Original Article

Antiviral Efficacy of Entecavir versus Entecavir plus Adefovir for Hepatitis B Virus rtA181V/T Mutants Alone

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ABSTRACT

Background/Aims: Hepatitis B virus (HBV) rtA181V/T mutants developed by long-term nucleos(t)ide analogue therapy are known to present cross-resistance for other nucleos (t) ide analogues, except entecavir (ETV). Some studies reported that HBV rtA181V/T mutants could induce cross-resistance to ETV and showed incomplete response as well as persistence of HBV DNA, despite rescue therapy by ETV. This study aimed to investigate the antiviral efficacy of ETV monotherapy and ETV plus adefovir (ADV) as rescue therapy for HBV rtA181V/T single mutation. **Patients and Methods:** A total of 30 patients who received ETV alone (1.0 mg/day, $n = 16$) or ETV plus ADV (10.0 mg/day, $n = 14$) over 48 weeks between April 2008 and October 2011 were enrolled. Virological, biochemical, and serological response at 48 weeks of rescue therapy were investigated retrospectively. **Results:** No significant difference in baseline characteristics was observed between the ETV group and the ETV plus ADV group. Virological response showed complete response (62.5 vs. 42.9%), partial response (6.3 vs. 28.6%), non-response (25.0 vs. 28.6%), and virological breakthrough (6.3 vs. 0%) in the two groups, respectively. Virological response did not statistically differ between both groups ($P = 0.278$). No significant difference in the mean reduction of serum HBV DNA and biochemical response was observed between both groups (4.3 ± 2.9 vs. 4.1 ± 1.8 $\log_{10}$ IU/ml; $P = 0.294$ and 88.9 vs. 100%; $P = 1.000$, respectively). In addition, no significant difference in HBeAg loss or seroconversion was observed between the two groups (26.7 vs. 28.6%; $P = 1.000$). **Conclusions:** ETV monotherapy and ETV plus ADV therapy were clinically effective and comparable as rescue therapy for HBV rtA181V/T mutants alone.

Key Words: Adefovir, entecavir, hepatitis B virus, rescue therapy, rtA181V/T mutants

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Treatment of hepatitis B virus (HBV) infection has improved significantly due to development of nucleos (t) ide analogues (NA), including lamivudine (LMV), adefovir (ADV), telbivudine (LdT), clevudine (CLV), entecavir (ETV), and tenofovir (TDF).[1-3] Durable suppression of serum HBV DNA through NA therapy can prevent progression of serious HBV-related liver diseases such as cirrhosis and hepatocellular carcinoma.[4,5] However, treatment of HBV infection with NA has a fatal weakness in that NA cannot remove covalently closed circular DNA in the nucleus of infected hepatocytes and the rate of virological relapse is high when NA therapy is discontinued.[6] Consequently, an indefinite therapeutic duration is essential in NA therapy for chronic hepatitis B. Prolonged treatment with NA inevitably results in development of drug resistance mutation, although it is rare in ETV or TDF therapy.[7,8]

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Of NA-related HBV mutants, HBV rtA181V/T mutants develop as a result of mutation of the HBV reverse transcriptase gene at position 181, where an alanine (A) is substituted with a valine (V) or threonine (T). HBV rtA181V/T mutants have been reported in chronic hepatitis B patients who received antiviral treatment with LMV, ADV, LdT, or CLV, and have been known to present cross-resistance against other NA, except ETV.[9-11] Thus, ETV with high susceptibility *in vitro* has been used primarily as rescue therapy for HBV rtA181V/T mutants.[12] However, some studies reported that HBV rtA181V/T mutants could even induce cross-resistance to ETV.[13,14] In practice, a clinical investigation reported that HBV rtA181V/T mutants might present persistence of HBV DNA and showed an

association with incomplete response, despite rescue therapy by ETV.[15]

In general, the majority of HBV rtA181V/T mutants are known to be induced after ADV therapy, along with the rtN236T mutant. Many guidelines published in Korea, the United States, and Europe recommend ETV plus TDF (or ADV if TDF is unavailable) as rescue therapy for HBV rtA181V/T mutants.[1-3] However, there is no specific therapeutic recommendation or clinical study on HBV rtA181V/T single mutation. Thus, antiviral therapy should be determined to support the decision on which rescue therapy, add-on therapy with ETV or switch to ETV monotherapy, is to be applied in patients who received prior ADV therapy for HBV rtA181V/T mutants alone. In other words, the aim of this study is to investigate the antiviral efficacy of ETV alone and in combination with ADV for HBV rtA181V/T single mutation.

PATIENTS AND METHODS

Study subjects

This research was conducted as a retrospective cohort study. Sequencing analysis of the HBV reverse transcriptase genome was performed in 797 adult patients (≥18 years in age) with virological breakthrough during antiviral therapy for chronic hepatitis B, from April 2008 to October 2011. Among the 797 patients, mutations of the HBV reverse transcriptase genome were detected in 557 patients. Of the 557 patients, HBV rtA181T/V mutants were found in 136 patients. Of these, a total of 30 patients who received ETV (1.0 mg/day) monotherapy or ETV plus ADV (10.0 mg/day) therapy over 48 weeks as rescue therapy against HBV rtA181V/T mutants only without other concomitant mutations were enrolled in this study. The enrolled subjects were divided into the ETV group (n = 16) and the ETV plus ADV group (n = 14) [Figure 1].

The study was conducted according to the principles of the Declaration of Helsinki of 1975. All patients submitted their written informed consent prior to enrollment in the study. Details of this study were approved by the Institutional Review Board of Yeungnam University Hospital (YUH-13-0319-O9).

Study assessments

Serum HBV DNA levels, alanine aminotransferase (ALT) levels, and the status of HBeAg at baseline and at 48 weeks of rescue therapy for HBV rtA181V/T mutants were investigated through review of medical records. Assessments of virological, biochemical, and serological responses as well as reduction of serum HBV DNA were performed at 48 weeks. In addition, reduction of serum HBV DNA was analyzed at week 24 of rescue therapy in order to investigate the trend of change.

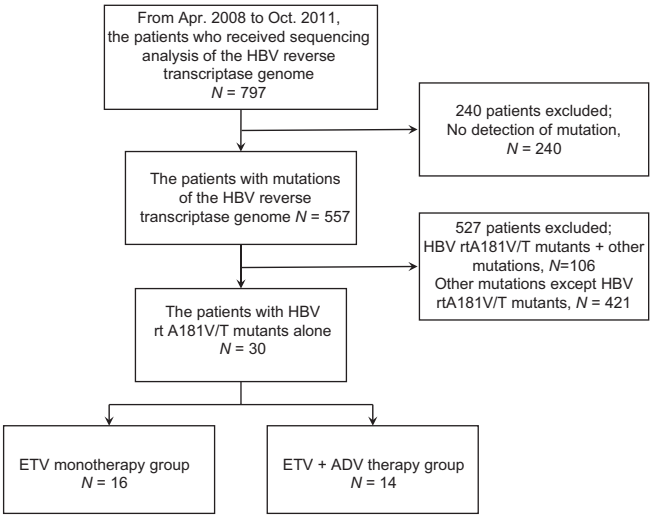

Figure 1: Flow chart of the enrolled patients

Sequencing analysis of the HBV reverse transcriptase polymerase gene was performed using polymerase chain reaction (PCR) and direct sequencing of HBV isolated from all individuals [Applied Biosystems (ABI) 3730 DNA Analyzer; Applied Biosystems, Foster City, CA, USA]. The HBV DNA load was tested using a commercially available quantitative real-time PCR assay (Cobas Taqman HBV-DNA Test; Roche Diagnostics Systems, Pleasanton, CA, USA) with a linear dynamic detection range of 20–1.7 × 10^8 IU/ml. Serological markers, including HBsAg, HBeAg, and anti-HBe, were determined using routine commercially available enzyme immunoassays (Architect i2000SR; Abbott Diagnostics, Abbott Park, IL, USA). Serum ALT, albumin, total bilirubin, platelet levels, and prothrombin time were measured using standard laboratory procedures.

Definitions

Virological responses to NA therapy were divided into complete virological response, partial virological response, non-response, and virological breakthrough, according to the guidelines of the Korean Association for the Study of the Liver for treatment of chronic hepatitis B.[3] Complete virological response was defined as HBV DNA undetectable by quantitative real-time PCR at 48 weeks of rescue therapy (serum HBV DNA level <20 IU/ml). Partial virological response was defined as a decrease of HBV DNA of ≥2 $\log_{10}$ IU/ml, but detected by real-time PCR assay. Virological breakthrough was defined as rebound of HBV DNA greater than 1 $\log_{10}$ IU/ml, compared to the on-treatment nadir (the lowest value). Non-response was defined as a decrease in HBV DNA of <2 $\log_{10}$ IU/ml at 48 weeks of rescue therapy. Biochemical response was defined as normalization of serum ALT level. The upper limit of normal ALT was defined as 40 IU/l. Serological response was defined as HBeAg loss or seroconversion. Liver cirrhosis was defined by means of histologic, clinical, or radiologic

modalities. Clinical diagnosis of liver cirrhosis included lower platelet level ($<1.4 \times 10^5$ cell/μl) with splenomegaly, existence of ascites or varices, or hepatic encephalopathy.

Evaluation of antiviral efficacy

The primary endpoint was virological response after 48 weeks of rescue therapy. The secondary endpoints included reduction of serum HBV DNA, normalization of serum ALT levels, HBeAg loss or seroconversion, and complications, including mortality and morbidity, after 48 weeks of rescue treatment.

Statistical analysis

Categorical data or frequency data were presented as absolute values and percentages, whereas continuous variables were summarized as mean ± standard deviation (SD) or median including range. Between-group comparisons were performed using the Mann–Whitney *U* test for continuous variables, and the Chi-square test or Fisher's exact test for categorized variables, as appropriate. A two-sided *P* value of less than 0.05 was considered statistically significant. Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) was used for organization of all data, and PASW statistics version 18.0 for Windows (SPSS Inc., Chicago, IL, USA) was used in performance of data analysis.

RESULTS

The baseline characteristics of the ETV group and the ETV plus ADV group are shown in Table 1. Most parameters of demographic and laboratory characteristics, including age; gender; cirrhosis; Child–Pugh class; serum ALT, total bilirubin, albumin, and platelet level; prothrombin time; and the rates of HBeAg positivity, did not differ significantly between the two groups ($P > 0.05$). Baseline serum HBV DNA levels in the ETV and ETV plus ADV groups were 4.8 ± 1.7 $\log_{10}$ IU/ml and 4.1 ± 1.8 $\log_{10}$ IU/ml, respectively ($P = 0.193$). Previous history of NA therapy prior to rescue therapy with ETV monotherapy or combination therapy of ETV plus ADV was analyzed. Of the enrolled total patients, 14 patients had no experience of ADV therapy (14/30, 46.7%). The 14 patients had received prior LMV monotherapy ($n = 6$), CLV monotherapy ($n = 6$), and sequential therapy with CLV after LMV therapy ($n = 2$). The ADV-experienced patients in the ETV group and the ETV plus ADV group numbered 6 and 10, respectively. Statistically, there was no difference in experience of ADV therapy ($P = 0.081$). Duration of prior antiviral therapy in the ETV plus ADV group was relatively longer compared with the ETV group (307.1 ± 180.8 vs. 188.6 ± 157.3 weeks); however, no statistical difference was observed ($P = 0.064$).

Data on the mean changes in serum HBV DNA at 24 weeks and 48 weeks are shown in Figure 2 and Table 2. The mean reduction of serum HBV DNA at 24 weeks did not differ significantly between the ETV group (4.0 ± 3.4 $\log_{10}$ IU/ml) and the ETV plus ADV group (3.7 ± 2.5 $\log_{10}$ IU/ml) ($P = 0.400$). In addition, no statistical difference in the mean decline of serum HBV DNA at 48 weeks was observed

Table 1: Baseline characteristics of patients

Variables	ETV group (*n*=16)	ETV plus ADV group (*n*=14)	*P*
Age*, years	44.1±11.7	42.4±12.2	0.766
Male, *n* (%)	12 (75.0)	10 (71.4)	1.000
HBeAg positivity, *n* (%)	15 (93.8)	14 (100)	1.000
Liver cirrhosis, *n* (%)	1 (6.3)	2 (14.3)	0.586
Child-Pugh class, *n* (%)			1.000
A	1 (100)	1 (50.0)	
B	0	1 (50.0)	
Prior ADV experienced, *n* (%)			0.081
No	10 (62.5)	4 (28.6)	
Yes	6 (37.5)	10 (71.4)	
Duration of prior antiviral therapy*, weeks	188.6±157.3	307.1±180.8	0.064
Serum HBV DNA*, $\log_{10}$ IU/ml	4.8±1.7	4.1±1.8	0.193
Serum ALT*, IU/l	337.5±611.1	212.1±478.0	0.075
Serum total bilirubin*, mg/dl	1.1±0.6	2.0±2.4	0.780
Serum albumin*, g/dl	4.6±0.5	4.5±0.7	0.906
Platelet count*, ×10^3 cells/μl	253.0±67.4	214.8±70.9	0.119
Prothrombin time*, s	11.0±1.0	12.2±3.1	0.761

*The value was expressed as mean±deviation. ETV: Entecavir, ADV: Adefovir, HBV: Hepatitis B virus, ALT: Alanine aminotransferase

Table 2: Summary of efficacy measures after rescue therapy

Variables	ETV group (*n*=16)	ETV plus ADV group (*n*=14)	*P*
Reduction in serum HBV DNA level*, $\log_{10}$IU/ml			
At 24 weeks	4.0±3.4	3.7±2.5	0.400
At 48 weeks	4.3±2.8	4.1±1.8	0.294
Virological responses at 48 weeks, *n* (%)			0.278
Complete response	10 (62.5)	6 (42.9)	
Partial response	1 (6.3)	4 (28.6)	
Non-response	4 (25.0)	4 (28.6)	
Virological breakthrough	1 (6.3)	0	
Normalization of serum ALT level†, *n* (%)	8 (88.9)	4 (100)	1.000
Serological responses‡, *n* (%)			
HBeAg loss	4 (26.7)	4 (28.6)	1.000
HBeAg seroconversion	2 (13.3)	4 (28.6)	0.401

*The value was expressed as mean±deviation. †Patients with initial serum ALT level >40 IU/l numbered nine in the ETV group and four in the ETV plus ADV group. ‡Patients with initial HBeAg seropositivity numbered 15 in the ETV group and 14 in the ETV plus ADV group. ETV: Entecavir, ADV: Adefovir, HBV: Hepatitis B virus, ALT: Alanine aminotransferase

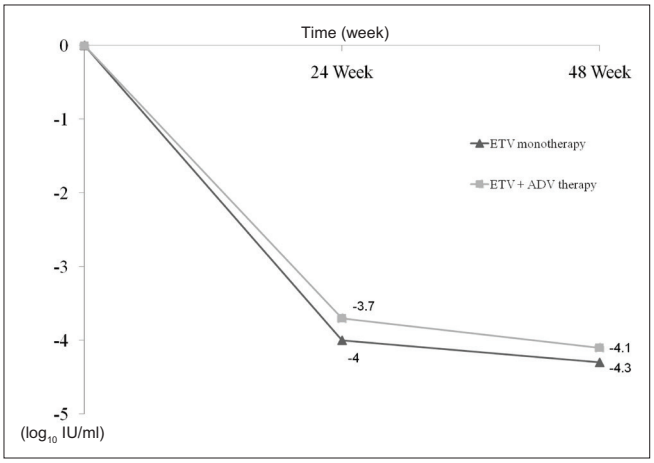

Figure 2: Comparison of the mean reduction of serum HBV DNA at 24 weeks and 48 weeks between ETV monotherapy and ETV plus ADV therapy

between the two groups (4.3 ± 2.8 vs. 4.1 ± 1.8 $\log_{10}$ IU/ml; $P = 0.294$).

Virological response at 48 weeks of rescue therapy showed complete virological response (62.5 vs. 42.9%), partial virological response (6.3 vs. 28.6%), non-response (25.0 vs. 28.6%), and virological breakthrough (6.3 vs. 0%) in the ETV group and the ETV plus ADV group, respectively. No significant difference in virological response was observed between the two groups ($P = 0.278$).

At baseline, serum ALT levels over the upper limit of normal were detected in nine patients and four patients in the ETV group and the ETV plus ADV group, respectively. Normalization of serum ALT level occurred after 48 weeks of rescue therapy in eight patients (8/9, 88.9%) in the ETV group and four patients (4/4, 100%) in the ETV plus ADV group. Biochemical response did not differ significantly between the two groups ($P = 1.000$).

Among the patients who were HBeAg-positive at baseline, 26.7% (4/15) in the ETV group and 28.6% (4/14) in the ETV plus ADV group achieved HBeAg loss at 48 weeks. No significant difference was observed in the rates of HBeAg loss ($P = 1.000$). In addition, the rates of HBeAg seroconversion at 48 weeks did not differ statistically between the two groups (13.3 vs. 28.6%; $P = 0.401$). There was no occurrence of complications, including mortality and morbidity, related to HBV with rtA181T/V mutants during the 48 weeks of rescue therapy. In addition, newly developed hepatocellular carcinoma was not observed among these subjects.

DISCUSSION

Development of NA with a high genetic barrier, such as ETV and TDF, has revolutionized our ideas regarding treatment of HBV. The optimal therapeutic goal or complete suppression of HBV DNA can be achieved in many CHB patients through ETV or TDF therapy. However, NA used prior to the introduction of ETV or TDF can hardly be free from the problem of development of drug resistance related to long-term medication. Above all, the emergence of cross-resistance or multidrug resistance of HBV can be more problematic due to sequential monotherapy for treatment of chronic hepatitis B.[11,16,17] Cross-resistance of HBV, including HBV rtA181V/T mutants, can result in development of serious liver-related diseases such as hepatic failure or progression to liver disease.[11,15,18,19] HBV rtA181V/T mutants are more important in practice because a single mutation can confer multidrug resistance against the L-nucleoside LMV, CLV, and LdT, as well as the alkyl phosphonates ADV and TDF.[10-12] As a result, the standard of care for HBV rtA181V/T mutants has not been established and clinical evidence of optimal treatment for HBV rtA181V/T mutants is also lacking. Although many authorized guidelines have recommended the combination therapy of a nucleoside and a nucleotide analogue for multidrug resistance of HBV, the efficacy has not been confirmed in practice.[1-3] Thus, to the best of our knowledge, this research appears to be valuable as it provides rare clinical evidence of treatment of HBV rtA181V/T mutants alone.

In this study, all therapeutic responses of HBV rtA181V/T mutants alone, including decline of serum HBV DNA, virological, biochemical, and serological responses did not differ significantly between the ETV monotherapy group and the ETV plus ADV group ($P > 0.05$). In addition, a study on multidrug resistance of HBV reported a similar result showing that antiviral efficacy of ETV plus ADV combination therapy was not clinically superior to that of ETV monotherapy.[20]

In our study, virological breakthrough occurred in one patient belonging to the ETV monotherapy group. The patient had received CLV therapy prior to detection of the HBV rtA181V mutant. ETV alone was administered as rescue therapy for the HBV rtA181V mutant. Reduction of serum HBV DNA by more than 2 $\log_{10}$ IU/ml was observed at 24 weeks. However, virological breakthrough occurred at 48 weeks. At 48 weeks, sequencing analysis for the reverse transcriptase gene of HBV was performed in order to detect the cause of the virological breakthrough. Newly developed HBV rtM204I and rtP237H mutants were detected, and the pre-existing HBV rtA181V mutant disappeared completely through rescue therapy. The patient received ETV plus TDF combination therapy after the introduction of TDF in Korea, and partial virological response has been shown. Although liver failure or serious complications related to HBV did not happen, occurrence of virological breakthrough by ETV monotherapy might be problematic. The appearance of mutations other than HBV rtA181V/T mutants in this case may be associated with

sequential antiviral monotherapy, as mentioned above, and is a problem to be solved before agreement on use of ETV monotherapy for HBV rtA181V/T mutants.

In our study, the mean duration of previous antiviral therapy prior to rescue therapy was longer in patients of the ETV plus ADV group. The reason may be associated with the fact that physicians are concerned about the increased risk of development of drug resistance to HBV according to therapeutic duration.[2] Therefore, there was a high probability of intentional selection of ADV combination therapy. However, no statistically significant difference in therapeutic results was observed between the methods of rescue therapy. In addition, although there was no statistical significance, more ADV-experienced patients were recruited in the ETV plus ADV group. It seemed that more add-on therapy with ETV was intentionally selected in the patients who received prior ADV therapy.

Response to antiviral therapy differed according to genotype.[21] A recent study reported that drug susceptibility of HBV rtA181V/T mutants was different, and the difference might be explained by a difference in HBV genotypes.[12] In addition, an investigation conducted in China reported that the genotype-dependent polymorphism feature of HBV reverse transcriptase sequences in treatment-naïve chronic hepatitis B patients would be an important basis for understanding evolution of NA resistance.[22] As a result, the antiviral efficacy of ETV or ETV plus ADV as rescue therapy for HBV rtA181V/T mutants may be related to the genotypes of HBV. However, the genotypes of HBV in most Korean patients were known to be genotype C2; therefore, in this study, HBV genotypes of the enrolled patients were not investigated.[23] Consequently, there is a limitation to application of our results for other genotypes of HBV, except for the predominant genotype C in Korea. Clinical researches for rescue therapy for HBV rtA181V/T mutants should be performed considering the discrepancy in genotypes of HBV.

There were some limitations in this study. This research was conducted retrospectively, with an insufficient number of subjects. Thus, there might be a limitation of selection bias. In addition, the duration of rescue therapy was relatively short term. The outcomes can change through a longer follow-up period. After rescue therapy for rtA181V/T mutants alone, newly developed mutants or disappearance of HBV rtA181V/T mutants were not evaluated in total. Finally, TDF, which is known to be a potent HBV inhibitor with a higher barrier to resistance, has recently been used in Korea.[24,25] As a substitute of ADV, antiviral efficacy of TDF for HBV rtA181V/T mutants is still unclear in practice. Further investigation of rescue therapy, including TDF, for HBV rtA181V/T mutants alone will be necessary.

CONCLUSION

In conclusion, findings of this study demonstrated that ETV monotherapy and ETV plus ADV therapy were clinically effective and comparable as rescue therapy for HBV rtA181V/T mutants alone. However, occurrence of virological breakthrough by ETV monotherapy may be problematic. Large-scale, long-term studies of rescue therapy for HBV rtA181V/T mutants alone should be conducted, and therapeutic plans for achievement of further antiviral efficacy for HBV rtA181V/T mutants alone should be established and recommended.

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