------|
| AZT+ddI+IDV   | Jun-02| 100,000<          | 463.7          | 0.7     |
|               | Jul-02| n/a               | n/a            | n/a     |
|               | Aug-02| 7,900             | 478.8          | 1       |
|               | Sep-02| 2,900             | 530.6          | 1.3     |
|               | Oct-02| 1,100             | 434.5          | 1.3     |
|               | Dec-02| 800               | 501.7          | 1.4     |
|               | Feb-03| 1,500             | 446.5          | 1.3     |
|               | Mar-03| 3,400             | 431.9          | 1.2     |
| d4T+3TC+EFV   | Apr-03| 2,300             | 451.4          | 1.3     |
|               | May-03| undetectable      | 595            | 1.3     |
|               | June-05| undetectable   | 637.8          | 1.2     |
|               | Oct-05| 620               | 582.6          | 0.9     |
|               | Nov-05| 691               | n/a            | n/a     |
| LPV+RTV+ddI   | Dec-06| 1,700             | n/a            | n/a     |
|               | Jan-06| undetectable      | n/a            | n/a     |
|               | Feb-06| undetectable      | 765.6          | 1.2     |

n/a: not applicable

---

Intern Med 54: 585-590, 2015 DOI: 10.2169/internalmedicine.54.2038
**Figure 1A.** Overview of the major events in the patient’s clinical course. The vertical bars represent the AST level; the triangles (▲) represent the HBeAg titer; the diamonds (◇) represent the anti-HBe titer, the squares (■) represent the HBV DNA level. The numbers from 1 to 5 indicate the time points for which full length sequence analyses were performed. The timing of hospitalizations and lamivudine therapy are highlighted in the graph. The levels of HBsAg at the time points of the full length sequence analyses are indicated at the bottom.

**Figure 1B.** Additional overview of the major events in the patient’s clinical course. The vertical bars represent the CD4 level; the squares (■) represent the HBV DNA level, the asterisks (★) represent the HBcrAg titer. The timing of lamivudine therapy is highlighted in the graph.
Abbott Laboratories) and hepatitis B core-related antigens (HBcAg) (Lumipulse® HBcAg, Fujirebio, Tokyo, Japan) were examined using chemiluminescent or enzyme immunoassays. HBV DNA was detected quantitatively using a PCR-based assay (COBAS TaqMan HBV auto v2.0, Roche Molecular, Pleasanton, USA; detection limit from 2.1 to 9.0 log copies/mL). The predominant nucleotide (nt) sequences of full-length HBV DNA at each time point were determined according to the PCR direct sequencing method. Briefly, HBV DNA was extracted from 200 μL of serum stored at -20°C using the QIAamp DNA Mini kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). The first round of PCR used the following primers, which were previously designed by Gunther et al. (12): FullIS (5'-CCG GAA AGC TTG AGC TCT TCT TTA CCT CTG CCT AAT CA-3', nt 1821 to 1841); FullAS (5'-CCG GAA AGC TTG AGC TCT TCA AAA AGT TGC ATG GTG CTG G-3', nt 1823 to 1806). The second round of PCR reactions used three sets of primers to obtain mutually overlapping HBV DNA fragments. The following primers, previously designed by Kanada et al. (13) were used: BF1s (5'-TTT TTC ACC CCT GCA TCA-3', nt 1821 to 1841), BR5 (5'-AAC TGG AGC ACA CAG CAG GA-3', nt 74-55), BF4 (5'-GTC ACC ATA TTG GGT AG-3', nt 2816 to 2835), BR7 (5'-GGG TTC AAA TGT ATA CCC AG-3', nt 839-820), BF7 (5'-TAT TGG GGG CCA AGT CTG TA-3', nt 752 to 771) and BR1s (5'-AAA AAG TTG CAT GGT GCT GG-3', nt 1825 to 1806). All PCR reactions were performed using the TaKaRa Ex Taq™ Hot Start Version system (Takara Bio, Shiga, Japan). After purification, the DNA fragments were directly sequenced using the GenomeLab™ Dye Terminator Cycle Sequencing with the Quick Start Kit and the CEQ8000 DNA sequencer (Beckman Coulter, Tokyo, Japan). The cycle sequencing reaction was conducted using primers designed in a previous report (13): BF2 (5'-CAG ACA ACT ATT GTG GGT TC-3', nt 2191 to 2210), BF3 (5'-TCT TTA ATC TCG AGT GGC AA-3', nt 2512 to 2531), BF5 (5'-AAG AGA CAG CAG TCA TCC TCA GA-3' nt 3183 to 3202), BF6 (5'-CCT CCA ATT TGT CCT GCA TA-3', nt 350 to 369), BF8 (5'-TTT ACC CCG TTG CCC GGC A-3', nt 1142 to 1160), BR2 (5'-CAG AGT ATC GTT CCT GAG TG-3', nt 2080 to 2061), BR3 (5'-TTC CCA AGA TTG AGA TCT CTC CT-3', nt 3040 to 3021) and BR6 (5'-GAG CAG GGG TCC TAG GAA TCA-3' nt 193 to 174), as well as the above-mentioned primers, BF1s, BF4, BF7, BR5, BR7 and BR1s. This sequence-based method allowed for the detection of the mutation at an HBV concentration of approximately 9 to 3 log copies/mL. We compared five fully sequenced nucleo-
tide sequences obtained at different time points and determined which nucleotides differed from that observed baseline. Fig. 2. We subsequently translated the nucleotide sequence to the amino acid sequence in the polymerase region, including the RT, S, core and X regions; the differences from baseline are presented in Fig. 2. Mutations were seen in 13 nucleotides (Fig. 2a), with seven mutations in the RT region (Fig. 2b). The lamivudine mutation rtL180M was noted starting at time point 3, and a direct sequence analysis indicated the presence of the lamivudine mutation rtM204V as a second peaked wave at time points 1 and 3 and as the main peaked wave at time points 4 and 5. Meanwhile, three mutations were detected in the S region (Fig. 2c). The mutation s1195M in the S region was identified corresponding to the lamivudine resistance rtM204V mutation in the RT region at time points 1 and 3 and as the main peaked wave at time points 4 and 5, whereas the sF134L mutation in the S region was noted starting at time point 3 in the ‘a’ determinant region. In the core region, three mutations of cP50A, cE77Q and cV149I were observed (Fig. 2d). HBeAg seroclearance subsequently occurred after fluctuations in the HBV viral load. However, there were no precore (G1896A) mutations in the S region at time points 1 and 3 and as the main peaked wave at time points 4 and 5. Meanwhile, three mutations were detected in the S region (Fig. 2c). The mutation s1195M in the S region was identified corresponding to the lamivudine resistance rtM204V mutation in the RT region at time points 1 and 3 and as the main peaked wave at time points 4 and 5, whereas the sF134L mutation in the S region was noted starting at time point 3 in the ‘a’ determinant region. In the core region, three mutations of cP50A, cE77Q and cV149I were observed (Fig. 2d). HBeAg seroclearance subsequently occurred after fluctuations in the HBV viral load. However, there were no precore (G1896A) or basal core promoter (A1762T, G1764A) mutations from points 1 to 5.

The data were retrospectively obtained from the patients’ records, and informed consent was obtained for this study. The study protocol was carried out in accordance with the Declaration of Helsinki.

Discussion

We herein experienced a patient whose chronic hepatitis B virus stabilized due to HBeAg and HBsAg seroclearance without treatment involving any additional nucleotide analogues. We searched for viral mutations starting before the second episode of acute exacerbation and at several time points at which the viral load was elevated. Consequently, the lamivudine mutation rtL204V was recognized as a second peaked wave starting at time point 1. Furthermore, the rtL180M mutation emerged at time point 3 in correlation with the sF134L mutation, which corresponds to the S gene sF134L mutation. Because the S gene is overlapped by the polymerase gene, whenever a nucleotide mutation occurs in the polymerase gene, a concomitant nucleotide mutation develops in the S gene (14). The mutation in sF134L/rtS143T emerged based on the following two main possible explanations: the sF134L mutation induced by host immune pressure forced the development of the rtS143T mutation and the lamivudine-resistant rtS143T mutation forced the development of the sF134L mutation. In this case, the former possibility is that lamivudine reduced the viral load of HBV DNA, HBs antigens and HBC antigens and consequently activated host immune responses to HBV, thus allowing preexisting M204V mutations to become dominant. The sF134L mutation may be induced under immune pressure (15). Finally, the patient’s immune responses inducing the core mutations resulted in HBs seroclearance. As to the latter scenario, there were no S gene mutations initially and the rtS143T mutations emerged at time point 3 concomitantly with the rtL180M mutation, a lamivudine mutation; thus, the rtS143T mutation associated with lamivudine may have forced the development of the sF134L mutation. Although we cannot deny the possibility that both amino acid changes were significant and not due to viral persistence, the sF134L mutation and core mutation are key mutations in this case. However, further in vitro studies are needed to elucidate the role of the sF134L mutation.

In addition, although we did not detect sG145R in this case, Sheldon et al. reported that some mutations associated with lamivudine resistance may cause premature stop codons in the S gene, resulting in the impaired secretion of HBsAg (10). The sF134L mutation exists in the ‘a’ determinant (amino acids 124 to 147), which is the major target of neutralizing antibodies. This mutation, which exists in the ‘a’ determinant, may also impair the secretion of HBsAg. The level of HBsAg decreased dramatically after the emergence of the sF134L mutation and subsequently remained low (Fig. 1A, bottom), while the level of HBcAg decreased in correlation with the decline in the HBV DNA titer. The HBcAg titer reflects the amount of HBcAg, HBeAg and p22cr translated from mRNA transcribed from intrahepatic covalently closed circular DNA. These results indicate that both the impaired secretion of HBsAg and immune reaction were associated with the reduction in HBV DNA. Moreover, from the time point of 4, core mutations occurring in the cP50A [T helper T1 (C1-C20), T2 (C50-C69), T3 (C117-C131), B cell (C130-C138) and T-cytotoxic: CTL (C18-C27)] epitope associated with HBV clearance have been reported (16), and the cP50A mutation is present in the helper T2 epitope. This mutation may also have contributed to the patient’s viral clearance in this case. Furthermore, in the current case, the core mutation may have induced the increased clearance of HBV due to the immune reaction. Following the suppression of HIV RNA with successful treatment for HIV, the CD4 level became elevated and the HBV DNA titer increased. Therefore, the patient’s immune reaction and impaired secretion of HBsAg may account for the dramatic decrease in the HBsAg level and ultimate clearance of HBsAg. Moreover, there is a paper in which a mutation near the ‘a’ determinant and a core mutation were found to interact with respect to HBV virion secretion (17). Further investigation is therefore required to clarify whether immune pressure on the core region and various mutations in the ‘a’ determinate region, such as sF134L or both, increases the probability of HBsAg or HBeAg seroclearance in a large number of chronic hepatitis B patients with lamivudine resistance. In addition, the further accumulation of longitudinal data as well as virological analyses of the surface and core region and T-cell proliferation analyses are also needed.

We were consulted on this case prior to the patient’s second episode of acute exacerbation, at which point we decided to restart the dose of lamivudine in order to control
the wild-type HBV infection. Since the liver biopsy showed relatively mild fibrosis (A2/F1), guidelines for treating HBV/HIV coinfected patients state that drugs active against HIV, such as emtricitabine, lamivudine, tenofovir and entecavir, were not suitable for HIV treatment in this case (18); rather, adeovir and tellbuvudine, which have not been proven to exhibit an antiviral activity against HIV, are preferred (19). However, at that time, we were unable to use adeovir and tenofovir due to health insurance restrictions in Japan. Moreover, since the administration of interferon-based treatment was unlikely to be successful due to the patient’s high HBV viral load at the time, we restarted the treatment with lamivudine in order to suppress the wild-type HBV infection.

Resistance to lamivudine develops in the majority of HBV/HIV-coinfected patients after prolonged use. Adding tenofovir to the treatment regimen is one of the most promising strategies in such cases, as the antiviral efficacy of tenofovir is not influenced by the presence of lamivudine resistance (20). The introduction of tenofovir and emtricitabine into the HBV armamentarium for HBV/HIV-coinfected patients provides another treatment option (4). However, since HBsAg seroclearance is rare, long-term maintenance therapy and regimens containing multiple drugs may be required in patients with HBV and more so in those with HBV/HIV coinfecion. If the rate of HBV replication is reduced and the ALT level is within the normal range, it is preferable not to use unnecessary antiviral agents in order to prevent the potential for additional mutations in both HBV and HIV. The details of the present case shed light on some of the mechanisms associated with HBsAg and HBeAg seroclearance.

The authors state that they have no Conflict of Interest (COI).

References

1. Lai CL, Dienstag J, Schiff E, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 36: 687-696, 2003.
2. Allen MI, Deslauriers M, Andrews CW, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 27: 1670-1677, 1998.
3. McMahon MA, Jilek BL, Brennan TP, et al. The HBV drug entecavir: effects on HIV-1 replication and resistance. N Engl J Med 356: 2614-2621, 2007.
4. Nunez M, Soriano V. Management of patients co-infected with hepatitis B virus and HIV. Lancet Infect Dis 5: 374-382, 2005.
5. Puoti M, Torri C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. J Hepatol 44 (1 Suppl): S65-S70, 2006.
6. Benhamou Y. Antiretroviral therapy and HIV/hepatitis B virus co-infection. Clin Infect Dis 38 (Suppl 2): S98-S103, 2004.
7. Miallhès P, Trabaud MA, Pradat P, et al. Impact of highly active antiretroviral therapy (HAART) on the natural history of hepatitis B virus (HBV) and HIV coinfection: relationship between prolonged efficacy of HAART and HBV surface and early antigen seroconversion. Clin Infect Dis 45: 624-632, 2007.
8. Sheldon J, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. J Antimicrob Chemother 61: 766-768, 2008.
9. Torresi J, Earnest-Silveira L, Cividino G, et al. Restoration of replication phenotype of lamivudine-resistant hepatitis B virus mutants by compensatory changes in the “fingers” subdomain of the viral polymerase selected as a consequence of mutations in the overlapping S gene. Virology 299: 88-99, 2002.
10. Sheldon J, Rodes B, Zoulim F, Bartholomeusz A, Soriano V. Mutations affecting the replication capacity of the hepatitis B virus. J Viral Hepat 13: 427-434, 2006.
11. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAIVIR Cooperative Study Group. Hepatology 24: 289-293, 1996.
12. Gunther S, Li BC, Miska S, Kruger DH, Meisel H, Will H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunsuppressed patients. J Virol 69: 5437-5444, 1995.
13. Kanada A, Takehara T, Ohkawa K, et al. Type B fulminant hepatitis is closely associated with a highly mutated hepatitis B virus strain. Intervirology 50: 394-401, 2007.
14. Yeh CT. Development of HBV S gene mutants in chronic hepatitis B patients receiving nucleotide/nucleoside analogue therapy. An- tivir Ther 15: 471-475, 2010.
15. Hou J, Wang Z, Cheng J, et al. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. Hepatology 34: 1027-1034, 2001.
16. Bozuya H, Ayola B, Lok AS. High rate of mutations in the hepatitis B core gene during the immune clearance phase of chronic hepatitis B virus infection. Hepatology 24: 32-37, 1996.
17. Chaub PW, Wang RG, Lin MH, Masuda T, Suk FM, Shih C. Reduced secretion of virions and hepatitis B virus (HBV) surface antigen of a naturally occurring HBV variant correlates with the accumulation of the small S envelope protein in the endoplasmic reticulum and Golgi apparatus. J Virol 79: 13483-13496, 2005.
18. Joshi D, O’Grady J, Dieterich D, Gazzard B, Agarwal K. Increasing burden of liver disease in patients with HIV infection. Lancet 377: 1198-1209, 2011.
19. EASL Clinical Practice Guidelines. management of chronic hepatitis B. J Hepatol 50: 227-242, 2009.
20. de Vries-Slujs TE, Reijnders JG, Hansen BE, et al. Long-term therapy with tenofovir is effective for patients co-infected with human immunodeficiency virus and hepatitis B virus. Gastroenterology 139: 1934-1941, 2010.

© 2015 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imonline/index.html