



Adefovir plus Entecavir Therapy in Chronic Hepatitis B Patients with Treatment Failure to Lamivudine-Entecavir Sequential Therapy: Outcome at 2 Years

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Abstract

The efficacy of adefovir add-on therapy in treatment-experienced patients with chronic hepatitis B (CHB) is debatable. This study aimed to evaluate the efficacy of adefovir add-on therapy in CHB patients with antiviral resistance to lamivudine/entecavir sequential therapy. CHB patients who exhibited documented resistance to lamivudine and switched to entecavir 1.0 mg monotherapy were evaluated and 19 of them showed active viral replication (HBV DNA levels $\geq 10^5$ copies/mL) or a history of treatment failure to lamivudine/entecavir sequential therapy. Adefovir 10 mg/day has been added to these 19 patients and the virologic parameters were monitored every three months for 96 weeks. A primary responder was defined as patient who had a decline in serum HBV DNA ≥ 1 log₁₀ copies/mL after 12 weeks of therapy, compared with the pretreatment value. In 19 CHB patients, 10(52.6%) patients were HBeAg positive, 7 (36.8%) had cirrhosis. The mean duration of previous entecavir therapy was 84.4 ± 22.5 weeks. The mean HBV DNA levels and ALT at baseline were 6.17 ± 0.96 log₁₀ copies/mL, 53 ± 35 IU/L. The reduction of serum HBV DNA levels from baseline was 2.27 ± 1.34 , 2.77 ± 1.41 , and 3.09 ± 1.37 log₁₀ copies/mL, at 24, 48 and 96 weeks, respectively. The rate of undetectable serum HBV DNA was 10.5% (2/19), 26.3% (5/19), and 31.5% (6/19) and ALT levels were normalized in 5 (55.6%), 6(66.7%), and 6(66.7%) of 9 patients with elevated ALT at baseline. Initial HBV DNA level was the only independent factor that was inversely associated with serum HBV DNA negativity at 96 weeks. Among 7 primary non-responders, 6 patients achieved serum HBV-DNA level < 4 log₁₀ copies/mL at 96 weeks. Until 96 weeks, viral breakthrough was not detected in 19 patients. The adefovir add-on therapy may be helpful, even if not sufficient enough, for CHB patients with antiviral resistance to lamivudine/entecavir sequential therapy.

Keywords: Adefovir; Entecavir; Lamivudine; Antiviral resistance

Introduction

While oral antiviral agents with low resistance and high potency are available for the treatment-naïve patients in the current clinical setting,

limitations have been persistent in the treatment of patients with lamivudine (LAM) resistance.

Currently, the treatment of choice for LAM-resistant hepatitis B virus (HBV) is combination therapy based on adefovir dipivoxil (ADV) or tenofovir, with add-on medications such as LAM, telbivudine, and entecavir (ETV) [1-4]. An adequate antiviral effect can be expected in only 20 to 30% of patients when they are treated with ADV or ETV monotherapy. If those monotherapies are continued in patients showing an inadequate treatment response, there is a high risk of developing a mutant strain with resistance to second-line treatment; the risk is 10% per year [5-10]. In case of tenofovir, there is a high probability of therapeutic success, even with monotherapy, but the accumulated evidence is not enough. On the other hand, there have been studies on combination therapy that resulted in reduced resistance to second-line treatment and long-term efficacy in terms of virus reduction [11].

However, combination therapy carries a high cost, and there is a lack of evidence regarding the advantage of combination therapy in the early guidelines of treatment for resistance. Therefore, ETV monotherapy has been recommended as a treatment option and was frequently used in clinical practice, so there are still patients showing an inadequate response as mentioned above until now. Furthermore, the evidence for a third-line treatment is very limited, and there is no clinical study regarding the efficacy of ADV+ETV combination therapy in case of treatment failure to ETV monotherapy in LAM resistance. Hence, this study aimed to determine the efficacy of ADV +ETV combination therapy in patients who did not respond to LAM-ETV sequential treatment.

Materials and Methods

Second-line treatment with ETV 1.0 mg had been given to CHB patients with resistance to LAM. For the patients with treatment failure to second-line treatment, third-line treatment was given as a combination of ETV 1.0 mg and ADV 10 mg for 96 week. Patients who were co-infected with hepatitis A, hepatitis C, hepatitis D, or human immunodeficiency virus and those with other concomitant causes of chronic liver diseases were excluded. All patients were known to have been hepatitis B s antigen (HBsAg)-positive for more than 6 months. Patients were monitored at 3-month intervals using clinical, biochemical, and virologic assessments of serum alanine transaminase (ALT), serum HBV DNA load as the measurement of virologic response, and virologic breakthrough.

Patients

Second-line treatment with ETV 1.0 mg had been given to CHB patients with resistance to LAM. For the patients with treatment failure to second-line treatment, third-line treatment was given as a combination of ETV 1.0 mg and ADV 10 mg for 96 week. Patients who were co-infected with hepatitis A, hepatitis C, hepatitis D, or human immunodeficiency virus and those with other concomitant causes of chronic liver diseases were excluded. All patients were known to have been hepatitis B s antigen (HBsAg)-positive for more than 6 months. Patients were monitored at 3-month intervals using clinical, biochemical, and virologic assessments of serum alanine transaminase (ALT), serum HBV DNA load as the measurement of virologic response, and virologic breakthrough.

Serology

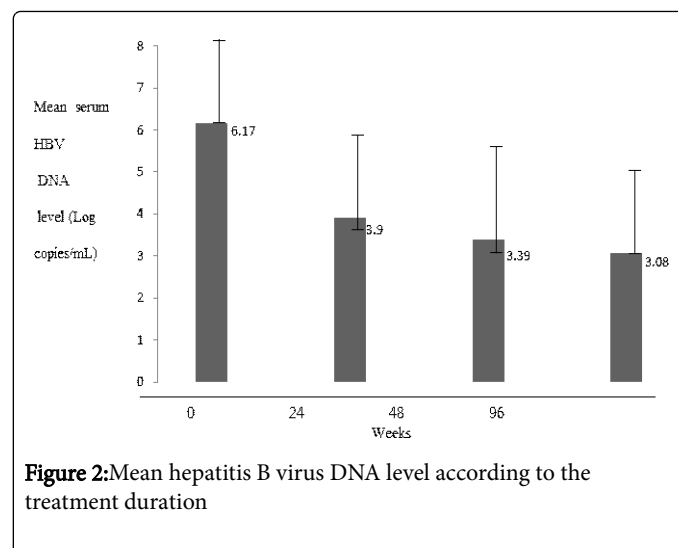
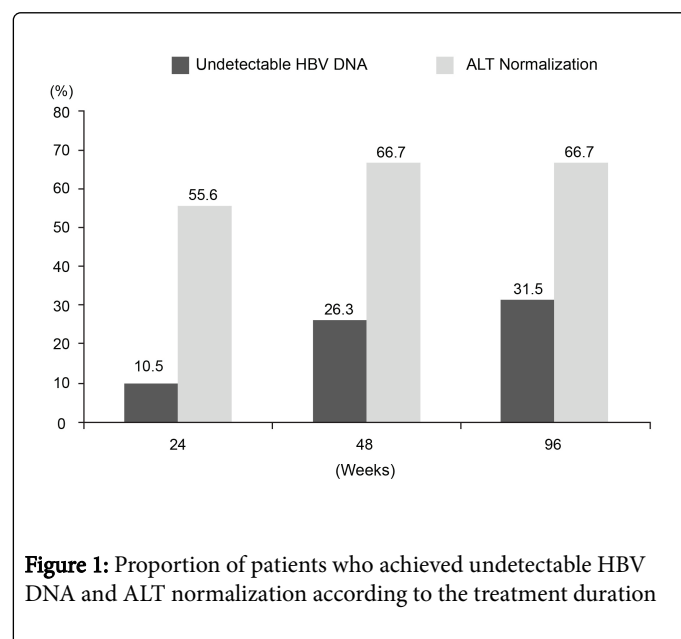
Serum aspartate aminotransferase (AST), ALT, total bilirubin and albumin were measured using standard laboratory procedures. HBsAg, anti-HBs, hepatitis B e antigen (HBeAg), and anti-HBe were detected using commercially available enzyme immunoassays (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Serum levels of HBV DNA were quantified using the COBAS Amplicor Monitor 2.0 HBV assay (Roche Diagnostic Systems Inc., Indianapolis, IN, USA), which has a lower limit of detection at 116 copies/mL.

Definition

Treatment failure to ETV was defined as either 1) a failure in the reduction in HBV DNA to less than 5 log copies/mL after 6 months of treatment compared with the HBV DNA value prior to treatment or 2) an increase in HBV DNA to more than 1 log from nadir during treatment. On the other hand, primary responders were defined as those with reductions in serum HBV DNA $\geq 1 \log_{10}$ copies/mL at week 12 of treatment compared with the pretreatment value. Viral breakthrough was defined as occurring when the HBV DNA value increased more than 1 log from nadir during treatment on more than 2 consecutive occasions.

Statistical analysis

Values are expressed as the means \pm standard deviation (SD). Continuous variables were analyzed with the two-tailed Student's t-test; categorical variables were analyzed with χ^2 test or Fisher's exact test, and univariate analysis was used to investigate the factor affecting the viral response at week 96. P value < 0.05 was considered to indicate statistical significance. All calculations were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA).



Results

Baseline characteristics

A total of 19 patients were included for the analysis of combination therapy for up to 96 weeks. The patients' baseline characteristics are presented in Table 1. The mean age was 53 ± 11 years, the male-to-female ratio was 14/5, and 7 patients (36.8%) had cirrhosis. The initial serum ALT levels were 53 ± 35 IU/L, the initial serum HBV DNA levels were $6.17 \pm 0.96 \log_{10}$ copies/mL, and there were 10 (52.6%) HBe Ag-positive patients.

LAM-resistant mutant strain was detected in 100% (19/19) of patients, and the genotype was M204V/I \pm L180M. ETV-resistant mutant strain was detected in 21.0% (4/19) of patients, with the genotype N202G. In addition, 5.2% (1/19) of patients had the genotype T184I/L. The mean treatment duration with ETV monotherapy prior to combination therapy was 84.4 ± 22.5 weeks.

Biochemical and virological response to the combination therapy

Biochemical response: Prior to combination therapy, 9 (47.4%) patients had serum ALT levels higher than the upper limit of normal. The rate of ALT normalization increased with continuation of therapy: 55.6% (5/9), 66.7% (6/9), and 66.7% (6/9) at weeks 24, 48, and 96 weeks, respectively (Figure 1). The median interval to ALT normalization from combination therapy was 16 weeks (range: 4-96 weeks). A half (2/4) of HBeAg-positive patients and 80% (4/5) of HBeAg-negative patients achieved normalization of serum ALT levels at 96 weeks. Meanwhile, serum ALT levels were maintained within normal range in 10 patients with normal ALT levels at baseline.

Virological response: The serum HBV DNA loss was observed in 10.5% (2/19), 26.3% (5/19), and 31.5% (6/19) of patients at 24, 48, and 96 weeks, respectively (Figure 1). The reduction in serum HBV DNA levels from the baseline were 2.27 ± 1.34 , 2.77 ± 1.41 , and $3.09 \pm 1.37 \log_{10}$ copies/mL at 24, 48, and 96 weeks, respectively. The mean HBV DNA levels were 3.9 ± 1.12 , 3.39 ± 1.42 , and $3.08 \pm 1.34 \log_{10}$ copies/mL, respectively (Figure 2). The primary response was seen in 12 of the total 19 patients (63.1%). The serum HBV DNA loss was noted in 4(33.3%) and 5(41.6%) of 12 primary responders versus in

only 1(14.2%) and 1(14.2%) of 7 primary non-responders at 48 weeks and 96weeks, respectively.

In terms of viral reduction, the serum HBV DNA level decreased less than 4 log₁₀ copies/mL in 4 (57.1%) and 6 (85.7%) of primary non responder versus in 10(83.3%) and 10(83.3%) of primary responder at 48 and 96 weeks, respectively. Up to week 96 of combination therapy, viral breakthrough was not observed in 19 patients.

At 96 weeks, univariate analysis was conducted to identify the pretreatment or on treatment factors that affect the serum HBV DNA loss. Age, gender, HBeAg-positive status, serum ALT levels prior to combination therapy, and primary response on treatment were not statistically significant (Table 2). The initial HBV DNA levels prior to combination therapy was the only predictive factor [OR=0.060; 95% CI (0.005-0.735); P=0.041; Table 2].

Variables	Values
Sex (No.[%])	
Male	14 (73.6)
Female	5 (26.3)
Age (years)	53 11
Serum HBV DNA level (log ₁₀ copies/mL)	6.17 0.96
HBeAg-positive (No.[%])	10(52.6)
Serum AST level (IU/L)	42 13
Serum ALT level (IU/L)	53 35
Serum total bilirubin level(mg/dL)	1.05 ± 0.81
LC (No.[%])	7 (36.8)
LAM resistance mutation pattern (No.[%])	
M204V + L180M	11 (57.8)
M204I + L180M	3 (15.7)
M204V/I + L180M	4 (21.0)
M204I	1 (5.2)
NA	0 (0.00)
ETV resistance mutation pattern (No.[%])	
N202G	4 (21.0)
T184I/L	1 (5.2)
Negative	5 (26.3)
NA	9 (47.3)

Table 1: Baseline characteristics of 19 chronic hepatitis B patients

Discussion

Current therapy for CHB aims to administer long-term antiviral agent to minimize the serum HBV levels and prevent the progression and exacerbation of liver disease. This practice has continued for the last 15 years, from the beginning of antiviral therapy with oral agent to the present.

The most significant problem with long-term antiviral therapy is the development of drug resistance. Recently, antiviral agents with substantially low resistance have been clinically available, but tenofovir, which is estimated as effective even in resistant HBV, has a limited availability in many counties.

Across the world, there has been substantial improvement with regard to the treatment of CHB, but drug resistance is still an important problem due to the limitations of antiviral efficacy in cases represented by LAM resistance and due to inadequate research evidence for the early stage of treatment.

In this study, CHB patients who had developed LAM resistance and did not respond to second-line treatment with ETV were given as third-line treatment; a combination of ETV and ADV for 96 weeks, and medical records were retrospectively analyzed to estimate its efficacy.

	Valuables	Proportion (%)	OR (95%CI)	P-value
Age (years)	≤40	0/3(0)	0.0(-)	0.517
	> 40	6/16(37.5)		
Sex	Male	4/12(33.3)	0.800(0.105-6.105)	1.000
	Female	2/7(28.6)		
HBeAg status	Positive	3/10(30.0)	0.857(0.124-5.944)	1.000
	Negative	3/9(33.3)		
HBV DNA level (Log10copies/mL)				
	≤6	5/8(62.5)	0.060(0.005-0.735)	0.041*
	>6	1/11(9.1)		
Serum ALT	≤40	4/10(40.0)	0.429(0.057-3.223)	0.628
	>40	2/9(22.2)		
Primary response	Responder	5/12(41.7)	4.286(0.386-47.627)	0.333
	Non-responder	1/7(14.3)		

Table 2: Analysis of baseline or on-treatment factors predictive of serum HBV DNA negativity after 96weeks of adefovir add-on therapy.

There are few studies with regard to the efficacy of ADV+ETV combination therapy in nucleoside-refractory CHB, and this study is different from previous report of ADV+ETV combination therapy in the treatment strategy. Cho *et al.* [12] investigated the efficacy of ADV+ETV combination therapy in non-responders to monotherapy with LAM-ADV sequential treatment, and Lim *et al.* [13]. Evaluated non-responders to sequential treatment with LAM followed by ADV+LAM combination therapy. Chae *et al.* [14] conducted a study in patients who underwent various treatment methods prior to ADV+ETV combination therapy, and among a total of 25 patients, only 1 had the same treatment regimen (from LAM-ETV to ETV+ADV combination therapy) as in this study.

In previous studies, combination therapy with ADV and ETV in the presence of LAM resistance was initiated as the third-line treatment when there was no response to ADV monotherapy or combination with LAM. However, in this study, the difference is that data were collected and analyzed after adding ADV in non-responders to ETV monotherapy as the second-line treatment.

With regard to treatment-related viral and biochemical responses, the reduction in virus was about 2.7 log copies/mL, HBV DNA loss occurred in 26.3%, and the biochemical response was observed in 66.7% after 1 year of treatment. In comparison with other studies, similar results were observed for the same treatment duration; in the study by Lim *et al.* [15], the reduction in HBV DNA was approximately 3.2 log, the HBV DNA loss rate was 25.6%, and the biochemical response rate was 74.4%. However, the efficacies of therapies with LAM to ADV to ADV+ETV and LAM to ETV to ADV+ETV were analyzed without differentiation; such a difference should be taken into account when drawing comparisons with this study.

Biochemical response was not achieved in 3 among 9 patients who had serum ALT levels higher than the upper limit of normal prior to combination therapy. However, virologic response was achieved in 2

among these 3 patients. So other factors but virologic response should be screened in patients failed to achieve biochemical response.

In this study, however, all but only 1 patient, who did not have the primary response, had HBV DNA reduction less than 4 log with continuous treatment. During 2 years of combination treatment, the viral breakthrough was not observed in any of the patients. Advantages of continuous treatment would be expected, although the evidence is not enough.

On the one hand, the initial HBV DNA levels were the only predictive factor for viral response at 96 weeks. The viral response was observed in about two-thirds of patients if the initial HBV DNA levels were less than 6 log₁₀ copies/mL, and loss of serum HBV DNA was achieved 2 times higher in these patients. There was no statistical significance in the virus loss effect of long-term treatment on the primary responders, which assessed viral response at week 12 of treatment as the on-treatment factor, but this could be due to the small sample size; in addition, the viral response tends to be higher in the primary responder.

These data might be translated into the probability that the serum HBV DNA loss can be expected in cases of low initial HBV DNA (less than 6 log₁₀ copies/mL) prior to treatment with ADV+ETV combination therapy in patients with antiviral resistance to LAM-ETV sequential therapy. Control of the HBV levels would be expected with a longer treatment period, without viral breakthrough in patients who continue treatment for more than 1 year, although there was no initial complete viral response.

There were limitations in this study, such as the small sample size and retrospective design. However, this study is the first to investigate the effect of ADV add-on combination therapy in patients with LAM resistance and response to ETV monotherapy. The long-term efficacy was evaluated over a relatively long period of 96 weeks, and these features of the study are considered to be meaningful. Further clinical

studies are anticipated with regard to the efficacy of tenofovir, which is known to be more effective in drug-resistant chronic HBV [16-20].

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References

1. Lok AS, McMahon BJ (2009) Chronic hepatitis B: update 2009. *Hepatology* 50: 661-662.
2. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, et al. (2016) AASLD guidelines for treatment of chronic hepatitis B. *Hepatology* 63: 261-283.
3. European Association For The Study Of The Liver (2009) EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 50: 227-242.
4. European Association For The Study Of The Liver (2012) EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 57: 167-185.
5. Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, et al. (2006) Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 130: 2039-2049.
6. Suzuki F, Toyoda J, Katano Y, Sata M, Moriyama M, et al. (2008) Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 23: 1320-1326.
7. Reijnders JG, Pas SD, Schutten M, de Man RA, Janssen HL (2009) Entecavir shows limited efficacy in HBeAg-positive hepatitis B patients with a partial virologic response to adefovir therapy. *J Hepatol* 50: 674-683.
8. Cho SW, Koh KH, Cheong JY, Lee MH, Hong SP, et al. (2010) Low efficacy of entecavir therapy in adefovir-refractory hepatitis B patients with prior lamivudine resistance. *J Viral Hepat* 17: 171-177.
9. Reijnders JG, Deterding K, Petersen J, Zoulim F, Santantonio T, et al. (2010) Antiviral effect of entecavir in chronic hepatitis B: influence of prior exposure to nucleos(t)ide analogues. *J Hepatol* 52: 493-500.
10. Shim JH, Suh DJ, Kim KM, Lim YS, Lee HC, et al. (2009) Efficacy of entecavir in patients with chronic hepatitis B resistant to both lamivudine and adefovir or to lamivudine alone. *Hepatology* 50: 1064-1071.
11. Sheng YJ, Liu JY, Tong SW, Hu HD, Zhang DZ, et al. (2011) Lamivudine plus adefovir combination therapy versus entecavir monotherapy for lamivudine-resistant chronic hepatitis B: a systematic review and meta-analysis. *Virol J* 8: 393.
12. Cho Y, Lee DH, Chung KH, Jin E, Lee JH, et al. (2013) The efficacy of adefovir plus entecavir combination therapy in patients with chronic hepatitis B refractory to both lamivudine and adefovir. *Dig Dis Sci* 58: 1363-1370.
13. Lim YS, Lee JY, Lee D, Shim JH, Lee HC, et al. (2012) Randomized trial of entecavir plus adefovir in patients with lamivudine-resistant chronic hepatitis B who show suboptimal response to lamivudine plus adefovir. *Antimicrob Agents Chemother* 56: 2941-2947.
14. Chae HB, Kim MJ, Seo EG, Choi YH, Lee HS, et al. (2012) High efficacy of adefovir and entecavir combination therapy in patients with nucleoside-refractory hepatitis B. *Korean J Hepatol* 18: 75-83.
15. Lim YS, Lee TH, Heo NY, Shim JH, Lee HC, et al. (2012) Entecavir plus adefovir combination therapy for chronic hepatitis B patients after failure of nucleoside/nucleotide analogues. *Antivir Ther* 17:53-60.
16. Berg T, Marcellin P, Zoulim F, Moller B, Trinh H, et al. (2010) Tenofovir is effective alone or with emtricitabine in adefovir-treated patients with chronic-hepatitis B virus infection. *Gastroenterology* 139: 1207-1217.
17. Lok AS, Trinh H, Carosi G, Akarca US, Gadano A, et al. (2012) Efficacy of entecavir with or without tenofovir disoproxil fumarate for nucleos(t)ide-naïve patients with chronic hepatitis B. *Gastroenterology* 143: 619-628.
18. Yip B, Chaung K, Wong CR, Trinh HN, Nguyen HA, et al. (2012) Tenofovir monotherapy and tenofovir plus entecavir combination as rescue therapy for entecavir partial responders. *Dig Dis Sci* 57: 3011-3016.
19. Choi K, Lee HM, Jun BG, Lee SH, Kim HS, et al. (2015) [Efficacy of tenofovir-based rescue therapy for patients with drug-resistant chronic hepatitis B]. *Korean J Gastroenterol* 65: 35-42.
20. Kim HJ, Cho JY, Kim YJ, Gwak GY, Paik YH, et al. (2015) Long-term efficacy of tenofovir disoproxil fumarate therapy after multiple nucleos(t)ide analogue failure in chronic hepatitis B patients. *Korean J Intern Med* 30: 32-41.