ORIGINAL ARTICLE

Gut and Liver, Vol. 9, No. 1, January 2015, pp. 103-108

Long-Term Outcomes and Dynamics of Mutants Associated with Lamivudine-Adefovir Rescue Therapy in Patients with Lamivudine-Resistant Chronic Hepatitis B

Jihyun Kim, Sae Hwan Lee, Hong Soo Kim, Kanghyug Choi, Soung Won Jeong, Sang Gyune Kim, Jae Young Jang, Young Seok Kim, and Boo Sung Kim

Department of Internal Medicine, Soonchunhyang University College of Medicine, Cheonan, Korea

Background/Aims: To investigate the association between the baseline profiles and dynamics of hepatitis B virus (HBV) DNA polymerase gene mutations and the long-term virological response of lamivudine (LAM)-adefovir (ADV) combination therapy in patients with LAM-resistant chronic hepatitis B. **Methods:** Seventy-five patients who received LAM-ADV combination therapy for more than 12 months were analyzed. Restriction fragment mass polymorphism assays were used to detect and monitor the dynamics of LAM- and ADV-resistant mutations. **Results:** The median duration of LAM-ADV combination therapy was 26 months (range, 12 to 58 months). The baseline mutation profiles, rtM204I (p=0.992), rtM204I/V (p=0.177), and rtL180M (p=0.051), were not correlated with the cumulative virological response, and the baseline HBV DNA level (p=0.032) was the only independent predictive factor for cumulative virological response. Tests for LAM- and ADV-resistant mutations were performed in 12 suboptimal responders in weeks 48 and 96. The population of rtM204 mutants persisted or increased in 8 of 12 patients, and rtA181T mutants newly emerged as a minor population in four patients until 96 weeks. Nevertheless, the viral loads progressively decreased during rescue therapy, and these dynamics did not correlate with virological response. **Conclusions:** The baseline profile and dynamics of LAM-resistant mutations during LAM-ADV combination therapy are not associated with a virological response. **(Gut Liver 2015;9:103-108)**

Key Words: Hepatitis B virus; Lamivudine resistance; Restriction fragment mass polymorphism; Mutation

INTRODUCTION

Lamivudine (LAM) has been used as a first-choice therapy for chronic hepatitis B because of its potency and safety.[1,2] However, the efficacy of long-term LAM therapy is compromised by resistance to the drug, and LAM-resistant mutation increases by 16% to 32% at 1 year and up to 70% at 4 years of therapy.[3,4] The emergence of this mutation has become a persistent problem in patients under long-term LAM monotherapy, thus making the management of LAM resistance is a major issue in the treatment of chronic hepatitis B.

Adefovir dipivoxil (ADV), an oral pro-drug of adefovir, has an antiviral activity not only in treatment-naive patients but also in those with LAM-resistant mutation.[5,6] LAM-ADV combination therapy remarkably reduces the emergence of ADV-resistant mutation compared with ADV monotherapy in patients with LAM-resistant chronic hepatitis B. Thus, this combination therapy has been recommended as one of standard treatment plan for such patients.[7-9] However, long-duration of LAM-ADV combination for achieving virological response was needed in patients with LAM-resistant chronic hepatitis B and it does not always guarantee complete response. A high level of hepatitis B virus (HBV) DNA, a lower level of alanine transaminase (ALT), the presence of hepatitis B e antigen (HBeAg), and the presence of liver cirrhosis were found to be associated with poor virological response in long-term LAM-ADV combination therapy.[10-12]

Various mutations in the HBV DNA polymerase gene have caused antiviral agent resistance and rtM204 mutations were strongly contributed to LAM-resistance. Mutation profiles emerged during LAM therapy was associated with clinical

Correspondence to: Hong Soo Kim
Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, 31 Suncheonhyang 6-gil, Dongnam-gu, Cheonan 330-721, Korea
Tel: +82-41-570-3837, Fax: +82-41-574-5762, E-mail: khskhs@schmc.ac.kr
Received on January 15, 2014. Revised on March 23, 2014. Accepted on April 7, 2014. Published online on October 7, 2014
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl14018
Jihyun Kim and Sae Hwan Lee contributed equally to this work as first authors.

outcome of rescue therapy and rtM204I were associated with favorable outcome during ADV rescue therapy in several reports.[13,14] However, this result was not constantly revealed in other studies.[10,11] Furthermore, these initial mutants were persistently detected in patients with suboptimal response to ADV add-on therapy but association between clinical significance of these dynamics of mutants and clinical outcomes was not conclusively identified in previous studies.[15-17]

The aims of this study were to investigate the association between the baseline mutation profile in HBV DNA polymerase gene and the population dynamics of mutants, and the virological response of LAM-ADV combination therapy in patients with LAM-resistant HBeAg-positive chronic hepatitis B.

MATERIALS AND METHODS

1. Study population

Between October 2006 and September 2010, 83 LAM-resistant HBeAg-positive chronic hepatitis B patients were consecutively enrolled in Soonchunhyang University Hospital, Cheonan, Korea. All 83 patients had experienced virological breakthrough and documented LAM-resistant mutations using restriction fragment mass polymorphism (RFMP) assay. A follow-up evaluation was performed every 3 months, wherein serum biochemistry was examined and tests for HBeAg, anti-HBe antibody, and HBV-DNA level were performed. The exclusion criteria were as follows: (1) insufficient follow-up time (<12 months); (2) coinfection with hepatitis C or human immunodeficiency virus; (3) presence of other causes of chronic liver disease including hepatotoxic drug, autoimmune hepatitis, Wilson's disease, or primary biliary cirrhosis; (4) presence of hepatocellular carcinoma beyond the Milan criteria; (5) presence of any clinical evidence of hepatic decompensation such as esophagogastric variceal bleeding, uncontrolled ascites, and hepatic encephalopathy; and (6) heavy alcohol consumption (>60 g of alcohol per day over 6 months). Of the 83 subjects, eight were excluded from the study because of loss to follow-up (n=6) and withdrawal of informed consent (n=2). This study was approved by the Institutional Ethics Committee of Soonchunhyang University Hospitals, Cheonan, Korea.

2. Methods and definitions

HBsAg, HBeAg, and anti-HBe antibody were detected using microparticle enzyme immunoassays available as commercial kits (Abbott, Wiesbaden, Germany). Serum HBV DNA levels were monitored every 3 months with the COBAS AmpliPrep/COBAS TaqMan HBV kit (Roche Diagnostics, Mannheim, Germany), which has a lower detection limit of 100 copies/mL. Virological response and biochemical response were defined as undetectable serum HBV DNA levels (<100 copies/mL) during treatment and a decrease of the serum ALT levels to within the normal range, respectively. Virological breakthrough was defined as an increase in the serum HBV DNA level by 1 log10 above the nadir on at least two consecutive occasions, and suboptimal response was defined as residual HBV DNA levels of 4 $\log_{10}$ copies/mL or higher at week 48.[18] Liver cirrhosis was diagnosed by histology or imaging features along with the presence of thrombocytopenia, esophageal varices, ascites or encephalopathy.[19]

3. Detection of HBV polymerase mutations

LAM-associated mutations including rtL180M and rtM204I/V were identified at the baseline by using the RFMP assay, which was performed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry as described previously.[20] The RFMP assay showed a high sensitivity for detection of minor mutant populations in mixtures of wild-type and mutant viruses. It had showed a clear correlation between the peak ratios and relative genotype concentrations of different YMDD motif-containing plasmids in mixed populations, so the changes of relative population of mutants can be assessed by RFMP assay during antiviral therapy. Serial blood samples were collected from patients every 3 months during the LAM-ADV combination therapy and stored at 70°C. For evaluation of the dynamics of mutants during rescue therapy, rtL180, rtA181, rtM204, and rtN236 mutations were tested using RFMP assay at baseline, 48 weeks, and 96 weeks of LAM-ADV combination therapy.

4. Statistical analysis

All data are expressed as median (range). The chi-square and Fisher exact tests were used to compare variables between groups. The Mann-Whitney U-test was used for continuous variables. The cumulative probability of virological response was calculated using the Kaplan-Meier method and differences between groups were calculated using log-rank test. Cox regression was applied to evaluate the effect of each clinical variable on cumulative virological response. Statistical significance was set at $p<0.05$. All analyses were performed using the SPSS software version14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Baseline characteristics of the study subjects

The median age of the subjects was 45 years (range, 20 to 81 years). The median baseline serum HBV DNA level was 7.4 $\log_{10}$ copies/mL (range, 4.2 to 9.7 $\log_{10}$ copies/mL). The median duration of LAM-ADV combination therapy was 26 months (range, 12 to 58 months). The rtM204I mutation was identified in 37 (49%), rtM204V in 27 (36%), and both mutations in 11 patients (15%). The rtL180M mutation was detected in 51 patients (68%) (Table 1). Thirty-three patients (87%) with rtM204V mutation and 24 patients (50%) with rtM204I mutation were accompanied with rtL180M mutation.

2. Clinical outcomes of LAM-ADV combination therapy

The cumulative virological response rates were 8%, 21%, and 36% after 24, 48, and 96 weeks, respectively (Fig. 1). The cumulative biochemical response rates were 77%, 84%, and 86% at 24, 48, and 96 weeks, respectively. The HBeAg seroconversion rates were 1.3% and 5.7% at 48 and 96 weeks, respectively. Two patients experienced ADV-resistant mutation with virological breakthrough at 84 and 96 weeks of LAM-ADV combination therapy.

3. Predictive factors for virological response

In univariate analysis, lower baseline HBV DNA level (p=0.017) was significantly associated with cumulative virological response but age (p=0.864), gender (p=0.083), cirrhosis (p=0.166), and higher baseline ALT (p=0.578) were not related with cumulative virological response. Baseline mutation profiles, rtL180M (p=0.051), rtM204I (p=0.992), and rtM204I/V (p=0.177) were not correlated with cumulative virological response (Table 2). Multivariate analysis revealed that only lower baseline HBV DNA level (p=0.032) still remained as a significant independent predictive factor for cumulative virological response during LAM-ADV combination therapy.

Table 1. Baseline Characteristics of the Study Subjects

Characteristic	Value
No. of patients	75
Age, yr	45 (20–81)
Sex, male/female	50/25
Diagnosis, chronic hepatitis/cirrhosis	67/8
HBeAg positivity	75 (100)
ALT, IU/L	82 (14–1,864)
Creatinine, mg/dL	0.8 (0.4–1.1)
HBV DNA, $\log_{10}$ copies/mL	7.4 (4.2–9.7)
Duration of lamivudine therapy, mo	25 (9–96)
Duration of lamivudine-adefovir therapy, mo	26 (12–58)
Lamivudine-resistant mutation	
rtM204V/I	75 (100)
rtM204I	37 (49)
rtM204V	27 (36)
rtM204I+V	11 (15)
rtL180M	51 (68)

Data are presented as median (range) or number (%).
HBeAg, hepatitis B e antigen; ALT, alanine aminotransaminase; HBV, hepatitis B virus.

4. Dynamics of LAM- and ADV-resistant mutations in patients with suboptimal virological response

In 32 patients with suboptimal response during 48 weeks of LAM-ADV combination therapy, serial blood samples from baseline to week 96 with 12-week intervals were available in 12 patients. LAM-resistant (rtL180M and rtM204I/V) and ADV-resistant (rtA181V/T and rtN236T) mutation profiles were followed through RFMP assay at weeks 48 and 96 of the combina-

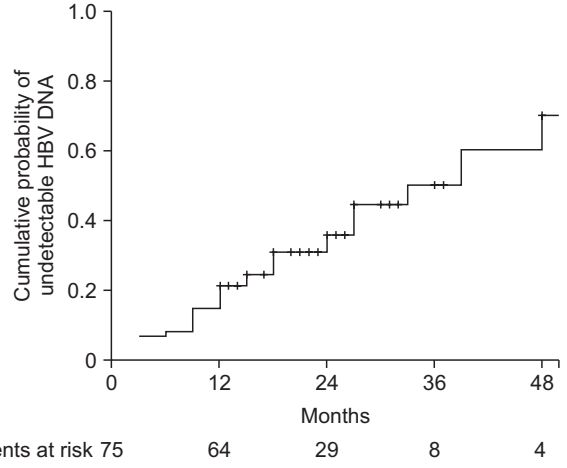

Fig. 1. Cumulative virological response rate after LAM-ADV combination therapy.
HBV, hepatitis B virus; LAM, lamivudine; ADV, adefovir.

Table 2. Univariate and Multivariate Analysis of the Baseline Factors Associated with Cumulative Virological Responses

	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (<40 yr)	0.93 (0.42–2.04)	0.864	-	-
Gender (male)	0.51 (0.24–1.09)	0.083	0.81 (0.36–1.81)	0.611
Cirrhosis (no)	0.46 (0.15–1.37)	0.166	0.51 (0.16–1.61)	0.248
ALT (>200 IU/mL)	1.26 (0.55–2.89)	0.578	-	-
Baseline HBV-DNA (<7 $\log_{10}$ copies/mL)	2.45 (1.17–5.12)	0.017	2.33 (1.07–5.07)	0.032
rtL180M mutation (yes)	2.61 (0.99–6.81)	0.051	2.44 (0.91–6.55)	0.077
rtM204I (versus rtM204V+rtM204I/V)	1.01 (0.48–2.06)	0.992	-	-
rtM204I/V (versus rtM204I+rtM204V)	0.37 (0.08–1.56)	0.177	0.28 (0.66–1.23)	0.094

OR, odds ratio; CI, confidence interval; ALT, alanine aminotransaminase; HBV, hepatitis B virus.

Table 3. Hepatitis B Virus DNA Level and Mutation Profiles during Lamivudine-Adefovir Combination Therapy

Patients	Before the combination therapy					After 48 weeks of combination therapy					After 96 weeks of combination therapy				
	HBV DNA*	rt180 L:M†	rt204 M:I:V	rt181 A:T	rt236 N:T	HBV DNA	rt180 L:M	rt204 M:I:V	rt181 A:T	rt236 N:T	HBV DNA	rt180 L:M	rt204 M:I:V	rt181 A:T	rt236 N:T
1	8.09	1:0	1:2:0	1:0	1:0	5.31	2:1	0:7:1	1:0	1:0	3.16	3:1	0:7:1	1:0	1:0
2	5.78	1:0	0:1:0	1:0	1:0	6.12	1:0	1:8:0	1:0	1:0	6.11	1:0	1:1:0	2:1	1:0
3	6.82	1:0	2:2:1	1:0	1:0	5.46	9:1	7:1:0	1:1	1:0	5.94	1:0	1:0:0	5:1	1:0
4	9.01	1:0	0:1:0	1:0	1:0	4.98	7:1	1:1:0	6:1	1:0	4.61	1:0	6:1:0	9:1	1:0
5	7.01	0:1	0:1:0	1:0	1:0	4.76	1:3	1:7:0	1:0	1:0	5.61	1:1	1:1:0	3:1	1:0
6	7.85	1:2	0:1:0	1:0	1:0	5.37	2:1	0:1:0	1:0	1:0	4.93	2:1	0:1:0	1:0	1:0
7	8.91	1:0	0:1:0	1:0	1:0	5.42	1:0	0:1:0	1:0	1:0	5.61	1:0	0:1:0	1:0	1:0
8	8.81	1:0	0:1:0	1:0	1:0	5.47	1:0	0:1:0	1:0	1:0	4.36	1:0	0:1:0	1:0	1:0
9	9.01	0:1	0:0:1	1:0	1:0	5.74	0:1	0:0:1	1:0	1:0	6.08	0:1	0:0:1	1:0	1:0
10	8.89	1:3	0:1:1	1:0	1:0	5.01	1:5	0:1:4	1:0	1:0	4.98	1:4	0:1:4	1:0	1:0
11	7.71	1:0	0:1:0	1:0	1:0	6.31	1:0	0:1:0	1:0	1:0	3.61	1:0	0:1:0	1:0	1:0
12	8.93	0:1	0:0:1	1:0	1:0	5.76	0:1	0:0:1	1:0	1:0	5.38	0:1	0:0:1	1:0	1:0

HBV, hepatitis B virus.
*Log_{10} copies/mL; †Ratio of wild-type to mutants.

tion therapy. None of these 12 patients had ADV-resistance at the start of the combination therapy. Baseline rtL180 mutation was found in five patients, and this mutation was persisted until week 96 in five patients. The rt180 mutation newly emerged in three patients at week 48 and detected in one of them at week 96 (Table 3). The LAM-resistant mutation, rtM204 persisted in all of 12 patients after 48 weeks and 11 of 12 patients at 96 weeks of combination therapy.

The relative population of wild-type and mutant virus was analyzed by RFMP assay at baseline, weeks 48 and week 96. The relative numbers of rtM204 mutants was increased in one (patient 1), decreased in four (patient 2–5), and sustained in seven (patient 6–12) of 12 suboptimal responders (Table 3). Nine of the 12 patients persistently showed suboptimal response at week 96, but none of the 12 patients experienced virological breakthrough. However, HBV DNA levels during 96 weeks of rescue therapy of the 12 suboptimal responders were progressively decreased and not correlated with the trend of the viral population (Fig. 2). The ADV-resistant mutation, rtN236T was not found in 12 patients at 48 and 96 weeks of rescue therapy; however, rtA181T mutants emerged as a minor population in two and four patients at weeks 48 and 96, respectively. However, none of these four patients who developed the rtA181T mutation showed virological breakthrough during 96 weeks of LAM-ADV combination therapy.

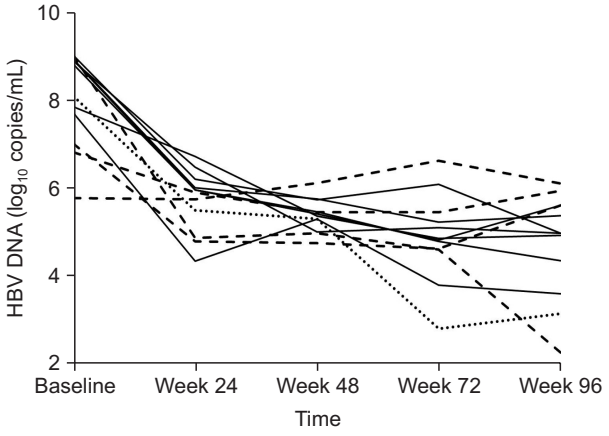

Fig. 2. Serum hepatitis B virus (HBV) DNA levels during 96 weeks of lamivudine-adefovir combination therapy in patients with suboptimal responses. Dotted line, patients with increasing mutant populations; dashed lines, patients with decreasing mutant populations; and solid lines, patients with sustained mutant populations.

DISCUSSION

The emergence of mutations in the HBV polymerase gene has become a major problem during LAM therapy in patients with chronic hepatitis B, and many studies have demonstrated the superiority in treatment efficacy and drug-resistance prevention of on-going LAM to ADV rescue therapy.[9,10,21] Although this add-on therapy was one of the standard treatments for LAM-resistant chronic hepatitis B, its efficacy was limited in HBeAg-positve patients.[10–12] In this study, mutation profiles of rtL180 and rtM204 prior LAM-ADV rescue therapy and dynamics of mutants in several suboptimal responders were not associated with cumulative virological response in HBeAg-positive LAM-resistant chronic hepatitis B patients.

In this study, in 11 of 12 suboptimal responders, rtM204 mutants were persistently detected by RFMP assay during 96 weeks of LAM-ADV combination therapy. Several studies reported that

LAM-resistant mutation were prolonged in patients with poor response to LAM-ADV combination therapy.[15,16] Because the clinical dose of ADV 10 mg was insufficient for controlling HBV replication, ADV has shown poorer clinical outcomes compared with other antiviral agents in treatment-naive patients with chronic hepatitis B. This is the primary reason for the persistence of LAM-resistant mutation in suboptimal responders during LAM-ADV combination therapy. Another possible reason is on-going LAM to prevent the emergence of ADV-resistant mutations, and LAM maintenance itself confers a strong selective pressure toward the emergence of LAM-resistant mutations.[9,22,23]

The rtL180M could compensate for the impact of a mutation in the YMDD motif on viral replication *in vitro*.[24] In the present study, baseline rtL180M was not associated with cumulative virological response. Furthermore, rtL180M mutations were newly emerged in four patients as minor population at 48 weeks of LAM-ADV combination therapy but they were restored as baseline mutation profile at 96 weeks. It is also observed in the trends of YMDD mutations during combination therapy. Therefore, LAM-resistant mutation, rtL180 and rtM204 profiles, was persistently detected with minor evolution by RFMP assay in assessable subjects of this study and this trends were not associated with virological response during 96 weeks of LAM-ADV combination therapy.

The relative population of mutants and wild-type virus could be evaluated through RFMP assay. Even though long-term LAM-ADV rescue therapy guarantee favorable outcome in several patients, the mutant virus was the major population in more than half of the suboptimal responders. In this study, rtA181 mutants emerged in four patients who showed rtM204 mutants gradually decreased during rescue therapy. However, there is no association between the newly emerging mutants and virological response in these four suboptimal responders. Furthermore, near all of 12 suboptimal responders had experienced gradual decrease of HBV DNA level, the changes of relative population of mutants was not compatible with these HBV DNA changes. Interestingly, in patient 1, the total viral load progressively decreased, but the rtM204 mutant population increased with that having a newly emerged compensatory mutation, rtL180, during the LAM-ADV combination therapy. Thus, we observed that viral evolution during LAM-ADV rescue therapy was not significantly correlated with clinical outcome.

A study from Italy reported the rtA181 substitution, which was detected at the initiation of LAM-ADV combination therapy, was associated with poor virological response.[25] In the present study, the rtA181 mutation emerged during 96 weeks of LAM-ADV combination therapy in four suboptimal responders who did not have the mutation at baseline. Both LAM and ADV can be a selection pressure on emerging of rtA181 mutation, thus, the exact cause for the emergence of this mutation was not clear during the combination therapy.[26] However, the relative population of the rtA181 mutants was smaller than that of the wild-type virus, and these four patients with rtA181 mutation did not experience virological breakthrough during 2 years of LAM-ADV combination therapy. Several studies have reported that patients with a minor population of HBV polymerase gene mutation rarely show viral breakthrough during prolonged antiviral therapy.[27-29]

Some of the limitations of this study include methods the assay work in mutation detection. RFMP assay is very sensitive and reproducible in the clinical field. However RFMP assay has to design target site before assay and it could detect limited domain. Therefore, this assay could not evaluate various or entire region of polymerase gene, unlikely direct sequencing or cloning assay. Second is analysis for dynamics of mutants had not done in all suboptimal responders and subjects with virological breakthrough because available blood samples were limited for this study.

In summary, baseline mutation profile in HBV DNA polymerase was not associated with virological response to long-term LAM-ADV combination therapy in patients with HBeAg-positive LAM-resistant chronic hepatitis B. Most of the rtM204 mutants persisted up to 96 weeks of rescue therapy, and the evolution of LAM-resistant mutations existing before the combination therapy showed a stationary trend. ADV-resistant mutants were newly emerged, but they remained as a minor population with clinical significance.

In conclusion, baseline profiles and the dynamics of mutations were not correlated with virological response during LAM-ADV combination therapy.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

We want to warmly thank Dr. Seul Ki Min for counseling in statistical analysis.

This study was supported in part by the Soonchunhyang University Research Fund.

REFERENCES

1. Lai CL, Chien RN, Leung NW, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339:61-68.
2. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256-1263.
3. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy.

Hepatology 2001;33:1527-1532.
4. 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 2003;36:687-696.
5. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006;131:1743-1751.
6. Peters MG, Hann HW, Martin P, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology. 2004;126:91-101.
7. Lee YS, Suh DJ, Lim YS, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology 2006;43:1385-1391.
8. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology 2007;45:307-313.
9. Gaia S, Barbon V, Smedile A, et al. Lamivudine-resistant chronic hepatitis B: an observational study on adefovir in monotherapy or in combination with lamivudine. J Hepatol 2008;48:540-547.
10. Yatsuji H, Suzuki F, Sezaki H, et al. Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. J Hepatol 2008;48:923-931.
11. Hosaka T, Suzuki F, Suzuki Y, et al. Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine. J Gastroenterol 2007;42:368-374.
12. Kurashige N, Hiramatsu N, Ohkawa K, et al. Factors contributing to antiviral effect of adefovir dipivoxil therapy added to ongoing lamivudine treatment in patients with lamivudine-resistant chronic hepatitis B. J Gastroenterol 2009;44:601-607.
13. Suzuki F, Kumada H, Nakamura H. Changes in viral loads of lamivudine-resistant mutants and evolution of HBV sequences during adefovir dipivoxil therapy. J Med Virol 2006;78:1025-1034.
14. Kim HJ, Park JH, Park DI, et al. The influence of YMDD mutation patterns on clinical outcomes in patients with adefovir add-on lamivudine combination treatment. Liver Int 2012;32:303-310.
15. Aizawa M, Tsubota A, Fujise K, et al. Clinical course and predictive factors of virological response in long-term lamivudine plus adefovir dipivoxil combination therapy for lamivudine-resistant chronic hepatitis B patients. J Med Virol 2011;83:953-961.
16. Wu S, Fukai K, Imazeki F, et al. Initial virological response and viral mutation with adefovir dipivoxil added to ongoing Lamivudine therapy in Lamivudine-resistant chronic hepatitis B. Dig Dis Sci 2011;56:1207-1214.
17. Inoue J, Ueno Y, Wakui Y, et al. Four-year study of lamivudine and adefovir combination therapy in lamivudine-resistant hepatitis B patients: influence of hepatitis B virus genotype and resistance mutation pattern. J Viral Hepat 2011;18:206-215.
18. van Bömmel F, de Man RA, Wedemeyer H, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology 2010;51:73-80.
19. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology 1988;8:493-496.
20. Hong SP, Kim NK, Hwang SG, et al. Detection of hepatitis B virus YMDD variants using mass spectrometric analysis of oligonucleotide fragments. J Hepatol 2004;40:837-844.
21. Kim HJ, Park JH, Park DI, et al. Rescue therapy for lamivudine-resistant chronic hepatitis B: comparison between entecavir 1.0 mg monotherapy, adefovir monotherapy and adefovir add-on lamivudine combination therapy. J Gastroenterol Hepatol 2010;25:1374-1380.
22. Zoulim F. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antiviral Res 2004;64:1-15.
23. Ijaz S, Arnold C, Dervisevic S, et al. Dynamics of lamivudine-resistant hepatitis B virus during adefovir monotherapy versus lamivudine plus adefovir combination therapy. J Med Virol 2008;80:1160-1170.
24. Ono SK, Kato N, Shiratori Y, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest 2001;107:449-455.
25. Moriconi F, Colombatto P, Coco B, et al. Emergence of hepatitis B virus quasispecies with lower susceptibility to nucleos(t)ide analogues during lamivudine treatment. J Antimicrob Chemother 2007;60:341-349.
26. Yim HJ. Hepatitis B virus genetic diversity and mutant. Korean J Hepatol 2008;14:446-464.
27. Lee CH, Kim SO, Byun KS, et al. Predominance of hepatitis B virus YMDD mutants is prognostic of viral DNA breakthrough. Gastroenterology 2006;130:1144-1152.
28. Yeon JE, Yoo W, Hong SP, et al. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. Gut 2006;55:1488-1495.
29. Lee SH, Kim HS, Byun IS, et al. Pre-existing YMDD mutants in treatment-naive patients with chronic hepatitis B are not selected during lamivudine therapy. J Med Virol 2012;84:217-222.