Response to Adefovir Depends on Mutation Patterns in Precore Region, Basal Core Promoter and Reverse Transcriptase, and On-Treatment Responses in Lamivudine-Resistant Chronic Hepatitis B Patients

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Key Words

Chronic hepatitis B • Lamivudine resistance • Adefovir •
Precore region • Basal core promoter

Abstract

Objective: In vitro studies showed that mutations in the basal core promoter (BCP) or precore (PC) region restore the replication inefficiency of the lamivudine-resistant mutant. The aim of this study was to clarify the effect of molecular characteristics on the antiviral response to adefovir in patients with lamivudine-resistant chronic hepatitis B (CHB). **Methods:** Sixty-six lamivudine-resistant patients who were treated with adefovir monotherapy were studied. Sequences of BCP, PC region and reverse transcriptase were determined before adefovir therapy. In patients with virologic breakthrough, reverse transcriptase sequencing was performed. **Results:** The cumulative probabilities of virologic response were 23.3, 46, 52.7 and 59.5% at years 1, 2, 3 and 4, respectively. PC mutation, the absence of compensatory mutations (rtL80I/V or rtV173L), and a decrease in serum hepatitis B virus (HBV) DNA by 3 log or greater at 6 months were independent predictors of virologic response. The cumulative

probabilities of virologic breakthrough were 0, 12.9, 30.7 and 44.5% at years 1, 2, 3 and 4, respectively. BCP mutation and a less than 3 log decrease in serum HBV DNA at 6 months were 2 independent risk factors for virologic breakthrough. **Conclusion:** Response to adefovir depends on mutation patterns in the BCP, PC region and reverse transcriptase, and on-treatment decreases in serum HBV DNA in lamivudine-resistant CHB patients.

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Introduction

With the introduction of oral nucleoside/nucleotide analogs (NA), NA treatment has prevented the progression of chronic hepatitis B (CHB) and the development of hepatocellular carcinoma (HCC) [1, 2]. In spite of this advancement, resistance to NA is still a major challenge for antiviral therapy.

Lamivudine is one of the widely used NA. However, long-term use of lamivudine increases the risk of drug resistance [3, 4]. Lamivudine resistance harbors the rtM204V/I mutation. In addition, compensatory muta-

tions of rtL180M, rtL80I/V and rtV173L are also present [5–10].

Adefovir has been reported to be effective in suppressing hepatitis B virus (HBV) DNA in NA-naïve and lamivudine-resistant patients [11]. It has been reported that resistance to adefovir is high in lamivudine-resistant patients in Korea [12, 13], suggesting that some geographical and virologic factors may affect antiviral responses to adefovir therapy.

The HBV genotype might have clinical relevance in terms of natural course and antiviral response, especially in those treated with interferon [14, 15], and most studies have reported that NA did not affect the antiviral response in terms of HBeAg loss and resistance [16, 17]. Most CHB patients in Korea are infected with genotype C [18, 19], which frequently harbors mutation A1762T/G1764A in the basal core promoter (BCP) [19–21]. In vitro studies showed that mutations in the precore (PC; G1896A) or in the BCP region of HBV restore the replication inefficiency of lamivudine-resistant mutants [22, 23]. In addition, compensatory mutations of rtL80I/V are usually accompanied by rtM204I [24, 25]. In contrast, rtV173L is usually accompanied by an rtM204V mutation [5]. These compensatory mutations restore the replication competency of lamivudine-resistant viruses similar to the level of wild-type HBV [5, 6]. These observations suggest that molecular characteristics of the mutant virus may affect the antiviral response in lamivudine-resistant patients.

Therefore, the aim of this study was to clarify the effect of molecular characteristics of mutant viruses on the antiviral response to adefovir in lamivudine-resistant CHB patients.

Patients and Methods

In this retrospective cohort study, data were collected from 66 of 70 consecutive, compensated, lamivudine-resistant CHB patients treated with sequential adefovir monotherapy between October 2003 and August 2006.

Patients were included in the study if they were 18 years or older and had compensated liver disease (Child-Pugh class A). Patients were excluded if they had HCC, a liver transplantation, received immunotherapy or chemotherapy, or if they were coinfecting with human immunodeficiency virus, or hepatitis C or D viruses. Patients with hepatic decompensation (hepatic encephalopathy, recent variceal bleeding, uncontrolled ascites, serum bilirubin of >2.0 mg/dl and serum albumin of <3.0 g/dl) and a serum creatinine concentration of >1.4 mg/dl were also excluded. Four patients were excluded from this analysis because serum was not available before the beginning of the adefovir therapy.

HBsAg, anti-HBs, HBeAg and anti-HBe were tested by third-generation microparticle enzyme immunoassays using commercial kits (Abbott, North Chicago, Ill., USA). Serum HBV DNA levels were measured using Cobas Amplicor HBV Monitor kits with a detection limit of 60 IU/ml (Roche Molecular Systems, Pleasanton, Calif., USA). Before August 2005, HBV DNA was measured using a Digene Hybrid Capture assay (Digene Corp., Gaithersburg, Md., USA). Therefore, serum HBV DNA levels in these periods were retested by Cobas Amplicor HBV Monitor, using stored serum at the time of analysis. The HBV genotype was determined by the S gene sequence, and sequences of PC, BCP and reverse transcriptase were determined before the beginning of the adefovir therapy in all patients, as previously reported [19].

Virologic response was defined as an undetectable serum HBV DNA (<60 IU/ml) during treatment, and biochemical response was defined as a decrease in serum alanine transaminase (ALT) levels within the normal range. Virologic breakthrough was defined as an increase in serum HBV DNA by >1 log₁₀ above the nadir while on treatment [26].

During the study period, the Korean national insurance system only covered the use of adefovir monotherapy as a rescue therapy in lamivudine-resistant CHB patients, based on the study by Peters et al. [11]. Therefore, all patients were treated with sequential adefovir monotherapy before December 2007. After that, most patients were recommended to receive a combination therapy of adefovir and lamivudine as most guidelines for CHB recommended add-on combination therapy [26, 27]. Therefore, data collection ended in November 2007. The patients were followed up at 1- to 3-month intervals.

The serum samples were collected by written informed consent from the patients, and the study protocol was approved by the ethics committees of our institutions.

Statistical Analysis

Differences between the categorical variables were analyzed by the χ^2 test. For the continuous variables, the Student t test was used. The cumulative probability of virologic response and the virologic breakthrough rate were calculated by the Kaplan-Meier method, and the difference was determined by the log-rank test. In multivariate analysis, Cox's proportional hazard model was used. $p < 0.05$ (two-tailed) was considered to be statistically significant.

Results

The baseline characteristics of the 66 study patients are shown in table 1. All patients were infected with genotype C, especially subgenotype C2. rtM204I, rtM204V, rtM204I/V mutations and the wild type were found in 36 patients (54.5%), 25 patients (37.9%), 4 patients (6.1%) and 1 patient (1.5%), respectively, prior to adefovir therapy. rtL80I/V mutation was observed in 27 patients. This mutation was observed in 24 patients with the rtM204I mutant, and in 3 patients with the rtM204I/V mixed-type mutant. rtV173L mutation was observed in 9 patients. This mutant was observed in 6 patients with the rtM204V,

Fig. 1. Cumulative rate of virologic response depending on HBV DNA levels. It was higher in patients with serum levels of HBV DNA of $<10^7$ IU/ml than in those with serum levels of HBV DNA of $\geq 10^7$ IU/ml (40, 62.4, 70 and 70% vs. 13.2, 33.2, 37.7 and 50% at years 1, 2, 3 and 4, respectively; $p = 0.018$).

Fig. 2. Cumulative rate of virologic response depending on PC mutation. It was higher in patients with PC mutation than in those without (50, 72.2, 81.5 and 81.5% vs. 7.5, 29.7, 36.1 and 46.8% at years 1, 2, 3 and 4, respectively; $p = 0.001$).

in 2 patients with the rtM204I/V, and in 1 patient with the rtM204I mutant.

During sequential adefovir monotherapy, the cumulative probabilities of virologic response were 23.3, 42.1, 52.7 and 59.5% at years 1, 2, 3 and 4, respectively. A biochemical response was achieved in 62.7, 88, 88 and 88% of the patients at years 1, 2, 3 and 4, respectively. Among 55 HBeAg-positive CHB patients, HBeAg loss was achieved by 7.2, 15, 20.7 and 20.7% at years 1, 2, 3 and 4, respectively. The cumulative rate of virologic response was higher in patients with serum levels of HBV DNA of $<10^7$ IU/ml (mean value of the study) than in those with serum levels of HBV DNA of $\geq 10^7$ IU/ml (40, 62.4, 70 and 70% vs. 13.2, 33.2, 37.7 and 50%; $p = 0.018$) (fig. 1). It was also higher in patients with PC mutation than in those without (50, 72.2, 81.5 and 81.5% vs. 7.5, 29.7, 36.1 and 46.8% at years 1, 2, 3 and 4; $p = 0.001$) (fig. 2). Patients without compensatory mutations of rtL80I/V or rtV173L had a higher probability of virologic response compared to those with these mutations ($p = 0.046$) (fig. 3). There was no difference in virologic response rate according to age, sex, serum ALT level or the pattern of rtM204/rtL180M mutation.

On treatment, the virologic response rate was higher in patients with a 3 log or greater decrease in HBV DNA at 6 months than in patients without (40.6, 76, 82 and 82% vs. 3, 19.1, 27.2 and 41.7% at years 1, 2, 3 and 4, respec-

Table 1. Baseline characteristics of study patients

Age, years	43.0 ± 12.1
Male/female gender, n	54/12
HBV genotypes (C2), n	66 (100%)
Serum ALT, IU/ml	177 (49–738)
HBeAg positive, n	59 (89.4%)
HBV DNA (log), IU/ml	6.96 ± 1.1 (4.4–10.4)
Primary rtM204 mutations, n	
rtM204I	36 (54.5%)
rtM204V	25 (37.9%)
rtM204I/V	4 (6.1%)
rtM204M	1 (1.5%)
Compensatory mutations, n	
rtL180M	46 (69.7%)
rtL80I/V	27 (40.9%)
rtV173L	9 (13.6%)
BCP mutation, n	52 (78.8%)
PC mutation, n	26 (39.4%)
Duration of ADV therapy, months	31.8 ± 10.3

Values are means ± SD unless stated otherwise.
ADV = Adefovir dipivoxil.

tively; $p < 0.001$) (fig. 4). In multivariate analysis, PC mutation, the absence of compensatory mutations (rtL80I/V or rtV173L), and a 3 log or greater decrease in HBV DNA levels at 6 months were independent predictive factors for virologic response (table 2).

Fig. 3. Cumulative rate of virologic response depending on compensatory mutation. Patients without compensatory mutations (rtL80L and rtV173V) had a higher probability of virologic response compared to those with these mutations (rtL80I/V or rtV173L; 31.2, 57.2, 71.4 and 71.4% vs. 17.6, 35.4, 40 and 48.5% at years 1, 2, 3 and 4, respectively; $p = 0.046$).

Fig. 4. Cumulative rate of virologic response depending on decrease in serum levels of HBV DNA at 6 months. The virologic response rate was higher in patients with a 3 log or greater decrease in HBV DNA at 6 months than in patients without (40.6, 76, 82 and 82% vs. 3, 19.1, 27.2 and 41.7% at years 1, 2, 3 and 4, respectively; $p < 0.001$).

Table 2. Independent predictive factors for virologic response during adefovir monotherapy in lamivudine-resistant CHB patients

	Odds ratio	p
PC mutation		0.004
No	1	
Yes	3.5 (1.8–8.1)	
Compensatory mutation (rt80/rt173)		0.025
No	1	
Yes	0.4 (0.2–0.9)	
Decrease in HBV DNA at 6 months		<0.001
<3 log IU/ml	1	
≥3 log IU/ml	4.9 (2.1–11.6)	
HBV DNA before adefovir therapy		0.8
<10 ⁷ IU/ml	1	
≥10 ⁷ IU/ml	0.8 (0.4–2.1)	

Values in parentheses denote 95% CI.

Virologic breakthrough occurred in 19 of the 66 patients. Seventeen of the 19 patients with virologic breakthrough harbored known adefovir-resistant mutants (rtA181T/V, $n = 10$; rtN236T, $n = 5$; rtA181T/V and rtN236T, $n = 2$), while 2 patients did not harbor any

known adefovir-resistant mutant. In a patient without a known adefovir-resistant mutant, a genotypic switch from genotype C to genotype A after virologic breakthrough was observed.

The cumulative probabilities of virologic breakthrough were 0, 12.9, 30.7 and 44.5% at years 1, 2, 3 and 4, respectively. As a result, persistent virologic response, defined as an undetectable HBV until the final follow-up, was achieved only in 27 of the 66 patients (40.1%).

The virologic breakthrough rate was higher in patients with the BCP mutation than in those without (0, 16, 36.3 and 55% vs. 0, 0, 10 and 10% at years 1, 2, 3 and 4; $p = 0.045$) (fig. 5). It was also higher in patients without a 3 log or greater decrease in serum HBV DNA at 6 months than in those with such a decrease (0, 15.4, 43 and 64.5% vs. 0, 10.1, 14 and 14% at years 1, 2, 3 and 4, respectively; $p = 0.018$) (fig. 6). It was also higher in patients with detectable serum HBV DNA at 1 year than in patients with undetectable serum HBV DNA at 1 year (0, 17.3, 39.6 and 54.55% vs. 0, 0, 6.2 and 6.2% at years 1, 2, 3 and 4, respectively; $p = 0.02$). There was no difference in virologic breakthrough rate according to age, gender, HBeAg, serum ALT, PC mutation, serum HBV DNA level or mutation pattern in reverse transcriptase, including rtL80I/V, rtV173L, rtL180M and rtM204. In multivariate analysis,

Fig. 5. Cumulative rate of virologic breakthrough depending on BCP mutation. It was higher in patients with BCP mutation than in those without (0, 16, 36.3 and 55% vs. 0, 0, 10 and 10% at years 1, 2, 3 and 4, respectively; $p = 0.045$).

Fig. 6. Cumulative rate of virologic breakthrough depending on HBV DNA response at 6 months. It was higher in patients without a 3 log or greater decrease in serum HBV DNA at 6 months than in those with such a decrease (0, 15.4, 43 and 64.5% vs. 0, 10.1, 14 and 14% at years 1, 2, 3 and 4, respectively; $p = 0.018$).

the presence of a BCP mutation and a decrease in serum HBV DNA levels at 6 months were 2 independent risk factors for virologic breakthrough (table 3).

Discussion

The goal of antiviral therapy for CHB is to improve the quality of life and survival by preventing the progression of the disease to liver cirrhosis, HCC or death. This goal can be achieved if HBV replication can be suppressed in a sustained manner [28], which could decrease the risk of progression to liver cirrhosis and the development of HCC [1].

In this study, the cumulative probabilities of virologic response and virologic breakthrough rate at year 4 were 59.5 and 44.5%, respectively. An on-treatment persistent virologic response, defined as an undetectable HBV until the final follow-up, was achieved in only 40.1% of the patients. The virologic response at 1 year, using the same definition (<60 IU/ml), was similar to that in previous reports of 22–33.8% [12, 24, 29].

In this study, independent predictive factors for virologic response were the presence of a PC mutation and the absence of any additional compensatory mutations of

Table 3. Independent risk factors for virologic breakthrough during adefovir monotherapy in lamivudine-resistant CHB patients

	Odds ratio	p
BCP mutation		0.05
No	1	
Yes	7.2 (1.0–54.6)	
Decrease in HBV DNA at 6 months		0.01
<3 log IU/ml	1	
≥3 log IU/ml	0.3 (0.13–0.8)	
HBV DNA before adefovir therapy		0.5
< 10^7 IU/ml	1	
≥ 10^7 IU/ml	1.4 (0.5–3.7)	
Values in parentheses denote 95% CI.		

rtL80I/V or rtV173L, in addition to a decrease in serum HBV DNA by 3 log or greater at 6 months. Previous studies suggest that the pretreatment level of serum HBV DNA is a predictive factor for virologic response [30–32]. The pretreatment level of HBV DNA was a predictive factor for virologic response in univariate analysis in this study, but showed marginal significance in multivariate

analysis. This result might be due to our small sample size. An on-treatment decrease in serum HBV DNA level was a good indicator for virologic response, similar to that in NA-naïve patients [33], even in CHB patients with lamivudine resistance. Therefore, on-treatment monitoring of serum HBV DNA is very important in the prediction of virologic response.

In vitro studies show that rtL80I/V, rtV173L, PC and BCP mutations restore the replication inefficiency of a lamivudine-resistant mutant [5, 6, 22, 23]. On this aspect, we hypothesized that these mutations might influence the antiviral response. In this study, rtL80I/V mutation was associated with rtM204I or mixed-type mutation (rtM204I/V). rtV173L mutation was associated with rtM204V or mixed-type mutation, which confirmed previous observations [5, 24, 25]. These results support those of the previous in vitro study that rtL80I or rtV173L enhances the replication efficiency of rtM204 mutant viruses. Therefore, a high replication efficiency of these mutants with rtL80I/V or rtV173L might influence the virologic response to adefovir.

PC mutation was a good indicator for virologic response, whereas BCP mutation did not influence virologic response. Previously, it has been reported that a reversion from PC mutation to the wild type has occasionally been observed in patients treated with lamivudine, suggesting an increased sensitivity to lamivudine in the PC mutant in vivo [34, 35]. However, this phenomenon has not yet been reported for adefovir therapy.

In this study, the cumulative rates of virologic breakthrough were 0, 12.9, 30.7 and 44.5% at years 1, 2, 3 and 4, respectively. Resistances to adefovir were 0, 3, 11, 18 and 29% at years 1, 2, 3, 4 and 5, respectively, in NA-naïve HBeAg-negative CHB patients [36]. Therefore, this study supports the previous report on the result that adefovir resistance increases in patients with lamivudine resistance compared with NA-naïve patients [12]. In this study, most cases of virologic breakthrough were associated with the development of adefovir resistance. Interestingly, a genotypic switch from genotype C to genotype A after virologic breakthrough was observed in 1 patient. Recently, Jardi et al. [37] reported that a selection of genotype A forms mixed strains during antiviral therapy. Therefore, these results might suggest that sensitivity to antiviral therapy might differ between genotypes.

Resistance to adefovir was reported to be 0–18% at 1 year in patients with lamivudine resistance [11–13, 30, 32, 38–40]. However, the virologic breakthrough rate at 1 year was relatively low in this study. This result might be

attributed to the methodological difference. Previous studies tested resistance profiles at 1 year regardless of virologic breakthrough. However, we only performed mutational analyses if the patients showed virologic breakthrough. Yeon et al. [13] reported that genotypic resistance to adefovir in lamivudine-resistant patients occurred in 6.4% of the cases at 1 year, and the virologic breakthrough rate was 0% at 1 year. In addition, Fung et al. [41] and Liu et al. [29] did not find any genotypic resistance to adefovir during 48 weeks of adefovir monotherapy in lamivudine-resistant CHB patients. Recently, Idilman et al. [30] reported that the cumulative probabilities of virologic breakthrough rate were 1.2, 15.1 and 37.3% at years 1, 2 and 3, respectively, which was very similar to the results of our study.

Several factors are associated with the development of antiviral resistance, but the key ones, based on our current understanding, are the potency of the antiviral agents, the replication fitness of HBV and the genetic barrier of the antiviral agent, as well as host factors [26, 42].

In previous studies, patients with a high baseline viral load were more likely to develop adefovir resistance [38, 39]; however, some studies did not show the same results [13, 31, 41]. Our study also denied the correlation between pretreatment serum HBV DNA level and virologic breakthrough. We showed that the levels of HBV DNA at 6 months and 1 year – these on-treatment responses usually reflect the drug potency in each individual – were associated with virologic breakthrough. This observation was in line with some previous studies [30, 36, 38, 43], suggesting that the on-treatment HBV response might be important to predict adefovir resistance.

Interestingly, the BCP mutation increased the probability of adefovir resistance regardless of the HBV DNA level and on-treatment response. In vitro studies showed that the presence of a BCP mutation increased viral replication above the wild-type baseline level in the wild type [44–47] and even in the lamivudine-resistant mutant viruses [23]. Therefore, the increasing replication fitness of the BCP mutation in lamivudine-resistant viruses might persist and eventually induce virologic breakthrough during adefovir treatment.

However, we observed different virologic responses and breakthroughs according to mutation patterns (rtL80I/V, rtV173L, PC and BCP mutations), even though these mutants have been reported to increase replication fitness in vitro [5, 6, 22, 23]. This finding suggests that other factors than replication fitness, such as immune systems, or host cellular enzymes necessary for converting prodrugs to their active compounds or for phosphor-

ylating antiviral agents to their triphosphates [42, 48], influence virologic outcomes.

The major limitation of this study is that sequential adefovir monotherapy is not recommended any more for lamivudine-resistant CHB patients according to many guidelines [26, 28]. Therefore, our data can be applied only to sequential adefovir monotherapy patients.

In conclusion, the response to adefovir therapy depends on mutation patterns in the PC region, BCP and

reverse transcriptase, and on the on-treatment decrease in serum HBV DNA in lamivudine-resistant CHB patients.

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