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Efficacy of 2 years of entecavir plus adefovir therapy in patients with chronic hepatitis B who had failed on prior nucleos(t)ide analog treatment

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ABSTRACT

Entecavir (ETV) plus adefovir (ADV) combination therapy may be a promising option for chronic hepatitis B (CHB) patients who have failed on prior nucleos(t)ide analog (NA) treatment. However, the long-term efficacy and safety of this combination are not well-defined. In a single-center, retrospective study, 104 patients (mean age 31.7 years; 88.5% male) with HBV DNA >10^3 IU/mL who had received one or multiple prior NAs for ⩾6 months (median 44.5 months) were treated for ⩾24 months with ETV (0.5 mg/day) plus ADV (10 mg/day). Among patients with available samples, 44/90 (48.9%) had drug-resistant mutations. At 2 years, HBV DNA levels were undetectable (<12 IU/mL) in 52/104 (50.0%) patients. The mean HBV DNA level was 2.0 ± 1.2 log 10 IU/mL, and it was decreased by 3.2 ± 2.0 log 10 IU/mL from the pre-combination treatment (V0) value. The 2-year HBeAg loss rate was 14.4% (13/90), HBeAg seroconversion rate was 10.0% (9/90), and ALT normalization rate was 75%. In multivariate analyses, the prior NA treatment duration, the V0 HBV DNA level, and the HBV DNA reduction at 1 year after ETV + ADV therapy were associated with the virological response after 2 years. No patients developed renal impairment, clinical decompensation or new HCC, and no relapses of HCC or deaths occurred. Thus, 2-year rescue therapy with ETV + ADV was effective and well-tolerated in CHB patients who had previously failed on multiple NA treatments. The HBV DNA level just before ETV + ADV combination therapy and the decrease of HBV DNA at 1 year could predict the efficacy of 2 years of ETV + ADV treatment.

1. Introduction

In China, about 93 million individuals have been infected with hepatitis B virus (HBV), and around 30 million have chronic hepatitis B (CHB) (Lu and Zhuang, 2009), some of whom progress to liver failure, decompensated cirrhosis and hepatocellular carcinoma (HCC). As is well-known, antiviral therapy is a key component of the management of HBV-related liver diseases. Clinical evidence suggests that anti-HBV therapy can delay disease progression, improve patients' quality-of-life, and prolong the survival time (Liaw, 2006; Liaw et al., 2004).

At present, two important classes of antiviral drugs are available for treatment of CHB: nucleos(t)ide analogs (NAs) and interferons (IFNs). In China, the available NAs include lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), and telbivudine (LDT), all of which are recommended as first-line anti-HBV therapies (Chinese Society of Hepatology and Chinese Society of Infectious Diseases, 2011). Because of viral persistence in infected hepatocytes, long-term antiviral therapy is needed in the majority of patients with HBV-related liver diseases (Ganem and Prince, 2004). The selection of potent NAs with a high barrier to resistance as first-line therapy such as ETV or tenofovir disoproxil fumarate (TDF) provides the best chance of achieving long-term treatment goals, and should be used wherever possible. However, the selection of treatments with a high barrier to resistance is not always possible in China. Thus, antiviral resistance and suboptimal virological responses have begun to emerge as important challenges for clinicians due to poor patient compliance, the pharmacologic properties of the particular drug(s), and individual genetic variations occurring during NA therapy (Fung and Lok, 2004; Carroué-e-Durantel et al., 2008; Zoulim and Locarnini, 2012).

Abbreviations: ADV, adefovir dipivoxil; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CHB, chronic hepatitis B; CK, creatine kinase; Cr, creatinine; Cys C, cystatin C; ETV, entecavir; GLB, globulin; HAV, hepatitis A virus; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HDV, hepatitis D virus; HEV, hepatitis E virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; IFN, interferon; LAM, lamivudine; LDT, telbivudine (L-deoxythymidine); LLD, lower limit of detection; NA, nucleos(t)ide analog; OR, odds ratio; PCR, polymerase chain reaction; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; V0, value at the initiation of combination therapy.

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With increasing knowledge of drug resistance and wide application of many antiviral therapy regimens, interventions for treatment failure have become an important issue. Theoretically, a combination of ETV and ADV or TDF should be a promising salvage treatment for patients who fail on various NA therapies as there is no cross-resistance between the two drugs. Recently, ETV + ADV combination therapy has been widely used as one of the regimens advocated for managing CHB patients in clinical practice after multi-drug failure (Kim et al., 2012; Yang et al., 2012; Jeon et al., 2012; Cho et al., 2013; Chae et al., 2012; Lim et al., 2012; Xu et al., 2013; Seo et al., 2014); however, the long-term efficacy and safety of this combination regimen are not well defined.

In this study, we retrospectively evaluated the long-term efficacy and safety of ETV + ADV combination therapy, and the factors influencing viral responsiveness in CHB patients who had failed on previous multiple sequential NA therapies, rather than instituting drug verification tests with various strict control standards.

2. Materials and methods

2.1. Study subjects

This single-center, retrospective investigator-initiated cohort study enrolled 158 patients with HBV-related liver diseases (including CHB, cirrhosis or HCC) who were switched to ETV (0.5 mg/day) and ADV (10 mg/day) combination therapy after the failure of sequential NA monotherapy or combination therapy (excluding ETV + ADV) regimens during the period July 2006 to September 2012. Of these, 104 patients who received 2 years of ETV + ADV combination therapy were included in final analysis. The Chinese Clinical Trial Registry Number of the study was ChiCTR-ONC-12002285. It was approved by the Ethical Committee of Southwest Hospital.

Eligible patients included those with HBV-related liver diseases who were serum hepatitis B surface antigen (HBsAg)-positive for at least 24 weeks and who had failed on single or multiple NA therapies (LAM/ADV/ETV/LDT) previously, which was defined as sequential NA therapy for more than 6 months with the persistence of serum HBV DNA levels $>10^3$ IU/mL. Patients were excluded if they were coinfected with other hepatitis viruses (HAV, HCV, HDV, HEV) or human immunodeficiency virus (HIV); had other concurrent autoimmune or metabolic liver diseases; or had a history of alcohol or substance abuse. Of the 158 patients who were initially enrolled, 54 were not included in the analysis as 1 was coinfected with HCV, 6 were lost to follow-up after 1 year of therapy; 3 withdrew or changed drugs for economic reasons after 1 year of therapy; 3 had incomplete data; and 41 received less than 2 years of therapy. The patient recruitment process is summarized in the flowchart displayed in Fig. 1.

2.2. Study procedures

Detailed clinical data were retrieved from patient's medical record. The patients' serum samples, which were collected at the initiation of ETV + ADV combination therapy and after 1 and 2 years and preserved at 80 °C, were tested for various laboratory markers, including alanine and aspartate aminotransferases (ALT, AST), total bilirubin (TBIL), albumin (ALB), globulin (GLB), blood urea nitrogen (BUN), creatinine (Cr), creatine kinase (CK), cystatin C (Cys C), HBV DNA, and quantitative HBsAg and HBeAg.

The primary study endpoints were the proportion of patients whose serum HBV DNA levels were <12 IU/mL and the mean decrease of HBV DNA at 1 and 2 years after initiation of ETV + ADV combination therapy. Secondary endpoints were the cumulative normalization rates of ALT after 1 and 2 years of combination therapy; the HBeAg loss rate and the seroconversion rate of HBeAg-positive patients; the viral breakthrough rate; and changes in serum biochemical markers such as CK, calcium, phosphorus, Cr, BUN, and early indicators of renal dysfunction (Cys C).

For the analysis of factors influencing the efficacy of the ETV + ADV regimen, which was based on serum HBV DNA levels after 2 years of combination therapy, patients were divided into two groups: (1) those who achieved a complete response (HBV DNA <12 IU/mL); and (2) those who had a suboptimal response (HBV DNA $\geqslant$12 IU/mL). In both groups, disease characteristics and biochemical and virological markers were analyzed statistically.

2.3. Laboratory assessments

Serum HBV DNA levels were determined by the COBAS® AmpliPrep/COBAS® TaqMan® HBV test (Roche Molecular Systems, Branchburg, NJ, USA), for which the lower limit of detection (LLD) is 12 IU/mL and lower limit of quantitation (LLQ) is 54 IU/mL. HBV DNA genotype and resistant profiles were identified by a genotype-specific primer pairs PCR system (Chen et al., 2007) and by HBV P region sequencing and phylogenetic analysis. A chemiluminescence system (Roche ELECSYS 2010) was used to detect HBV markers, and a latex-enhanced immunoturbidimetric method (Sichuan Maker Biotechnology Co., Ltd, Chengdu, China) was used to determine the Cys C concentration. Serum biochemical markers such as BUN, Cr, calcium (Ca^{2+}), phosphorus (P^{3+}) and CK were routinely assessed by standard laboratory detection (Sichuan Maker Biotechnology Co., Ltd, Chengdu, China).

2.4. Statistical analysis

All data were analyzed using SPSS 13.0 software. Logarithmic conversion of quantitative HBV DNA values was performed before statistical analysis; the log value was defined as 1.08 when below the LLD value. For analysis of factors influencing efficacy, a multivariate logistic regression model was used; for independent variables selection, the backward stepwise (likelihood ratio) method was used.

To assess the effect of HBV DNA resistant mutation profiles on the efficacy of combination therapy, Fisher's exact test was used. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the 104 patients analyzed are summarized in Table 1. The mean duration of NA treatment prior to ETV + ADV therapy was 44.5 months (range 6–108 months). The frequency of treatment regimen changes was $\geqslant$3 in 44 patients (42.3%); 53 patients (51.0%) had experienced brief discontinuations during previous NA treatments.

At the time of the initial NA treatment, the patients' mean baseline HBV DNA level was 7.2 ± 1.3 log 10 IU/mL and the median ALT level was 91 IU/L (range 18–1458 IU/L). During sequential NA therapy prior to ETV + ADV therapy, ALT levels in 18 patients were persistently normal, 29 had levels 1 to 2 times the upper limit of normal (ULN) fluctuation, and 50 had a peak at least 2 times the ULN fluctuation. In 43 patients, HBV DNA levels did not reach the LLD at any stage. In 7/104 patients, data were not available.

At the beginning of ETV + ADV therapy, the patients' mean serum HBV DNA level (V0) and median ALT level were 5.2 ± 1.9 - log 10 IU/mL and 41.5 IU/L (range 5–1335 IU/L), respectively. 90 patients (86.5%) were HBeAg-positive.

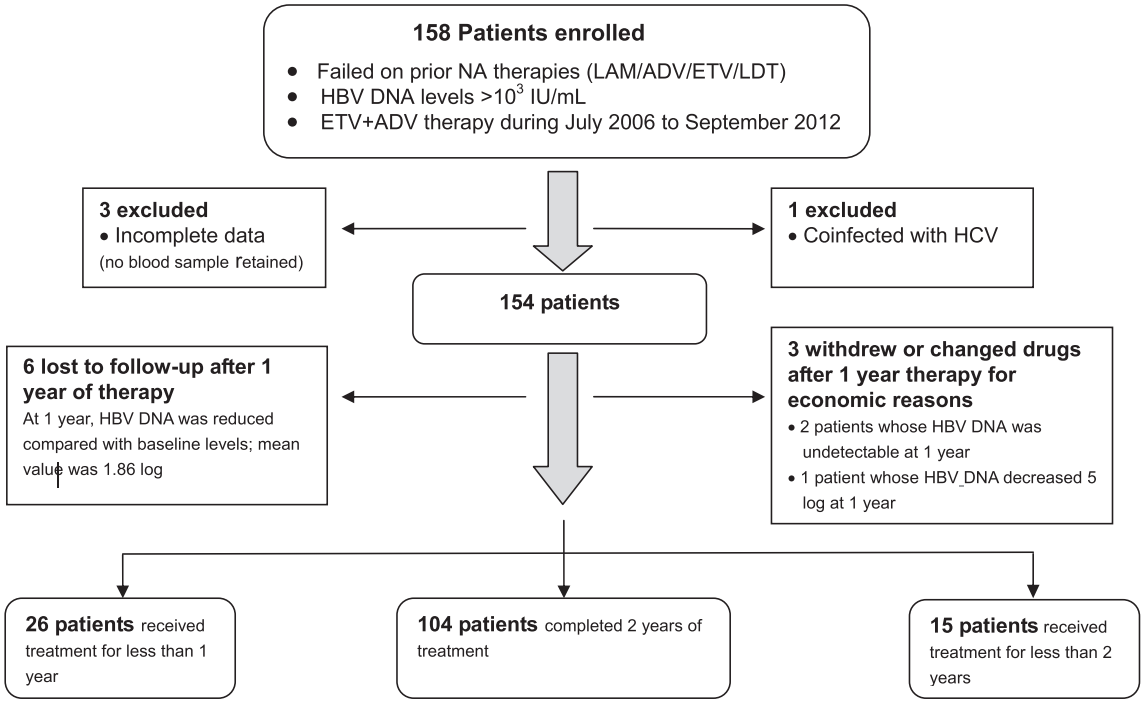

Fig. 1. Patient recruitment flowchart.

Table 1
Clinical characteristics of the patients (n = 104).

Characteristic	Value[a]
Male, *n* (%)	92 (88.5)
Mean age, years (mean ± SE)	31.7 ± 9.8
Course of the disease, years (mean ± SE)	8.9 ± 5.7
NA treatment duration prior to ETV + ADV, months (mean ± SE)	44.5 ± 26.3
Baseline HBV DNA, log10 IU/mL (mean ± SE)	7.2 ± 1.3
Baseline ALT level, IU/L [median (range)]	91 (18–1458)
V0 HBV DNA, log10 IU/mL (mean ± SE)	5.2 ± 1.9
V0 HBeAg-positive rate, *n* (%)	90 (86.5)
V0 ALT level, IU/L [median (range)]	41.5 (5–1335)
Genotype	
B, *n* (%)	44/90 (48.9)
C, *n* (%)	45/90 (50.0)
B + C, *n* (%)	1/90 (1.1)
HBV mutations prior to ETV + ADV, *n* (%)[b]	44/74 (59.5)
Previous NA treatment patterns	
Single NA, *n* (%)	24 (23.1)
Two NAs, *n* (%)	52 (50.0)
Three NAs, *n* (%)	26 (25.0)
Four NAs, *n* (%)	2 (1.9)
Patients whose HBV DNA never decreased to undetectable levels during prior NA treatment, *n* (%)	43 (41.3)
Patients whose ALT fluctuated during prior NA treatment, *n* (%)	80 (76.9)
Patients who had intermittent prior NA treatment, *n* (%)	53 (51.0)

[a] Values are numbers of patients (%), means (±SE), or medians (range).
[b] 30 patients did not undergo P region sequencing testing prior to ETV + ADV.
ALT, alanine aminotransferase; V0, at the initiation of ETV + ADV combination therapy.

3.2. Virological response during 2 years of ETV+ADV therapy

At 1 year after beginning ETV + ADV therapy, undetectable HBV DNA levels (<12 IU/mL) were achieved in 22.1% (23/104) of patients (Fig. 2). The mean HBV DNA level at 1 year was 2.6 ± 1.3 log10 IU/mL and it was decreased by 2.5 ± 2.2 log10 IU/mL compared with the V0 value (Fig. 3). The rates of HBeAg loss and seroconversion were 10.0% (9/90) and 6.7% (6/90), respectively.

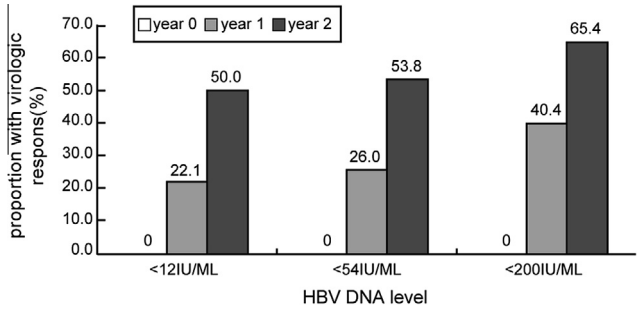

Fig. 2. Virological response rates during 2 years of ETV + ADV combination therapy.

At 2 years after beginning ETV + ADV therapy, undetectable HBV DNA levels (<12 IU/mL) were achieved in 50.0% (52/104) of patients (Fig. 2). The mean HBV DNA level at 2 years was 2.0 ± 1.2 log10 IU/mL and it was decreased by 3.2 ± 2.0 log10 IU/mL compared with the V0 value (Fig. 3). The rates of HBeAg loss and seroconversion were 14.4% (13/90) and 10.0% (9/90), respectively, and the 2-year viral breakthrough rate was 9.6% (10/104) [mean increase of HBV DNA, 1.9 log10 IU/mL]. In the 10 patients in whom viral breakthrough occurred, HBV DNA levels were transiently raised to a low level in 7 patients, but as therapy continued, they were maintained at undetectable levels during follow-up. However, in the other 3 patients, HBV DNA levels rose significantly and replacement therapy was required. ETV + TDF replacement therapy was administered in 2 of the 3 patients who had genotype C and rtM204I/V + rtL180M resistant mutants. The other patient (resistance data not available) received ADV + pegylated IFN therapy. All of these 3 patients finally achieved an undetectable HBV DNA level during follow-up.

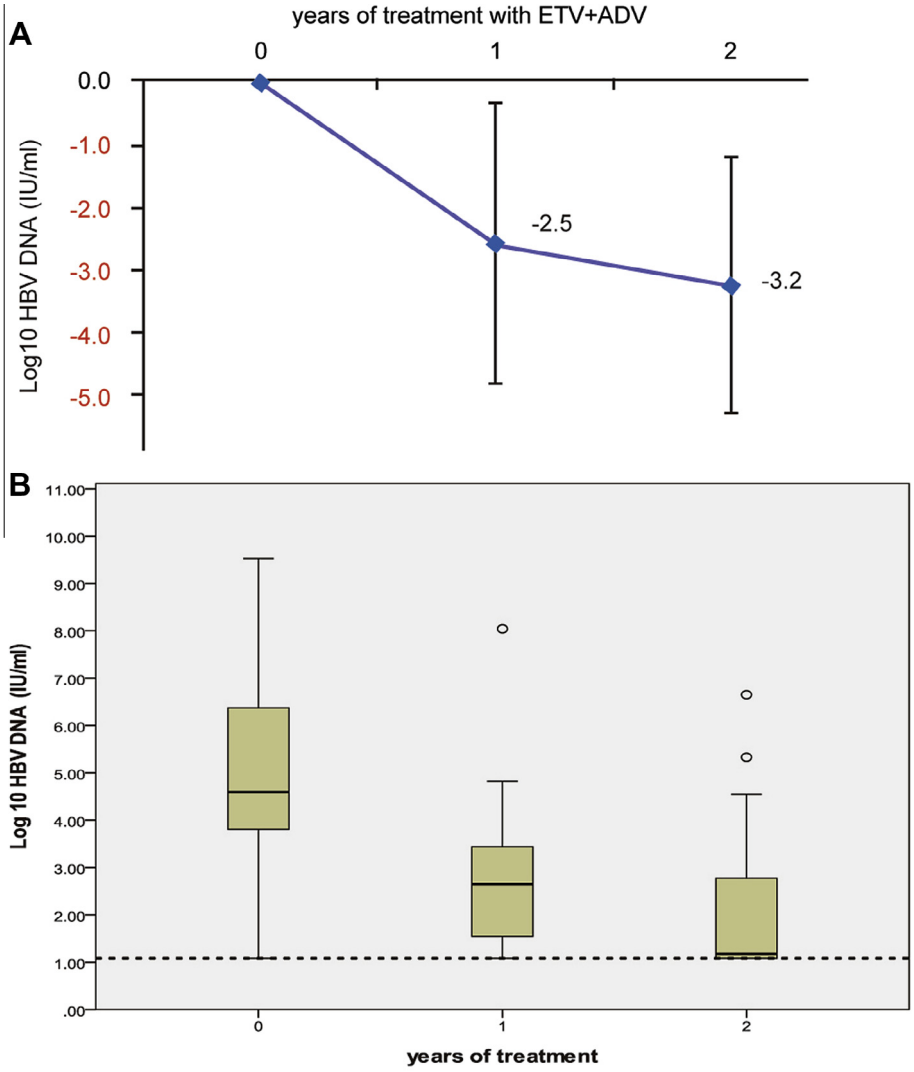

Fig. 3. HBV DNA levels during ETV + ADV combination therapy. (A) Magnitude of the reduction of HBV DNA levels. (B) Changes of HBV DNA levels. The boxes indicate the lower and upper quartiles, the central lines are the medians, and the bars are the highest and lowest values. The horizontal dotted line represents the lower limit of detection of HBV DNA by the COBAS® AmpliPrep/COBAS® TaqMan® HBV test. At 1 year of combination therapy, the HBV DNA level of 2 patients was increased significantly. Clinical records showed that 1 patient discontinued therapy for 5 months because of a traffic accident; the HBV DNA level in this patient was 8.04 log 10 IU/mL and ALT was 585 IU/L. Another patient discontinued therapy for 7 months because of recurrent oral ulcers; the HBV DNA level in this patient was 8.04 log 10 IU/mL and ALT was 1067 IU/L. At 2 years of combination therapy, there were 2 patients with obvious elevation of the HBV DNA level who had viral breakthrough.

3.3. Factors influencing the virological response to 2 years of ETV+ADV combination therapy

We analyzed the potential factors influencing virological responsiveness (HBV DNA levels decreased to below 12 IU/mL) to 2 years of ETV + ADV therapy, including gender, age, prior NA treatment duration, the baseline HBV DNA level, baseline ALT level, prior treatment discontinuations, HBV DNA level at the initiation of ETV + ADV combination therapy (V0), the V0 ALT level, the magnitude of the decrease of HBV DNA at 1 year after initiating ETV + ADV therapy, and resistance mutations. None of these factors showed significant differences in univariate analyses ($P \geqslant 0.05$). However, in multivariate analyses, 3 factors, the prior NA treatment duration ($P = 0.028$; OR = 1.022, 95% CI 1.002–1.042), the V0 HBV DNA level ($P = 0.000$; OR = 0.359, 95% CI 0.215–0.601), and the magnitude of the decrease of HBV DNA at 1 year after initiating ETV + ADV therapy ($P = 0.002$; OR = 1.931, 95% CI 1.276–2.922), showed significant differences (Table 2). The influence of resistance mutations on the efficacy of 2 years of ETV + ADV therapy was of borderline significance ($P = 0.050$), although this may have been a consequence of the limited sample size.

The resistance mutation profiles prior to ETV + ADV therapy in 44 patients are shown in Table 3. A single site mutation was found in 27 patients (61.4%), 2 site mutations in 13 (29.6%), and 3 or more site mutations in 4 (9.1%). In terms of single rtA181V/T mutations (18.2% of patients), there was no significant association with HBV DNA non-detectability and viral breakthrough after 2 years of ETV + ADV therapy (P values 0.184 and 0.628, respectively).

3.4. Biochemical responses and clinical outcomes during 2 years of ETV + ADV combination therapy

After 1 and 2 year of ETV + A DV therapy, the ALT normalization rate in the study cohort was 68.3% (71/104) and 75.0% (78/104), respectively. No cases of decompensated cirrhosis or new HCC cases were observed during the 2 years of treatment; in 6 patients who had concurrent HCC, no recurrent lesions were found.

Table 2
Analysis of factors influencing viral responsiveness during 2 years of combination ETV + ADV therapy.

Parameters	HBV DNA level		Univariate analysis	Multivariate analysis	
	⩽12 IU/mL (n = 52)	>12 IU/mL (n = 52)	P-value	P value	OR (95% CI)
Male, n (%)	47 (90.4)	45 (86.5)	0.539		
Mean age, years (mean ± SE)	31.0 ± 7.9	32.3 ± 11.2	0.481		
Prior NAs treatment duration, months (mean ± SE)	46.6 ± 26.6	42.3 ± 25.8	0.337	0.028	1.022 (1.002–1.042)
Baseline HBV DNA, log10 IU/mL (mean ± SE)	7.2 ± 1.4	7.1 ± 1.2	0.848		
Baseline ALT level, IU/L [median (range)]	100 (20–1457)	90 (18–1106)	0.608		
Prior treatment discontinuation, n (%)	21 (40.4)	29 (55.8)	0.116		
V0 ALT level, IU/L [median (range)]	38 (5–1335)	45 (5–484)	0.445		
V0 HBV DNA, log10 IU/mL (mean ± SE)	4.9 ± 2.0	5.5 ± 1.7	0.183	0.000	0.359 (0.215–0.601)
Magnitude of the change in HBV DNA level at year 1, log10 IU/mL (mean ± SE)	2.7 ± 2.1	2.4 ± 2.3	0.322	0.002	1.931 (1.276–2.922)
Resistance mutations[a], n (%)	19/38 (50.0)	25/36 (69.4)	0.050	0.051	0.523 (0.274 – 1.002)

V0, value at the initiation of ETV + ADV combination therapy; OR, odds ratio.
[a] Resistance testing was performed in 74 of the 104 patients.

Table 3
Resistance profile in 44 patients.

Resistance mutation	n (%)	
rtM204I	18 (40.91)	27 (61.36)
rtA181T	2 (4.54)	
rtA181V	6 (13.63)	
N236T236T,181T	1 (2.27)	
rtM204I + rtL180M	3 (6.82)	13 (29.55)
rtM204V + rtL180M	8 (18.18)	
N236T + rtA181T	2 (4.54)	
rtM204V + A180M + 173L	1 (2.27)	4 (9.09)
rtA181V + Q215H + N236T + rtM204V	1 (2.27)	
rtM204V + rtL180M + rtA181T + N236T	1 (2.27)	
rtM204V + rtL180M + Q215H	1 (2.27)	

3.5. Safety of 2 years of ETV + ADV therapy

From a retrospective analysis of data in 104 patients, the adverse events occurring during ETV + ADV therapy, summarized according to the National Cancer Institute (NCI) grading criteria, are shown in Table 4. Five patients (4.8%) experienced liver pain, 2 had oral mucositis, 1 had a transient increase in blood pressure, 1 had genital herpes, 1 had neurotic headache, and 1 had alopecia. In addition, laboratory tests showed that 5 patients (4.8%) patients had a high serum creatinine concentration, 6 (5.8%) had a low serum calcium concentration, and 2 (1.9%) had a low serum phosphate concentration. Most events were NCI grade 1 and were well tolerated by patients. No patient who discontinued therapy did so due to adverse events.

Table 4
Adverse events occurring in the 104 patients who received 2 years of ETV + ADV combination therapy.

Adverse events	NCI grade	No. of patients (%)
Liver pain	1	5 (4.8)
Oral mucositis	1	1 (1.0)
	2	1 (1.0)
Hypertension	1	2 (1.9)
Genital herpes	2	1 (1.0)
Neurotic headache	1	1 (1.0)
Alopecia	1	1 (1.0)
Increased serum creatinine	1	5 (4.8)
Hypophosphatemia	1	2 (1.9)
Hypercalcemia	1	13 (12.5)
	2	1 (1.0)
	3	2 (1.9)
Hypocalcemia	1	6 (5.8)
Abnormal cystatin C	NA	2 (1.9)

NA, not applicable; NCI grade, National Cancer Institute Common Terminology Criteria for Adverse Events.

4. Discussion

Cumulative evidence suggests that the HBV DNA level is an independent risk factor for disease progression of HBV-infected patient, and that achieving an undetectable serum HBV DNA level can delay progression (Chen et al., 2006; Papatheodoridis et al., 2005; Chang et al., 2010b). During antiviral therapy, maintenance of the serum HBV DNA reservoir at a low level indicates ongoing intrahepatic HBV replication, and in this situation, the emergence of drug-resistant strains or the development of multi-drug resistance is inevitable (Yim et al., 2006). Therefore, a fall in HBV DNA to below detectable levels is an important indicator for evaluating antiviral efficacy. In the present study, most patients had received multiple sequential NA therapies before ETV + ADV combination therapy was administered, and their serum HBV DNA was still at a detectable level. For these patients, there are limited antiviral therapeutic regimens that can be chosen in clinical practice. Recently, Bifano et al. (2007) and Delaney et al. (2004) reported that ETV and ADV were well tolerated, did not interact pharmacokinetically, and produced additive antiviral effects against hepatitis B virus in vitro when administered in combination. Thus, ETV + ADV therapy may be an useful option in this group, although it needs to be evaluated for its long-term efficacy in clinical practice.

In the study, we demonstrated that 50% of patients who were refractory to antiviral treatment had a complete virological response after 2 years of ETV + ADV therapy. The mean HBV DNA level exhibited an obvious downward trend from 5.2 to 2.0 log 10 IU/mL, and the mean decrease was 3.2 log 10 IU/mL. Although the HBeAg seroconversion rate was low in our patient cohort, the rate increased over time from 6.7% after 1 year of treatment to 10.0% after 2 years. Thus, 2 years of ETV + ADV therapy proved to be an effective regimen for patients who had suboptimal responses to previous multiple NA monotherapy or combination sequential therapy regimens (except ETV + ADV). In a randomized trial, Lim et al. (2012) reported that the undetectable HBV DNA rate (<60 IU/mL) after 1 year was 29% (13/45 patients) in LAM-resistant patients with suboptimal responses to LAM + ADV combination therapy who were switched to ETV + ADV therapy, as compared with 4% (2/45) with continuation of LAM + ADV therapy. Consequently, these authors showed that ETV + ADV provided a superior virological response, which was similar to our findings.

Of note, the viral breakthrough rate was also low. During 2 years after the initiation of ETV + ADV therapy, 10 patients showed viral breakthrough, with 7 showing a higher HBV DNA level than after 1 year of treatment, but the levels became undetectable with sustained therapy and their illness was eventually stable. The other 3 patients who showed viral breakthrough had their treatment replaced. Overall, the actual viral breakthrough rate in

our study was low during 2 years of ETV + ADV therapy, and detailed analysis of the viral breakthroughs showed that viral replication can occasionally occur under the pressure of antiviral agents. The frequency of this phenomenon, its pathogenesis, and its clinical significance should be investigated in future studies.

Analysis of various impact factors indicated that there was no significant effect of baseline HBV DNA and ALT levels on the undetectable HBV DNA rate after 2 years of ETV + ADV therapy, but a longer prior NA treatment duration was associated with a higher rate of undetectable HBV DNA. The reason for this may be related to the long-term interaction between NAs and the virus, which leads to a weakened virus fitness and improved immune status in the host. It has been reported that in patients who receive long-term NA antiviral therapy, the HBeAg seroconversion rate increases year-by-year (Leung et al., 2001; Marcellin et al., 2008; Liaw et al., 2009; Chang et al., 2010a). This reflects improvement of the immune status in the host, which supports the above view. Multivariate regression analysis showed when the HBV DNA level just prior to initiation of ETV + ADV therapy was lower and the magnitude of the reduction of HBV DNA levels at 1 year was larger, the possibility of undetectable HBV DNA after 2 years of ETV + ADV treatment was higher. This implies that these 2 parameters can be used as predictors of the efficacy of 2 years of combination therapy with ETV + ADV for patients who have failed on multiple NA monotherapy or sequential NA therapy (except ETV + ADV).

Drug-resistant mutations were found in 59.5% (44/74) of patients in our study, including a variety of common NA-associated resistance mutations (see Table 3). Statistical analysis showed that there was no significant association with the antiviral efficacy of ETV + ADV or with viral breakthrough. The rtA181T/V mutation was a shared mutation in patients with LAM and ADV resistance, but they were sensitive to ETV (Villet et al., 2008). The proportion of patients with the rtA181T/V mutation was 18.2% in our study, but on univariate analysis, this mutation had no significant effect on efficacy and viral breakthrough.

After 2 years of ETV + ADV therapy, no occurrences of disease progression were observed. ETV + ADV therapy was well tolerated, and no patient dropped out of the study due to adverse events. This suggests that 2 years of ETV + ADV therapy is safe for these patients.

Our study has some limitations. Firstly, the sample size was small, such that some parameters did not show statistical significance on multivariate analysis. Moreover, the absence of viral mutation data in some patients made the significance of NA-resistant mutations for ETV + ADV combination therapy uncertain. Finally, the study was retrospective, and a prospective study with more strict standards will be needed to confirm the long-term efficacy of ETV + ADV therapy.

In conclusion, 2 years of rescue therapy with a combination of ETV and ADV was effective and well-tolerated in CHB patients who had previously failed on multiple NA treatments. The HBV DNA level just prior to initiation of ETV + ADV combination therapy and the magnitude of the reduction of HBV DNA levels at 1 year could predict the efficacy of 2 years of ETV + ADV treatment. In future, it will be necessary to evaluate whether even greater efficacy can be achieved with prolonged ETV + ADV therapy.

Author contributions

X.W. was the principal investigator and was responsible for the design of the study, interpretation of the data, writing the manuscript, and obtaining funding. C.Z. undertook data assembly, analysis, and interpretation. Y.Z. undertook collection and assembly of the data; Y.X. provided technical and material support; and Y.W. undertook data collection and provided material support. All authors critically reviewed the manuscript and approved the final version for journal submission.

Conflicts of interest

None of the authors has any conflict of interest to disclose.

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References

Bifano, M., Yan, J.H., Smith, R.A., Zhang, D., Grasela, D.M., LaCreta, F., 2007. Absence of a pharmacokinetic interaction between entecavir and adefovir. J. Clin. Pharmacol. 47, 1327–1334.

Carrouée-Durantel, S., Durantel, D., Werle-Lapostolle, B., Pichoud, C., Naesens, L., Neyts, J., Trépo, C., Zoulim, F., 2008. Suboptimal response to adefovir dipivoxil therapy for chronic hepatitis B in nucleoside-naive patients is not due to pre-existing drug-resistant mutants. Antivir. Ther. 13, 381–388.

Chae, H.B., Kim, M.J., Seo, E.G., Choi, Y.H., Lee, H.S., Han, J.H., Yoon, S.M., Park, S.M., Youn, S.J., 2012. High efficacy of adefovir and entecavir combination therapy in patients with nucleoside-refractory hepatitis B. Korean J. Hepatol. 18, 75–83.

Chang, T.T., Lai, C.L., Kew Yoon, S., Lee, S.S., Coelho, H.S., Carrilho, F.J., Poordad, F., Halota, W., Horsmans, Y., Tsai, N., Zhang, H., Tenney, D.J., Tamez, R., Iloeje, U., 2010a. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology 51, 422–430.

Chang, T.T., Liaw, Y.F., Wu, S.S., Schiff, E., Han, K.H., Lai, C.L., Safadi, R., Lee, S.S., Halota, W., Goodman, Z., Chi, Y.C., Zhang, H., Hindes, R., Iloeje, U., Beebe, S., Kreter, B., 2010b. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 52, 886–893.

Chen, C.J., Yang, H.I., Su, J., Jen, C.L., You, S.L., Lu, S.N., Huang, G.T., Iloeje, U.H., REVEAL-HBV Study Group, 2006. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 295, 65–73.

Chen, J., Yin, J., Tan, X., Zhang, H., Zhang, H., Chen, B., Chang, W., Schaefer, S., Cao, G., 2007. Improved multiplex-PCR to identify hepatitis B virus genotypes A–F and subgenotypes B1, B2, C1 and C2. J. Clin. Virol. 38, 238–243.

Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association, 2011. The guideline of prevention and treatment for chronic hepatitis B (2010 version). Zhonghua Gan Zang Bing Za Zhi 19, 13–21.

Cho, Y., Lee, D.H., Chung, K.H., Jin, E., Lee, J.H., Cho, E.J., Yu, S.J., Kim, J.W., Jeong, S.H., Yoon, J.H., Lee, H.S., Kim, C.Y., Kim, Y.J., 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.

Delaney IV, W.E., Yang, H., Miller, M.D., Gibbs, C.S., Xiong, S., 2004. Combinations of adefovir with nucleoside analogs produce additive antiviral effects against hepatitis B virus in vitro. Antimicrob. Agents Chemother. 48, 3702–3710.

Fung, S.K., Lok, A.S., 2004. Management of hepatitis B patients with antiviral resistance. Antivir. Ther. 9, 1013–1026.

Ganem, D., Prince, A.M., 2004. Hepatitis B virus infection – natural history and clinical consequences. N. Engl. J. Med. 350, 1118–1129.

Jeon, J.W., Shin, H.P., Lee, J.I., Joo, K.R., Cha, J.M., Park, J.J., Lim, J.U., Lim, K., Kim, S., 2012. Efficacy of entecavir and adefovir combination therapy for patients with lamivudine- and entecavir-resistant chronic hepatitis B. Dig. Dis. Sci. 57, 1358–1365.

Kim, S.S., Cheong, J.Y., Lee, D., Lee, M.H., Hong, S.P., Kim, S.O., Cho, S.W., 2012. Adefovir-based combination therapy with entecavir or lamivudine for patients with entecavir-refractory chronic hepatitis B. J. Med. Virol. 84, 18–25.

Leung, N.W., Lai, C.L., Chang, T.T., Guan, R., Lee, C.M., Ng, K.Y., Lim, S.G., Wu, P.C., Dent, J.C., Edmundson, S., Condreay, L.D., Chien, R.N., Asia Hepatitis Lamivudine Study Group, 2001. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 33, 1527–1532.

Liaw, Y.F., 2006. Hepatitis B virus replication and liver disease progression: the impact of antiviral therapy. Antivir. Ther. 11, 669–679.

Liaw, Y.F., Sung, J.J., Chow, W.C., Farrell, G., Lee, C.Z., Yuen, H., Tanwandee, T., Tao, Q.M., Shue, K., Keene, O.N., Dixon, J.S., Gray, D.F., Sabbat, J., Cirrhosis Asian Lamivudine Multicentre Study Group, 2004. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N. Engl. J. Med. 351, 1521–1531.

Liaw, Y.F., Gane, E., Leung, N., Zeuzem, S., Wang, Y., Lai, C.L., Heathcote, E.J., Manns, M., Bzowej, N., Niu, J., Han, S.H., Hwang, S.G., Cakaloglu, Y., Tong, M.J., Papatheodoridis, G., Chen, Y., Brown, N.A., Albanis, E., Galil, K., Naoumov, N.V., GLOBE Study Group, 2009. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology 136, 486–495.

Lim, Y.S., Lee, J.Y., Lee, D., Shim, J.H., Lee, H.C., Lee, Y.S., Suh, D.J., 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.

Lu, F.M., Zhuang, H., 2009. Management of hepatitis B in China. Chin. Med. J. (Engl.) 122, 3–4.

Marcellin, P., Chang, T.T., Lim, S.G., Sievert, W., Tong, M., Arterburn, S., Borroto-Esoda, K., Frederick, D., Rousseau, F., 2008. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology 48, 750–758.

Papatheodoridis, G.V., Dimou, E., Dimakopoulos, K., Manolakopoulos, S., Rapti, I., Kitis, G., Tzourmakliotis, D., Manesis, E., Hadziyannis, S.J., 2005. Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. Hepatology 42, 121–129.

Seo, S.Y., Kim, I.H., Sohn, J.Y., Lee, S., Kim, S.H., 2014. Long-term efficacy of entecavir plus adefovir combination therapy versus entecavir monotherapy in adefovir refractory chronic hepatitis B patients with prior lamivudine resistance. Intervirology 57, 8–16.

Villet, S., Pichoud, C., Billioud, G., Barraud, L., Durantel, S., Trépo, C., Zoulim, F., 2008. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. J. Hepatol. 48, 747–755.

Xu, X.H., Li, G.L., Qin, Y., Li, Q., He, F.Q., 2013. Entecavir plus adefovir rescue therapy for chronic hepatitis B patients after multiple treatment failures in real-life practice. Virol. J. 10, 162–166.

Yang, H.J., Lee, J.H., Kim, Y.J., Yoon, J.H., Lee, H.S., 2012. Antiviral efficacy of combination therapy with entecavir and adefovir for entecavir/lamivudine-resistant hepatitis B virus with or without adefovir resistance. J. Med. Virol. 84, 424–430.

Yim, H.J., Hussain, M., Liu, Y., Wong, S.N., Fung, S.K., Lok, A.S., 2006. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 44, 703–712.

Zoulim, F., Locarnini, S., 2012. Management of treatment failure in chronic hepatitis B. J. Hepatol. 56 (Suppl. 1), S112–S122.