Long-Term Efficacy of Entecavir Plus Adefovir Combination Therapy versus Entecavir Monotherapy in Adefovir Refractory Chronic Hepatitis B Patients with Prior Lamivudine Resistance

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Key Words

Chronic hepatitis B · Resistance · Entecavir · Adefovir

Abstract

Objectives: We investigated the long-term efficacy of entecavir (ETV) + adefovir (ADV) combination therapy versus ETV monotherapy in lamivudine (LAM)-resistant chronic hepatitis B (CHB) patients who failed to respond to ADV rescue therapy. **Methods:** A total of 91 ADV refractory patients with prior LAM resistance received ETV (1.0 mg/day) + ADV (10 mg/day) combination therapy (group A, n = 45) or ETV (1.0 mg/day) monotherapy (group B, n = 46) for more than 48 weeks. **Results:** The rates of undetectable serum hepatitis B virus DNA levels (≤ 20 IU/ml) at weeks 48 and 96 were not significantly different between group A and group B (31.1 vs. 23.9% at week 48, $p = 0.442$, and 44.7 vs. 34.5% at week 96, $p = 0.457$). However, the incidence of virological breakthrough in group A was significantly lower than that in group B (0 vs. 17.4% at week 48, $p = 0.006$, and 2.6 vs. 44.8% at week 96, $p < 0.001$). ETV monotherapy was the only independent factor significantly associated with virologic breakthrough ($p = 0.015$). **Conclusions:** ETV + ADV combination therapy is a better therapeutic option than ETV monotherapy for ADV refractory CHB patients with prior LAM resistance.

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Introduction

The main goals of chronic hepatitis B (CHB) treatment are the long-term suppression of hepatitis B virus (HBV) replication to prevent disease progression and to prolong patient survival [1, 2]. Although nucleos(t)ide analogues (NA) showed potent inhibition of HBV replication with a good safety profile, a durable virologic response is seldom achieved after withdrawal of NA therapy, necessitating long-term maintenance treatment. During long-term NA therapy the sustained suppression of HBV DNA to very low or undetectable levels has been known to reduce the risk of disease progression, whereas an inadequate or suboptimal virologic response is a strong risk factor for antiviral resistance, which may lead to treatment failure and exacerbation of liver disease [3–5].

Lamivudine (LAM) was the first NA approved for the treatment of patients with CHB. It has been used worldwide for more than a decade and has an excellent safety record. However, the risk of drug resistance increases with the duration of LAM therapy, reaching 70% after 4 years of use [6]. Adefovir (ADV) has been known to be effective against both wild-type HBV- and LAM-resistant HBV mutants [7–9]. However, several studies have shown a higher probability of ADV resistance in patients with LAM resistance than in treatment-naïve patients [10–12].

Furthermore, a substantial population of LAM-resistant patients show inadequate or suboptimal responses to treatment with ADV, especially if rescue therapy is started after the emergence of LAM-resistant mutations and when serum HBV DNA levels are high [13, 14]. In many Asian countries, including Korea, many patients are treated with sequential LAM and ADV therapy because of the relatively low cost of treatment and provision by national medical insurance systems. Thus, the sequential failure of LAM and ADV treatment is a significant issue in these countries.

The optimal treatment strategy for LAM-resistant CHB patients who show suboptimal responses to ADV rescue therapy remains unclear. Entecavir (ETV) is a possible treatment option for these patients in the many Asian countries where tenofovir (TDF) is not available. ETV has been shown to be potent antiviral agent that is superior to LAM and ADV with a very low risk of antiviral resistance in naive patients [15–17]. However, the response of patients with preexisting LAM resistance to ETV may be compromised since approximately 50% of them will develop resistance to ETV in 5 years [18]. Furthermore, several recent studies have shown that the efficacy of ETV monotherapy is attenuated by the frequent emergence of ETV resistance in LAM/ADV refractory patients [19–23]. Thus, it is necessary to investigate potential combination therapies such as ETV combined with ADV. However, no data is available on the efficacy of long-term ETV + ADV combination therapy compared with ETV monotherapy.

The aim of this study was to compare the long-term antiviral efficacies and resistance rates of ETV + ADV combination therapy versus ETV monotherapy for up to 96 weeks in LAM-resistant CHB patients after failed ADV rescue therapy.

Materials and Methods

Patients and Study Design

In this retrospective study, LAM-resistant CHB patients who failed to respond to ADV rescue therapy (ADV alone or ADV + LAM combination) received either ETV (1 mg daily) + ADV (10 mg daily) combination therapy or ETV (1 mg daily) monotherapy for at least 12 months at Chonbuk National University Hospital between February 2007 and December 2011. All patients were 18 years of age or older, showed positive hepatitis B surface antigen (HBsAg) for at least 6 months and demonstrated a history of LAM resistance by documented genotypic resistance or virologic breakthrough at the initiation of ADV rescue therapy. Failed ADV rescue therapy was defined as a suboptimal response (a persistent serum HBV DNA level $\geq 2,000$ IU/ml after ADV therapy

for at least 6 months) or the development of resistance (an increase in serum HBV DNA level $\geq 1 \log_{10}$ IU/ml from nadir during ADV therapy, irrespective of documented ADV-resistant mutations). Patients with decompensated liver disease (ascites, variceal bleeding, encephalopathy) or renal impairment (serum creatinine ≥ 1.5 mg/dl) were excluded. Other exclusion criteria were as follows: prior treatment with anti-HBV NAs other than LAM and ADV; coinfection with human immunodeficiency virus, hepatitis C virus, or hepatitis delta virus; a history of alcohol or drug abuse within the preceding 2 years; documented or suspected HCC; prior organ transplantation, or pregnancy/lactation.

Routine blood and liver enzyme tests were performed every 3 months and adverse events were assessed at each visit. Liver panel, creatinine, HBeAg, anti-HBe, and quantitative HBV DNA were checked at the start of therapy and then once every 3 months in all patients. This study was conducted in compliance with the World Medical Association Declaration of Helsinki and was approved by the ethics committee of our institution. All patients provided written informed consent.

Serum Assays

HBsAg, anti-HBs, HBeAg, and anti-HBe were detected using commercial enzyme-linked immunosorbent assay (ELISA) kits (AXSYM; Abbott Laboratories, Chicago, Ill., USA). Serum HBV DNA levels were determined using polymerase chain reaction (PCR) assays, primarily the real-time PCR COBAS TaqMan HBV Test (Roche Diagnostics, Branchburg, N.J., USA; detection limit 13 IU/ml). Genotypic analysis of HBV DNA polymerase was performed in patients showing virologic breakthrough during therapy using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based genotyping assay, previously known as the restriction fragment mass polymorphism (RFMP) assay [24].

Efficacy Endpoints and Definitions

The primary endpoints of this study were undetectability of serum HBV DNA levels by PCR (<20 IU/ml), virologic breakthrough, and genotypic resistance at weeks 48 and 96. The secondary endpoints included a reduction in serum HBV DNA versus baseline, the distribution of serum HBV DNA levels (<20 , 20–2,000, and $\geq 2,000$ IU/ml), serum alanine aminotransferase (ALT) normalization (≤ 40 IU/l), and HBeAg loss or seroconversion at weeks 48 and 96. Virologic breakthrough, which is usually associated with drug resistance, was defined as a $\geq 1 \log_{10}$ IU/ml increase in serum HBV DNA levels from nadir in two consecutive samples 1 month apart while receiving treatment and after achieving an initial response in medically compliant patients on antiviral medication(s). Genotypic resistance was defined as the detection of HBV mutations known to confer antiviral resistance during antiviral therapy.

Statistical Analysis

Results are reported as means $\pm$ SD. HBV DNA levels were logarithmically transformed for analysis. Continuous variables were compared using the two-tailed Student's *t* test or the Mann-Whitney *U* test. Categorical data were analyzed using the χ^2 test or Fisher's exact test. Factors associated with virologic breakthrough were analyzed by univariate analysis and multivariate logistic regression analysis. All *p* values <0.05 were considered statistically

Table 1. Baseline characteristics of patients

	Total (n = 91)	ETV + ADV combination therapy (n = 45)	ETV monotherapy (n = 46)	p value
Age, years	46.6±9.3	45.6±10.1	47.7±8.4	0.281
Male sex, n (%)	73 (80.2)	35 (77.8)	38 (82.6)	0.563
Cirrhosis, n (%)	28 (30.8)	14 (31.1)	14 (30.4)	0.944
HBeAg-positive, n (%)	80 (87.9)	40 (88.9)	40 (87.0)	1.000
LAM-resistant mutations, n (%)	87 (95.6)	43 (95.6)	44 (95.7)	1.000
rtL180M	3 (3.3)	0 (0.0)	3 (6.5)	
rtM204I/V	23 (25.3)	16 (35.6)	7 (15.2)	
rtL180M + rtM204I/V	56 (61.5)	25 (55.6)	31 (67.4)	
NA	5 (5.5)	2 (4.4)	3 (6.5)	
Duration of previous ADV treatment, months	30.6±14.3	34.3±13.0	26.9±14.7	0.013
ADV-resistant mutations, n (%)	36 (39.6)	13 (28.9)	23 (50.0)	0.046
rtN236T	3 (3.3)	2 (4.4)	1 (2.2)	
rtA181T/V	22 (24.2)	9 (20.0)	13 (28.9)	
rtN236T + rtA181T/V	11 (12.1)	2 (4.4)	9 (19.6)	
Negative	51 (56.0)	31 (68.9)	20 (43.5)	
NA	4 (4.4)	1 (2.2)	3 (6.5)	
Both LAM- and ADV-resistant mutations, n (%)	24 (26.3)	8 (17.8)	16 (34.8)	0.104
rtM204I/V + rtN236T + rtL180M	2 (2.2)	1 (2.2)	1 (2.2)	
rtM204I/V + rtA181V/T ± rtL180M	15 (16.4)	6 (13.3)	9 (19.6)	
rtM204I/V + rtN236T + rtA181V/T + rtL180M	7 (7.7)	1 (2.2)	6 (13.0)	
Platelets, ×10 ³ /mm ³	170.2±70.8	179.6±79.8	161.0±60.3	0.212
ALT, IU/l	69.6±52.3	56.4±43.6	82.6±57.1	0.016
Total bilirubin, mg/dl	2.3±10.0	2.2±9.2	2.4±10.7	0.925
Albumin, g/dl	4.3±0.4	4.3±0.2	4.2±0.5	0.312
Serum HBV DNA level, log ₁₀ IU/ml	5.4±1.5	5.1±1.9	5.7±0.9	0.067
Duration of ETV treatment, months	31.5±12.5	32.6±9.6	30.4±14.8	0.401

Data is expressed as mean ± SD or number (%). NA = Not applicable.

significant. Data was collated in Microsoft Excel (Microsoft Excel 2003; Microsoft Corp., Redmond, Wash., USA) and analyzed using SPSS for Windows version 15.0 (SPSS, Inc., Chicago, Ill., USA).

Results

Baseline Characteristics of Patients

A total of 91 patients were included in this analysis. Overall, 28 (30.8%) patients had compensated cirrhosis, 80 (87.9%) patients were HBeAg-positive, and 87 (95.6%) patients had documented LAM-resistant mutations. The mean duration of previous ADV treatment was 30.6 months. Genotypic resistance for ADV was analyzed in 87 patients, out of whom 36 (39.6%) had ADV-resistant mutations and 51 (56.0%) did not.

Out of total 91 patients, 45 received ETV + ADV combination therapy and 46 received ETV monotherapy. On average, patients in the ETV + ADV combination therapy

group had a longer duration of previous ADV treatment, a higher proportion of ADV-resistant mutations, and lower baseline serum ALT and serum HBV DNA levels than the ETV monotherapy group. However, the two treatment groups were comparable with respect to demographics and baseline laboratory features, including age, sex, presence of cirrhosis, HBeAg positivity, duration of ETV therapy, and serum HBV DNA level (table 1).

Treatment Responses

After treatment was initiated, serum HBV DNA levels declined continuously in both treatment groups. The mean reduction in serum HBV DNA level from baseline to week 48 was greater in the ETV + ADV combination therapy group than in the ETV monotherapy group (−3.0 vs. −2.0 log₁₀ IU/ml, *p* = 0.022) (table 2). However, at week 96, the mean reduction in serum HBV DNA level from baseline was not significantly different between the two treatment groups (−3.3 vs. −3.2 log₁₀ IU/ml, *p* = 0.549)

Fig. 1. Distribution of serum HBV DNA levels at week 48 (a) and week 96 (b).

Table 2. Virologic, biochemical, and serologic responses at weeks 48 and 96

	ETV + ADV combination therapy	ETV monotherapy	p value
At week 48	(n = 45)	(n = 46)	
Serum HBV DNA, mean reduction from baseline, log ₁₀ IU/ml	-3.0±2.0	-2.0±2.0	0.022
Serum HBV DNA level ≤20 IU/ml, n (%)	14 (31.1)	11 (23.9)	0.442
ALT normalization, n (%)	27 (60.0)	28 (60.9)	0.932
HBeAg loss or seroconversion, n (%)	3/40 (7.5)	5/40 (12.5)	0.712
At week 96	(n = 38)	(n = 29)	
Serum HBV DNA, mean reduction from baseline, log ₁₀ IU/ml	-3.3±2.4	-3.0±2.1	0.549
Serum HBV DNA level ≤20 IU/ml, n (%)	17 (44.7)	10 (34.5)	0.457
ALT normalization, n (%)	23 (60.5)	18 (62.1)	0.898
HBeAg loss or seroconversion, n (%)	5/34 (14.7)	5/26 (19.2)	0.733

Data is expressed as mean ± SD or number (%).

(table 2). Similarly, the proportion of patients who achieved an undetectable serum HBV DNA level (≤20 IU/ml) at weeks 48 and 96 was not significantly different between the combination therapy group and the monotherapy group (31.1 vs. 23.9% at week 48, $p = 0.442$, and 44.7 vs. 34.5% at week 96, $p = 0.457$) (table 2). We analyzed the distribution of patients according to serum HBV DNA levels (≤20 vs. 20–2,000 vs. ≥2,000 IU/ml) at weeks 48 and 96 (fig. 1). The proportion of patients who exhibited an inadequate virologic response (serum HBV DNA ≥2,000 IU/ml) at weeks 48 and 96 was significantly lower in the ETV + ADV combination therapy group compared to the ETV monotherapy group (13.3 vs. 56.5%

at week 48, and 5.3 vs. 51.7% at week 96). The distribution of serum HBV DNA levels of patients in the ETV + ADV group fell in significantly lower ranges compared to that of the ETV monotherapy group at both weeks 48 and 96 ($p = 0.002$ and 0.004 at weeks 48 and 96, respectively) (fig. 1).

However, patients in the ETV + ADV combination therapy and the ETV monotherapy groups showed similar biochemical and serological responses. The rate of serum ALT normalization in the combination therapy group versus the monotherapy group was 60.0 vs. 60.9% at week 48 ($p = 0.932$) and 60.5 vs. 62.1% ($p = 0.898$) at week 96. The rate of HBeAg loss and/or HBeAg serocon-

Fig. 2. Rates of virologic breakthrough (a) and genotypic resistance (b) at weeks 48 and 96.

version in the combination therapy group versus the monotherapy group was 7.5 vs. 12.5% at week 48 ($p = 0.712$) and 14.7 vs. 19.2% at week 96 ($p = 0.733$) (table 2).

Virologic Breakthrough and Genotypic Resistance

The proportion of patients who developed virologic breakthrough was significantly lower in the ETV + ADV combination therapy group than in the ETV monotherapy group (0 vs. 17.4% at week 48, $p = 0.006$, and 2.6 vs. 44.8% at week 96, $p < 0.001$) (fig. 2). Mutations associated with resistance to ETV were analyzed using RFMP assays in all patients that developed virologic breakthrough during treatment. At week 48, ETV-resistant mutations were not detected in any patients in the ETV + ADV combination therapy group, whereas ETV-resistant mutations were found in 2 patients (4.3%) in the ETV monotherapy group ($p = 0.495$). Furthermore, at week 96, the rate of developing genotypic resistance to ETV was significantly lower in the ETV + ADV combination therapy group than in the ETV monotherapy group (0 vs. 27.6%, $p < 0.001$) (fig. 2). The followings were ETV-resistant mutations found in 8 patients of ETV monotherapy group; 3 patients with rtT184L, 1 patient with rtT184L ± rtT184M, 1 patient with rtT184I, 1 patient with rtI169T ± rtT184S/M ± rtT184I/A, 1 patient with rtT184I/A, and 1 patient with rtS202G.

Factors Associated with Virologic Breakthrough

Overall, 18 out of 91 patients developed virologic breakthrough during treatment with ETV with or without ADV at 96 weeks. We assessed the factors associated

with virologic breakthrough using uni- and multivariate analyses (tables 3, 4). Neither age, sex, presence of cirrhosis, HBeAg positivity, duration of previous ADV treatment, or LAM-resistant mutations were found to be associated with virologic breakthrough. Patients who developed virologic breakthrough had a higher proportion of ADV-resistant mutations, lower baseline serum ALT levels, and higher baseline serum HBV DNA levels, but these differences were not statistically significant. However, virologic breakthrough was found to be associated with baseline serum HBV DNA level $\geq 20,000$ IU/ml ($p = 0.034$), ETV monotherapy ($p < 0.001$), and serum HBV DNA level $\geq 2,000$ IU/ml ($p = 0.016$) (table 3). In the multivariate logistic regression analysis (table 4), treatment strategy (ETV monotherapy vs. ETV + ADV combination therapy) (odds ratio 5.88, 95% confidence interval 1.66–119.36; $p = 0.015$) was the only independent factor significantly associated with virologic breakthrough.

Discussion

To the best of our knowledge, this is the first study to date to directly compare the long-term efficacy (for up to 96 weeks) of ETV + ADV combination therapy versus ETV alone in LAM-resistant CHB patients who failed to respond to ADV rescue therapy. The results of our study showed that a higher proportion of patients in the ETV + ADV combination therapy group fell into the lower ranges of serum HBV DNA levels than those in the ETV monotherapy group. ETV + ADV combination therapy

Table 3. Factors associated with virologic breakthrough after up to 96 weeks of treatment (univariate analysis)

	With virologic breakthrough (n = 18)	Without virologic breakthrough (n = 73)	p value
Age, years	49.0±11.3	46.1±8.7	0.243
Male sex, n (%)	16 (88.9)	57 (78.1)	0.509
Cirrhosis, n (%)	5 (27.8)	23 (31.5)	1.000
HBeAg-positive, n (%)	16 (88.9)	64 (87.7)	1.000
Duration of previous ADV treatment, months	25.2±14.5	31.9±14.1	0.076
LAM-resistant mutations, n (%)	16 (88.9)	71 (97.3)	0.174
rtL180M	1 (5.6)	2 (2.7)	
rtM204I/V	2 (11.1)	21 (28.8)	
rtL180M + rtM204I/V	13 (72.2)	43 (58.9)	
NA	0 (0.0)	5 (6.8)	
ADV-resistant mutations, n (%)	10 (55.6)	26 (35.6)	0.055
rtN236T	0 (0.0)	3 (4.1)	
rtA181T/V	5 (27.8)	17 (23.3)	
rtN236T + rtA181T/V	5 (27.8)	6 (8.2)	
Negative	6 (33.3)	45 (61.6)	
NA	2 (11.1)	2 (2.7)	
Serum ALT, IU/l	68.4±47.5	69.9±53.7	0.055
Serum HBV DNA level at baseline, log ₁₀ IU/ml	6.0±1.1	5.2±1.5	0.055
Serum HBV DNA level at baseline, n (%)			0.034
<200,000 IU/ml	5 (27.8)	42 (57.5)	
≥200,000 IU/ml	13 (72.2)	31 (42.5)	
Treatment strategy, n (%)			<0.001
ETV + ADV combination therapy	1 (5.6)	44 (60.3)	
ETV monotherapy	17 (94.4)	29 (39.7)	
Serum HBV DNA level at week 24, n (%)			0.016
<2,000 IU/ml	5 (27.8)	45 (61.6)	
≥2,000 IU/ml	13 (72.2)	28 (38.4)	

Data is expressed as mean ± SD or number (%).

Table 4. Factors associated with virologic breakthrough after up to 96 weeks of treatment period (multivariate analysis)

	OR	95% CI	p value
ADV-resistant mutations, positive vs. negative	1.26	0.48–6.51	0.389
Serum HBV DNA level at baseline, log ₁₀ IU/ml ≥20,000 vs. <20,000 IU/ml	1.78	0.58–16.82	0.183
Treatment strategy, ETV monotherapy vs. ETV + ADV combination therapy	5.89	1.66–119.36	0.015
Serum HBV DNA level at week 24, ≥2,000 vs. <2,000 IU/ml	1.79	0.67–8.24	0.181

was associated with significantly lower incidences of virologic breakthrough and genotypic resistance compared to ETV monotherapy. Furthermore, the treatment strategy of ETV monotherapy was an independent factor associated with virologic breakthrough.

ETV is an antiviral agent that does not exhibit cross-resistance with ADV. In addition, ETV has been shown to be effective against both the rtA181T/V and rtN236T mutant HBV strains [25]. Accordingly, the 2008 Asian Pacific Consensus Statement on the Management of CHB

recommended ETV rescue therapy for LAM- and ADV-resistant patients [26]. Reijnders et al. [27] reported that ETV was effective in ADV-experienced patients including those with ADV resistance and that there was no difference in the efficacy of ETV between LAM-naïve and -experienced patients without a history of LAM resistance. Similarly, Sheen et al. [28] demonstrated that, by 12 months after switching to ETV treatment, complete viral suppression (serum HBV DNA <100 IU/ml) was achieved in the majority of both LAM-experienced and -naïve patients (68%) who had a suboptimal response to ADV. However, in LAM-resistant patients, ETV monotherapy reduced the probability of achieving a virologic response. Several recent Korean studies have also reported that ETV monotherapy has limited efficacy in patients who fail to respond to LAM and ADV [21–23]. Son et al. [23] compared the efficacy of LMV + ADV versus ETV in HBeAg-positive patients who experienced sequential treatment failure with LAM and ADV. Their results showed that neither LAM + ADV nor ETV was adequately effective, with no significant difference in virologic response between the two treatment methods at 12 months (22.6 vs. 19.5%). The antiviral efficacy of ETV monotherapy is further attenuated by the frequent emergence of ETV resistance in ADV refractory CHB patients with prior LAM resistance. For example, Kwak et al. [21] reported that virologic breakthrough occurred in 21.1 and 78.9% of patients at 48 and 96 weeks, respectively. In our study, ETV monotherapy yielded limited efficacy in viral suppression as defined as achieving undetectable HBV DNA levels, reaching 23.9% at week 48 and 34.5% at week 96. Meanwhile, there was a substantial increase in the incidence of virologic breakthrough (17.4% at week 48 and 44.8% at week 96) and genotypic resistance (4.3% at week 48 and 27.6% at week 96). These results indicate that ETV monotherapy is not the optimal treatment strategy for patients with LAM-resistant CHB who failed to respond to ADV rescue therapy.

For patients in whom treatment has failed, there is a strong virologic rationale for use of an ‘add-on’ strategy with a complementary drug to minimize the risk of multidrug resistance [29]. ETV and ADV offer complementary resistance profiles and can reduce the viral load in LAM-resistant HBV patients [30, 31]. Thus, ETV + ADV combination therapy could be a promising rescue therapy for patients after LAM and ADV treatment failure. In a recent randomized trial conducted by Lim et al. [32], ETV + ADV combination therapy showed a favorable resistance profile and resulted in a superior viral response compared with continuing LAM + ADV combination

therapy in LAM-resistant CHB patients who had a suboptimal response to LAM + ADV. At week 52, 29% of the ETV + ADV group achieved a serum HBV DNA level of <60 IU/ml ($p = 0.004$) compared to 4% of the LAM + ADV group. Moreover, no patients in the ETV + ADV group demonstrated additional genotypic resistance to ADV or ETV while resistance occurred in 15% of the LAM + ADV group ($p = 0.018$). Similarly, 31.1 and 44.7% of patients who received ETV + ADV combination therapy achieved undetectable serum HBV DNA levels (<20 IU/ml) at weeks 48 and 96, respectively. In addition, there was a very low incidence of virologic breakthrough (0% at week 48 and 2.6% at week 96) and no genotypic resistance up to week 96. Although the proportion of patients with undetectable serum HBV DNA levels (<20 IU/ml) was not significantly different between the ETV + ADV combination therapy and the ETV monotherapy group, the serum HBV DNA levels of patients in the ETV + ADV combination therapy group were distributed over significantly lower ranges than those of patients in the ETV monotherapy group. Furthermore, multivariate analysis confirmed that ETV + ADV combination therapy significantly reduced the development of antiviral resistance compared to ETV monotherapy. In spite of these findings, our study also showed that more than half of patients receiving ETV + ADV combination therapy still had detectable serum HBV DNA levels at 96 weeks. This is likely due to the combination of the relatively low antiviral potency of ADV and the reduced antiviral efficacy of ETV in LAM-resistant patients. Therefore, research into more potent rescue therapies is necessary in order to develop a strategy that can achieve complete virologic response in LAM-resistant CHB patients after ADV failure.

TDF has been shown to be superior to ADV in terms of viral suppression and drug resistance in naïve patients [33]. The efficacy of TDF is not influenced by the presence of mutations associated with LAM resistance [34]. TDF also effectively suppresses HBV replication in LAM-resistant patients who have failed to adequately respond to ADV as well as in patients who are resistant to both LAM and ADV [35]. However, *in vitro* studies have demonstrated that ADV-resistant mutations such as rtA181T and rtN236T are associated with a decrease in TDF sensitivity, indicating potential cross-resistance [29]. Therefore, add-on emtricitabine or LAM to TDF therapy may be more appropriate than TDF monotherapy in ADV-resistant patients. In a prospective study by Patterson et al. [35], TDF/LAM rescue therapy following the failure of both LAM and ADV achieved an undetectable level of HBV DNA (<15 IU/ml) in 46 and 64% of patients at 48

and 96 weeks of treatment, respectively. While not a direct comparison, their results were superior to what was achieved in our study, which showed that 31.1 and 44.7% of patients reached an undetectable HBV DNA level at weeks 48 and 96 of ETV + ADV combination therapy, respectively. Furthermore, a recent multicenter cohort study by Peterson et al. [36] suggested that rescue therapy with ETV and TDF was efficient, safe, and well tolerated in patients who harbor viral resistance patterns and those who showed only partial antiviral responses to previous therapies. Thus, TDF + ETV combination therapy could also be considered for patients resistant to both LAM and ADV.

We acknowledge that this study has several limitations. First, since it was a retrospective observational study without randomization, differences in baseline characteristics between the two treatment groups may have affected the treatment outcomes. Second, drug adherence, an important factor that affects virologic breakthrough, could not be thoroughly assessed. Finally, data on the rate of genotypic resistance among study participants was limited because genotypic resistance to study drugs was only analyzed in patients showing virologic breakthrough.

In summary, this study demonstrated that ETV + ADV combination therapy was associated with a significantly lower incidence of antiviral resistance compared to ETV monotherapy. ETV monotherapy was an independent risk factor for the development of virologic breakthrough. Although the majority of patients showed a partial viral response after ETV-based rescue therapy, a higher proportion of patients in the ETV + ADV combination therapy group had lower serum HBV DNA levels than those in the ETV monotherapy group. Thus, the results of this study suggests that ETV + ADV combination therapy is a better therapeutic option than ETV monotherapy for LAM-resistant CHB patients who failed to respond to ADV rescue therapy since ETV monotherapy selects for LAM-resistant strains that increase the risk of ETV resistance. Further investigation into more potent rescue therapies, including TDF, is needed in order to achieve better viral suppression outcomes.

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