Prolonged Entecavir Therapy Is Not Effective for HBeAg Seroconversion in Treatment-Naïve Chronic Hepatitis B Patients with a Partial Virological Response

Hyun Woong Lee,^a Jae-Cheol Kwon,^a In Soo Oh,^a Hye Young Chang,^a Young Joo Cha,^b Ik-Seong Choi,^c Hyung Joon Kim^a

Department of Internal Medicine^a and Department of Laboratory Medicine,^b Chung-Ang University College of Medicine, and LSK Global Pharma Services Co., Ltd.,^c Seoul, Republic of Korea

The aims of this study were to investigate the efficacy of prolonged entecavir (ETV) therapy in treatment-naïve chronic hepatitis B (CHB) patients and to determine whether continuous ETV therapy is feasible to achieve HBeAg seroconversion, particularly in patients with partial virological response (PVR). A total of 142 treatment-naïve patients with CHB were enrolled. The mean duration of treatment was 65 (range, 26 to 90) months, and 86 patients (60.6%) were HBeAg positive. PVR was defined as detectable hepatitis B virus (HBV) DNA (>116 copies/ml) at year 1. The cumulative incidence of virological response (VR) increased from 54.9% at year 1 to 98.2% at year 7. HBeAg positivity (odds ratio [OR], 4.146; $P = 0.001$) and initial alanine aminotransferase (ALT) (OR, 0.997; $P = 0.004$) were independent risk factors for PVR. Among the 64 patients with PVR, 47 patients (73.4%) achieved VR within 4 years after prolonged ETV therapy without treatment adaptation. Three patients (2.1%) experienced virological breakthrough and HBV variants with genotypic resistance. The cumulative rate of HBeAg seroconversion was significantly higher in the patients with VR than in the patients with PVR ($P = 0.018$). None of the PVR patients with HBV DNA at $\geq 5,000$ copies/ml at year 1 ever experienced HBeAg seroconversion. Multivariate analysis identified VR at year 1 as the only determinant of HBeAg seroconversion (hazard ratio [HR], 3.009; $P = 0.010$). In conclusion, although there were patients with PVR, prolonged ETV therapy showed excellent VR, with only 2.1% emergence of viral resistance during a 7-year follow-up. However, to achieve HBeAg seroconversion, drug modification is needed for HBeAg-positive patients with PVR (especially those with HBV DNA at $\geq 5,000$ copies/ml at year 1).

Currently, treatment guidelines for both HBeAg-positive and HBeAg-negative chronic hepatitis B (CHB) patients recommend treatment with pegylated interferon or nucleos(t)ide analogs (NAs) (1–4). Although pegylated interferon has advantages, including a finite treatment duration and the absence of viral resistance, adherence to treatment is poor due to frequent side effects and the need for frequent subcutaneous injections (5). Given the increased risk of virological failure from poor adherence to pegylated interferon treatment, NAs have been widely used in clinical practice. Among several NAs, entecavir (ETV) and tenofovir (TDF) are especially potent inhibitors of hepatitis B virus (HBV) reverse transcriptase and DNA polymerase with minimal or no drug resistance (6–8). Therefore, ETV has been confidently used as a first-line therapy. Despite its efficacy, about 10 to 20% patients treated with ETV showed partial virological response (PVR) (9, 10). Unfortunately, HBV cannot be eradicated, and NAs must be continued until HBsAg seroclearance. Sustained viremia may ultimately result in HBV variants with genotypic resistance and clinical breakthrough. Therefore, it is related to subsequent therapeutic failure. In contrast to the frequently described genotypic resistance to lamivudine, adefovir, and ETV, genotypic resistance associated with TDF has not been reported so far. This benefit of TDF is likely to lead to early TDF-based treatment to prevent multidrug resistance (11, 12).

PVR is usually defined as detectable HBV DNA after at least 6 (or 12) months of therapy with a low (or high)-potency drug in compliant patients (2). In PVR patients receiving a low-potency drug (lamivudine or telbivudine) with a low genetic barrier to resistance, changing to a higher-potency drug (ETV or TDF) without cross-resistance is recommended. However, in patients with

PVR to ETV, it is not clear whether continuous ETV therapy adds additional benefits. The optimal management of patients with PVR under a high-potency drug is currently under debate (2).

The aim of this study was to investigate the efficacy of prolonged ETV therapy for up to 7 years in treatment-naïve CHB patients and to determine whether continuous ETV therapy is feasible to achieve HBeAg seroconversion, particularly in patients with PVR.

MATERIALS AND METHODS

Study population. A total of 142 patients with chronic hepatitis B visited Chung-Ang University Hospital between January 2007 and January 2010. They were all followed for at least 2 years after ETV treatment and enrolled in the current study. The patients enrolled in the study met the following entry criteria: they were 20 to 75 years of age; they were positive for serum HBsAg for at least 6 months; they had elevated serum alanine aminotransferase (ALT) levels on two occasions at least 3 months apart, with a value greater than the upper limit of normal (ULN); and they were positive for

Received 28 April 2015 Returned for modification 18 May 2015

Accepted 10 June 2015

Accepted manuscript posted online 22 June 2015

Citation Lee HW, Kwon J-C, Oh IS, Chang HY, Cha YJ, Choi I-S, Kim HJ. 2015.

Prolonged entecavir therapy is not effective for HBeAg seroconversion in treatment-naïve chronic hepatitis B patients with a partial virological response. *Antimicrob Agents Chemother* 59:5348–5356. doi:10.1128/AAC.01017-15.

Address correspondence to Hyung Joon Kim, lhwdoc@cau.ac.kr.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.01017-15

FIG 1 Flow diagram for 142 treatment-naïve chronic hepatitis B patients treated with entecavir.

serum HBV DNA on two occasions at least 3 months apart. Additional requirements included a hemoglobin value of ≥ 10 g/dl, a platelet count of $\geq 100,000$ mm³, and normal renal function with normal serum creatinine levels. Candidates were required to have compensated liver disease with a prolonged prothrombin time of less than 4 s, a serum albumin level of ≥ 3.0 g/dl, a total bilirubin level of ≤ 4 mg/dl, and no history of hepatic encephalopathy or bleeding esophageal varices.

The patients should not have received interferon or NAs prior to the onset of the present study. Other exclusion criteria were as follows: a history of corticosteroid treatment within 6 months of entry; the presence of antibodies to human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis D virus (HDV); a history of other malignancy, except hepatocellular carcinoma (HCC), or evidence of other forms of liver disease; a history of intravenous drug abuse. Patients with other significant medical or psychiatric problems were also excluded.

The patients were treated with ETV monotherapy for virological response (VR). ETV was given at a dose of 0.5 mg per day for at least 2 years. A flow diagram of the 142 patients enrolled in this study is shown in Fig. 1. Serum HBeAg, anti-HBe, HBV DNA, and ALT were assessed every 3 or 6 months. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of Chung-Ang University College of Medicine [IRB no. C2015068(1526)], and written informed consent was obtained from each patient.

Definitions. VR was defined as undetectable HBV DNA (HBV DNA < 116 copies/ml) by a sensitive PCR assay during the treatment follow-up period. PVR was defined as a decrease in HBV DNA of more than 1 log₁₀ copies/ml (10-fold) but having detectable HBV DNA at 1 year of ETV therapy in compliant patients. Virological breakthrough (VB) was defined as an increase in serum HBV DNA of > 1 log₁₀ copies/ml (10-fold) above nadir after achieving VR during continued treatment. HBeAg seroconversion was defined as the loss of HBeAg and detection of anti-HBe in a patient who was previously HBeAg positive and anti-HBe negative.

Endpoints. The primary outcome was the cumulative incidence of VR during the treatment follow-up period. Secondary endpoints were the cumulative incidence of HBeAg seroconversion, emergence of HBV variants with genotypic resistance to ETV, and incidence of newly developed HCC.

Assay methodology. Commercially available chemiluminescence immunoassays (CLIA) (Abbott Laboratories, North Chicago, IL, USA) were used for the detection of serum HBsAg, anti-HBs, anti-HBc, HBeAg, and

anti-HBe. The serum HBV DNA concentration was determined by a commercially available quantitative assay (Lightcycler 2.0; Roche Diagnostics, Branchburg, NJ, USA). The lower limit of sensitivity of the assay is 116 copies/ml. The presence of mutations encoding amino acid alterations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif was investigated in patients with VB. Genotyping and analyzing of resistance to ETV were performed with restriction fragment mass polymorphism (RFMP) analysis (GeneMatix, Inc., Seoul, Republic of Korea).

Statistical analysis. All data are expressed as means (range) where appropriate. HBV DNA levels were logarithmically transformed for analysis. Follow-up times were calculated from the date of ETV treatment initiation to the date of the event or the last date of follow-up. Data from patients who were still taking medication without VR, HBeAg seroconversion, and development of HCC were censored at the last available observation.

Student's *t* test was used to compare mean age, pretreatment ALT levels, and HBV DNA levels among subject groups. A chi-square test or Fisher's exact test was used to compare the sex ratio and the presence of liver cirrhosis and HCC. Multivariate analyses were performed using logistic regression to identify factors associated with PVR to ETV monotherapy.

The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk confidence. Independent risk factors predicting achievement of HBeAg seroconversion were analyzed using Cox proportional hazards regression models. The cumulative rates of VR, HBeAg seroconversion, and newly developed HCC were estimated using the Kaplan-Meier method, and *P* values were calculated using the log-rank test. All statistical analyses were performed using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) version 12.0. A *P* value of less than 0.05 was considered significant.

RESULTS

Patient characteristics. One hundred forty-two patients were followed for at least 2 years after ETV treatment. The characteristics at baseline are shown in Table 1; 107 (75.4%) patients were male, the mean age was 50 (range, 24 to 75) years, and 86 (60.6%) patients were HBeAg positive. Forty-nine (34.5%) patients had liver cirrhosis, and 9 (6.3%) patients had HCC. The mean baseline serum ALT level was 218 (range, 43 to 742) IU/liter, and the mean baseline serum HBV DNA level was 7.7 (range, 3.6 to 10.6) log₁₀ copies/ml. The HBeAg-positive patients were younger and in-

TABLE 1 Clinical characteristics of 142 patients with chronic hepatitis B

Variable	Value ^a		P value
	HBeAg positive (n = 86)	HBeAg negative (n = 56)	
Male/female [no. (%)]	71/15 (82.6/17.4)	36/20 (64.3/35.7)	0.014
Mean age (yr)	47 (24–75)	54 (32–75)	0.001
Baseline serum ALT (IU/liter) ^b	240 (43–742)	183 (43–738)	0.057
Baseline HBV DNA (log ₁₀ copies/ml) ^c	8.1 (4.6–10.6)	7.1 (3.6–9.6)	<0.001
Liver cirrhosis [no. (%)]	23 (26.7)	26 (46.4)	0.016
Hepatocellular carcinoma [no. (%)]	2 (2.3)	7 (12.5)	0.029
Mean treatment duration (mo)	67 (26–90)	62 (26–90)	0.106
Mean follow-up duration (mo)	69 (36–90)	66 (36–90)	0.224

^a Values are given as mean (range) unless otherwise indicated.
^b The upper limit of normal ALT levels is less than 40 IU/liter.
^c The lower limit of detection for the HBV DNA test is 116 copies/ml.

cluded more males than the HBeAg-negative patients. The initial ALT and HBV DNA levels were higher in the HBeAg-positive patients than in the HBeAg-negative patients. The incidences of liver cirrhosis and HCC were higher in the HBeAg-negative patients than in the HBeAg-positive patients. The mean duration of ETV treatment was 65 (range, 26 to 90) months, and the mean total follow-up period was 68 (range, 36 to 90) months (Table 1). We followed 142, 142, 141, 129, and 30 patients for 1, 2, 3, 4, and 7 years, respectively (Fig. 1).

Long-term clinical outcomes. The cumulative rates of achieving VR increased from 54.9% at year 1 to 98.2% at year 7 in 142 treatment-naïve patients with CHB. For HBeAg-positive patients (n = 86), the cumulative rates of achieving VR at years 1, 2, 3, and 7 were 43.0, 60.5, 73.8, and 97.0%, respectively. For HBeAg-negative patients (n = 56), the cumulative rates of achieving VR at years 1, 2, 3, and 7 were 73.2, 96.4, 98.2, and 100%, respectively (Fig. 2). However, HBsAg loss did not occur.

During ETV treatment, HBeAg seroconversion developed in 28 HBeAg-positive patients, and sustained VR over 1 year continued in all HBeAg-negative patients. Most patients were continuously treated with ETV in spite of sustained VR. However, ETV therapy was discontinued in 8 patients who were HBeAg positive and 3 patients who were HBeAg negative after optimal additional treatment (more than 12 months) (1, 2, 13). All patients experienced viral relapse after cessation of ETV. Most relapse occurred within 1 year after discontinuation of ETV (mean, 6 months; range, 3 to 12 months). Among them, 8 patients showed low viremia (<4 log₁₀ copies/ml) and did not undergo any rescue therapy. However, 3 patients experienced high viremia with biochemical breakthrough (HBV DNA, 6.2, 6.0, and 7.5 log₁₀ copies/ml; ALT, 92, 120, and 156 IU/liter). TDF was used by two patients as a rescue therapy, and ETV was used by one. All three of these patients showed complete viral and biochemical suppression within 1 year (TDF, 6 and 9 months; ETV, 2 months).

Partial virological response. PVR occurred in 64 (45.1%) of 142 treatment-naïve patients and 49 (76.6%) patients were HBeAg positive. Among the 64 PVR patients, 47 (73.4%) achieved VR within 4 years after prolonged ETV therapy without treatment adaptation. Seven PVR patients achieved VR within 5 (n = 3), 6

FIG 2 Cumulative rates of virological response for the 142 treatment-naïve patients according to HBeAg status at baseline. *, P values were determined using log-rank testing; †, data from patients who were still taking medication without VR were censored at the last available observation for the cumulative rate of VR.

TABLE 2 Univariate and multivariate analyses for factors associated with partial virological response to ETV monotherapy

Variable	Value ^a		Univariate <i>P</i> value	Multivariate	
	VR ^b (<i>n</i> = 78)	PVR ^c (<i>n</i> = 64)		OR (95% CI)	<i>P</i> value ^e
Male/female [no. (%)]	58/20 (74.4/25.6)	49/15 (76.6/23.4)	0.762		
Mean age (yr)	52 (30–75)	50 (24–75)	0.059	0.999 (0.996–1.003)	0.068
Baseline serum ALT (IU/liter) ^d	250 (43–742)	178 (43–740)	0.017	0.997 (0.995–0.999)	0.004
Baseline HBV DNA (log ₁₀ copies/ml) ^e	7.5 (3.6–10.6)	7.9 (4.8–9.8)	0.085	1.112 (0.837–1.476)	0.464
HBeAg positive [no. (%)]	37 (47.4)	49 (76.6)	<0.001	4.146 (1.846–9.315)	0.001
Liver cirrhosis [no. (%)]	25 (32.1)	24 (37.5)	0.497		
Hepatocellular carcinoma [no. (%)]	7 (9.0)	2 (3.1)	0.185		
Mean treatment duration (mo)	64 (26–90)	66 (29–88)	0.420		

^a Values are given as mean (range) unless otherwise indicated.

^b VR is defined as undetectable HBV DNA at 1 year of therapy in compliant patients.

^c PVR is defined as a decrease in HBV DNA of more than 1 log₁₀ copies/ml (10-fold) but detectable HBV DNA at 1 year of therapy in compliant patients.

^d *P* value from logistic regression models.

^e The upper limit of normal ALT levels is less than 40 IU/liter.

^f The lower limit of detection for the HBV DNA test is 116 copies/ml.

(*n* = 3), and 7 (*n* = 1) years after continuous ETV therapy. However, 10 HBeAg-positive patients did not achieve VR at the end of the follow-up period. Among them, fluctuating viremia (126 to 5,701 copies/ml) was observed in 7 patients with PVR (range, 29 to 67 months). Unfortunately, 3 patients experienced VB with genotypic resistance to ETV (2.1%). HBeAg positivity (OR, 4.146; 95% CI, 1.846 to 9.315; *P* = 0.001) and initial ALT (OR, 0.997; 95% CI, 0.995 to 0.999; *P* = 0.004) were independent risk factors for developing PVR (Table 2).

Patients with PVR were stratified according to their viral load at year 1 (Fig. 3). The cumulative rate of achieving VR was significantly higher in patients with HBV DNA at <5,000 copies/ml at

year 1 than in patients with HBV DNA at ≥5,000 copies/ml at year 1 (*P* = 0.019).

HBeAg seroconversion according to virological response. HBeAg positivity was detected in 86 patients (60.6%) at baseline. The cumulative rates of HBeAg loss increased from 20.9% at year 1 to 49.0% at year 7. The HBeAg seroconversion rates at years 1, 2, 3, and 7 were 20.9, 23.3, 26.7, and 37.5, respectively. In patients with VR at year 1 after ETV therapy, the cumulative rates of HBeAg seroconversion increased from 35.1% at year 1 to 45.9% at year 7. In patients with PVR, the cumulative rates of HBeAg seroconversion increased from 10.2% at year 1 to 36.2% at year 7. The cumulative rate of HBeAg seroconversion was significantly higher

FIG 3 Cumulative rates of virological response in patients with partial virological response according to HBV DNA levels at year 1. *, *P* values were determined using log-rank testing; †, PVR is defined as a decrease in HBV DNA of more than 1 log₁₀ copies/ml (10-fold) but detectable HBV DNA at 1 year of therapy in compliant patients; ‡, data from patients who were still taking medication without VR were censored at the last available observation for the cumulative rate of VR.

FIG 4 Cumulative rates of HBeAg seroconversion in patients according to virological response. *, *P* values were determined using log-rank testing; †, VR is defined as undetectable HBV DNA at 1 year of therapy in compliant patients; ‡, PVR is defined as a decrease in HBV DNA of more than 1 log₁₀ copies/ml (10-fold) but detectable HBV DNA at 1 year of therapy in compliant patients; §, data from patients who were still taking medication without HBeAg seroconversion were censored at the last available observation for the cumulative rate of HBeAg seroconversion.

in patients with VR than in patients with PVR (*P* = 0.018) (Fig. 4). In other words, prolonged ETV therapy is more effective for HBeAg seroconversion in patients with VR than in patients with PVR.

Among the 37 HBeAg-positive patients with VR, 17 patients achieved HBeAg seroconversion, but of the 49 HBeAg-positive patients with PVR, only 11 achieved HBeAg seroconversion. Interestingly, none of the PVR patients with HBV DNA at ≥5,000 copies/ml at year 1 ever experienced HBeAg seroconversion. Multivariate analysis identified VR at year 1 as the only significant determinant of HBeAg seroconversion (hazard ratio [HR], 3.009; CI, 1.304 to 6.945; *P* = 0.010) (Table 3).

Resistance to entecavir treatment. Three patients (2.1%) showed VB and HBV variants with genotypic resistance to ETV during the treatment period (24, 60, and 86 months). The pa-

tients' baseline demographics and on-treatment responses are shown in Table 4. Patient 1 had been reported previously (14). Three patients were switched to adefovir (ADV) add-on therapy or TDF-based therapy (ETV plus TDF or TDF monotherapy). One patient showed persistently detectable HBV DNA (≥526 copies/ml) without VR. Although 2 patients experienced a transient VR during ETV medication (34 and 54 months), VB did finally develop. As a rescue therapy, one patient treated with ETV plus ADV showed a very slow decrease to an undetectable HBV DNA level. Two patients rapidly decreased to undetectable HBV DNA levels within 3 months after TDF-based therapy. Of these, one patient achieved HBeAg seroconversion at 6 months after TDF monotherapy.

Newly developed hepatocellular carcinoma according to virological response. New instances of HCC developed in 7 patients

TABLE 3 Univariate and multivariate analyses for factors associated with HBeAg seroconversion to ETV monotherapy

Variable	Value ^a for patients:		Univariate <i>P</i> value	Multivariate	
	With HBeAg seroconversion (<i>n</i> = 28)	Without HBeAg seroconversion (<i>n</i> = 58)		HR (95% CI)	<i>P</i> value ^b
Male/female [no. (%)]	23/5 (82.1/17.5)	48/10 (82.8/17.2)	0.944		
Mean age (yr)	47 (26–73)	47 (24–75)	0.968	0.989 (0.951–1.028)	0.577
Baseline serum ALT (IU/liter) ^c	235 (43–742)	243 (43–740)	0.870	0.999 (0.997–1.001)	0.211
Baseline HBV DNA (log ₁₀ copies/ml) ^d	8.1 (5.4–9.9)	8.1 (4.6–10.6)	0.919	1.007 (0.747–1.357)	0.966
Liver cirrhosis [no. (%)]	7 (25.0)	16 (27.6)	0.800		
Hepatocellular carcinoma [no. (%)]	2 (7.1)	0 (0.0)	0.103		
Mean treatment duration (mo)	64 (26–90)	68 (29–87)	0.317		
VR ^e at yr 1 [no. (%)]	17 (60.7)	20 (34.5)	0.021	3.009 (1.304–6.945)	0.010

^a Values are given as mean (range) unless otherwise indicated.
^b *P* value from Cox proportional hazards regression models.
^c The upper limit of normal ALT levels is less than 40 IU/liter.
^d The lower limit of detection for the HBV DNA test is 116 copies/ml.
^e VR is defined as undetectable HBV DNA at 1 year of therapy in compliant patients.

TABLE 4 Characteristics of three patients with virologic breakthrough and HBV variants with genotypic resistance to entecavir

Variable	Value for patient:		
	1 ^a	2	3
Age (yr)	50	43	48
Sex	Male	Female	Male
Starting point of entecavir treatment			
HBeAg status	Positive	Positive	Positive
ALT (IU/liter) ^b	82	83	69
HBV DNA (log ₁₀ copies/ml) ^c	9.02	8.95	7.69
Ultrasonographic findings	Liver cirrhosis	Chronic hepatitis	Liver cirrhosis
Viral load at maximum viral suppression (log ₁₀ copies/ml) ^c	2.7	Negative	Negative
Time to VR (mo)		34	54
Duration of VR (mo)		20	27
Time point of resistance			
Treatment duration (mo)	24	60	86
ALT (IU/liter) ^b	21	10	21
HBV DNA (log ₁₀ copies/ml) ^c	6.0	4.29	4.04
HBV variants with genotypic resistance to ETV	T184A, M204V, L180M	S202G, M204V, L180M	S202G, M204V, L180M
Compliance ^d	Good	Good	Good
Response to rescue therapy			
Rescue therapy	ETV + ADV	ETV + TDF	TDF
Duration of follow-up (mo)	60	12	12
Time to undetectable HBV DNA (mo)	53	3	3
ALT (IU/liter) ^b	25	12	24
HBV DNA at last follow-up (log ₁₀ copies/ml) ^c	Negative	Negative	Negative
HBeAg seroconversion (mo)	No	No	Yes (6)

^a Patient 1 was reported previously (14).^b The upper limit of normal ALT levels is less than 40 IU/liter.^c The lower limit of detection for the HBV DNA test is 116 copies/ml.^d Compliance was calculated using the following formula: (number of treatment days – number of missed doses)/number of treatment days. Good compliance was defined as more than 0.9.

(5.3%) during the 7 years of continuous ETV therapy (Table 5). Among them, 6 patients showed PVR and only 1 patient achieved VR at year 1. All the patients were diagnosed using computerized tomography. Three patients were HBeAg positive, and all the patients had been diagnosed with liver cirrhosis. The cumulative incidence of HCC was 1.7% at year 7 in patients with VR at year 1. In patients with PVR, the cumulative incidences of HCC increased from 1.6% at year 1 to 11.5% at year 7. The incidence of HCC was significantly higher in the patients with PVR than in the patients with VR ($P = 0.032$) (Fig. 5). Interestingly, 4 patients showed sustained viremia for 1 year before detection of HCC. Fortunately, all were diagnosed in the early stages of HCC and successfully underwent an operation or radiofrequency ablation.

DISCUSSION

This study provided long-term results with respect to antiviral potency, viral resistance, and disease progression for treatment-naïve CHB patients treated with ETV for up to 7 years. Effective viral suppression was achieved in 132 of 142 patients (93%), and only 3 patients (2.1%) experienced VB with viral resistance. Although a significant proportion of the patients (45.1%) showed a PVR, especially among HBeAg-positive patients (76.6%), prolonged ETV treatment for up to 7 years was also effective. About 84% of the patients with PVR achieved VR through prolonged

therapy without adaptation. These results were consistent with previous ETV therapy studies (15, 16). Our results support previous studies suggesting that continuous ETV therapy is an effective and promising choice (15, 16).

Zoutendijk et al. reported that high baseline HBV DNA and HBeAg positivity were the only independent risk factors for having a PVR (15). In our study, multivariate analysis identified that HBeAg positivity (OR, 4.146; 95% CI, 1.846 to 9.315; $P = 0.001$) and initial ALT (OR, 0.997; 95% CI, 0.995 to 0.999; $P = 0.004$) were independent risk factors for having a PVR. The baseline HBV DNA level was not a risk factor in this study. One reason for this was that HBeAg-negative patients had higher HBV DNA levels at baseline than patients in the previous reports (7.1 versus 6.2 and 6.7 log₁₀ copies/ml) (15, 16). Even if the impact of the ALT factor is much less than we expected, this finding means that the strength of the host immune system is as important as the capacity of viral replication (16).

Patients with a PVR were stratified according to their viral load at year 1. The cumulative rate of achieving VR was significantly higher in patients with HBV DNA at <5,000 copies/ml at year 1 than in patients with HBV DNA at ≥5,000 copies/ml at year 1 without treatment adaptation during prolonged ETV therapy ($P = 0.019$). In accordance with a previously published article,

TABLE 5 Characteristics of seven patients with newly developed hepatocellular carcinoma during entecavir therapy

Variable	Value for patient:						
	1	2	3	4	5	6	7
Age (yr)	66	59	75	65	56	42	60
Sex	Male	Female	Male	Female	Female	Male	Male
Starting point of entecavir treatment							
HBeAg status	Negative	Positive	Positive	Negative	Positive	Negative	Negative
ALT (IU/liter) ^a	54	43	65	175	43	152	94
HBV DNA (log ₁₀ copies/ml) ^b	7.33	7.48	6.20	5.45	8.31	4.84	5.99
Ultrasonographic findings	Cirrhosis	Cirrhosis	Cirrhosis	Cirrhosis	Cirrhosis	Cirrhosis	Cirrhosis
Achievement of VR ^c	Yes	No	No	No	No	No	No
Viral load at maximum viral suppression (log ₁₀ copies/ml) ^b	Negative	2.70	Negative	Negative	2.21	Negative	Negative
Time to undetectable HBV DNA (mo)	6		52	24		21	27
Viral load for 1 yr before detection of HCC (range) (log ₁₀ copies/ml)	Negative	2.70–5.21	Negative	2.45–3.85	2.21–2.79	2.65–3.44	Negative
Time at diagnosis of HCC							
Treatment duration of ETV (mo)	58	33	72	15	53	15	12
ALT (IU/liter) ^a	18	17	21	36	38	37	34
HBV DNA (log ₁₀ copies/ml) ^b	Negative	5.21	Negative	2.45	2.21	2.65	3.27
Status of HBeAg	Negative	Positive	Positive	Negative	Positive	Negative	Negative
HBeAg seroconversion		No	No		No		
Compliance	Good	Good	Good	Good	Good	Good	Good
Status of HCC ^d							
Location (segment)	7	2	3	5	6	5/6	2
No.	Single	Single	Single	Single	Single	Single	Single
Size of mass (cm)	1.4	2.5	2.0	1.6	2.2	3.0	2.2

^a The upper limit of normal ALT levels is less than 40 IU/liter.^b The lower limit of detection for the HBV DNA test is 116 copies/ml.^c VR is defined as undetectable HBV DNA at 1 year of therapy in compliant patients.^d The status of HCC was defined using computerized tomography.

prolonged therapy beyond year 1 was highly effective and induced VR in most patients with HBV DNA at <5,000 copies/ml (15).

In terms of HBeAg seroconversion as a more realistic endpoint, prolonged ETV therapy was effective in only about 36% of the patients with PVR by 7 years. In particular, there was no HBeAg seroconversion in HBeAg-positive patients with HBV DNA at ≥5,000 copies/ml at year 1. On the basis of our results, early adaptation of the therapeutic regimen is recommended for an HBeAg-positive patient with HBV DNA at ≥5,000 copies/ml at year 1 to achieve HBeAg seroconversion.

In addition, VB with genotypic resistance was observed in three HBeAg-positive patients with PVR. Two patients with VB experienced a temporary VR during ETV medication (34 and 54 months). Although viral suppression was sustained for 20 and 27 months, respectively, transient viral suppression was not a satisfactory endpoint (Table 4).

Likewise, 8 patients who had optimal consolidation therapy after HBeAg seroconversion also experienced viral relapse within 1 year after cessation of ETV. Therefore, while the ideal endpoint is HBsAg loss, as expected, it is rarely achievable with the currently available antiviral agents. There was also no HBsAg loss in our case.

For patients with PVR to NAs related to a high risk of resistance, like lamivudine, telbivudine, and adefovir, early adaptation is recommended to prevent sequential resistance, to maintain vi-

ral suppression, and to improve the cost-effectiveness of HBV therapy (17–20). For patients with PVR to NAs related to a low risk of resistance, like ETV and TDF, the effectiveness of early adaptation has not been established. During ETV or TDF therapy, an evidence-based roadmap concept for patients with PVR has not been generated. Even though the number of patients with VB was small in this study, we determined that modification to TDF induced rapid viral suppression within 3 months and HBeAg seroconversion at 6 months.

Overall, among the 64 PVR patients, 47 patients (73.4%) gradually achieved VR within 4 years, and all the HBeAg-negative patients achieved VR within 4 years. Therefore, it is desirable to reappraise the current criteria (detectable HBV DNA at year 1) for PVR in consideration of ETV- and TDF-induced viral kinetics (21).

As was previously reported, adjustment of ETV therapy is not necessary in most patients with PVR (15, 16, 22). Nevertheless, rapid viral suppression, maintenance, and HBeAg seroconversion are known to be determinants of long-term outcomes, including histological response and disease progression, because complete eradication of HBV infection is not a feasible goal (23, 24). Failure to suppress viral replication can lead to selection of antiviral resistance mutations. Liu et al. reported that quasispecies complexity increased in PVR during ETV therapy. Higher complexity means a broader and more

FIG 5 Cumulative incidences of HCC according to virological response. *, *P* values were determined using log-rank testing; †, VR is defined as undetectable HBV DNA at 1 year of therapy in compliant patients; ‡, PVR is defined as a decrease in HBV DNA of more than 1 log₁₀ copies/ml (10-fold) but detectable HBV DNA at 1 year of therapy in compliant patients; §, data from patients who were still taking medication without the development of HCC were censored at the last available observation for the cumulative incidence of HCC.

complex mutant spectrum. Finally, sustained viremia induces resistant variants to restore replication fitness (25). Therefore, TDF-based modification is suggested for HBeAg-positive patients with HBV DNA at $\geq 5,000$ copies/ml at year 1 or sustained viremia at more than 4 years.

Progress in antiviral agents with high potency and low resistance will prolong the life span in patients with CHB. Given the need for a long duration of antiviral therapy, new issues, including disease progression, delayed resistance, and side effects, may emerge as a major interest. Cho et al. reported that the cumulative incidence of HCC in the NA-incomplete-responder group was higher than in the NA-complete-responder group (26). Likewise, we found that patients with PVR had a significantly higher incidence of HCC development than patients with VR ($P = 0.032$). Fortunately, they were all diagnosed in the early stages of HCC and were successfully treated. Taken together, these results suggest that more careful HCC surveillance is required for patients with PVR.

Additionally, some adverse events have been reported during TDF or ETV therapy, including mild renal impairment and osteomalacia or lactic acidosis (27–29). Further studies should attempt to individualize antiviral therapy, anticipating that the HBeAg/HBsAg seroconversion rates can be maximized and viral resistance and adverse events can be minimized using the roadmap concept.

Our study has some limitations. First, the study was based on a retrospective, nonrandomized design and was done only in Korean patients, among whom genotype C is predominant. Second,

patients with VB and sustained viremia were the only ones who underwent analysis for genotypic resistance. It is possible that the prevalence of genotypic ETV resistance was underestimated. Third, the sample size of the study was still limited to evaluate the incidence of cirrhosis and HCC, despite the relatively long-term follow-up period. Lastly, data regarding alcohol consumption and body mass indexes of the patients would have been very informative and would have pointed to additional determinants of PVR versus VR and progression to HCC.

In conclusion, although there were patients with PVR, prolonged ETV therapy showed excellent VR, with only 2.1% emergence of viral resistance during a 7-year follow-up. However, to achieve HBeAg seroconversion, drug modification is needed for HBeAg-positive patients with PVR (especially those with HBV DNA at $\geq 5,000$ copies/ml at year 1). More careful HCC surveillance is needed for patients with PVR. Our data may help physicians to prepare drug modifications early on and prevent patients from developing disease progression.

ACKNOWLEDGMENTS

We have no conflicts of interest to disclose.

This research was supported by The GlaxoSmithKline Research Fund of the Korean Association for the Study of the Liver.

Hyun Woong Lee analyzed the clinical data and wrote the paper; Jae-Cheol Kwon, In Soo Oh, and Hye Young Chang provided clinical data and performed research. Young Joo Cha provided clinical samples. Hyung Joon Kim edited and reviewed the manuscript.

REFERENCES

- Lok AS, McMahon BJ. 2009. Chronic hepatitis B: update 2009. *Hepatology* 50:661–662. <http://dx.doi.org/10.1002/hep.23190>.
- 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. <http://dx.doi.org/10.1016/j.jhep.2012.02.010>.
- Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S, Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study of the Liver. 2008. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2:263–283. <http://dx.doi.org/10.1007/s12072-008-9080-3>.
- Korean Association for the Study of the Liver. 2012. KASL clinical practice guidelines: management of chronic hepatitis B. *Clin Mol Hepatol* 18:109–162. <http://dx.doi.org/10.3350/cmh.2012.18.2.109>.
- van Zonneveld M, Flink HJ, Verhey E, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Hansen BE, Schalm SW, Janssen HL, HBV 99-01 Study Group. 2005. The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B: predictive factors for dose reduction and treatment discontinuation. *Aliment Pharmacol Ther* 21:1163–1171. <http://dx.doi.org/10.1111/j.1365-2036.2005.02453.x>.
- Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. 2009. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 49:1503–1514. <http://dx.doi.org/10.1002/hep.22841>.
- Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weilert F, Kurdas OO, Shiffman ML, Trinh H, Gurel S, Snow-Lampart A, Borroto-Esoda K, Mondou E, Anderson J, Sorbel J, Rousseau F. 2011. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. *Gastroenterology* 140:132–143. <http://dx.doi.org/10.1053/j.gastro.2010.10.011>.
- van Bommel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, Erhardt A, Huppe D, Stein K, Trojan J, Sarrazin C, Bocher WO, Spengler U, Wasmuth HE, Reinders JG, Moller B, Rhode P, Feucht HH, Wiedenmann B, Berg T. 2010. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. *Hepatology* 51:73–80. <http://dx.doi.org/10.1002/hep.23246>.
- Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D, BEHOLD AI463022 Study Group. 2006. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 354:1001–1010. <http://dx.doi.org/10.1056/NEJMoa051285>.
- Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L, BEHOLD AI463022 Study Group. 2006. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 354:1011–1020. <http://dx.doi.org/10.1056/NEJMoa051287>.
- Lee HW, Chang HY, Yang SY, Kim HJ. 2014. Viral evolutionary changes during tenofovir treatment in a chronic hepatitis B patient with sequential nucleos(t)ide therapy. *J Clin Virol* 60:313–316. <http://dx.doi.org/10.1016/j.jcv.2014.03.018>.
- Berg T, Marcellin P, Zoulim F, Moller B, Trinh H, Chan S, Suarez E, Lavocat F, Snow-Lampart A, Frederick D, Sorbel J, Borroto-Esoda K, Oldach D, Rousseau F. 2010. Tenofovir is effective alone or with emtricitabine in adefovir-treated patients with chronic-hepatitis B virus infection. *Gastroenterology* 139:1207–1217. <http://dx.doi.org/10.1053/j.gastro.2010.06.053>.
- Lee HW, Lee HJ, Hwang JS, Sohn JH, Jang JY, Han KJ, Park JY, Kim DO, Ahn YSH, Paik YH, Lee CK, Lee KS, Chon CY, Han KH. 2010. Lamivudine maintenance beyond one year after HBeAg seroconversion is a major factor for sustained virologic response in HBeAg-positive chronic hepatitis B. *Hepatology* 51:415–421. <http://dx.doi.org/10.1002/hep.23323>.
- Lee HW, Kim HJ, Hong SP, Cha BK, Chang HY, Choi CH, Do JH, Kim JG, Chang SK. 2012. Simultaneous emergence of entecavir resistance mutations in a nucleoside-naïve chronic hepatitis B patient. *Intervirology* 55:380–384. <http://dx.doi.org/10.1159/000336561>.
- Zoutendijk R, Reijnders JG, Brown A, Zoulim F, Mutimer D, Deterding K, Petersen J, Hofmann WP, Buti M, Santantonio T, van Bommel F, Pradat P, Oo Y, Luetgehetmann M, Berg T, Hansen BE, Wedemeyer H, Janssen HL, VIRGIL Surveillance Study Group. 2011. Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naïve patients with a partial virological response. *Hepatology* 54:443–451. <http://dx.doi.org/10.1002/hep.24406>.
- Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Suzuki Y, Saitou S, Arase Y, Ikeda K, Kobayashi M, Watahiki S, Mineta R, Kumada H. 2012. Long-term continuous entecavir therapy in nucleos(t)ide-naïve chronic hepatitis B patients. *J Hepatol* 57:508–514. <http://dx.doi.org/10.1016/j.jhep.2012.04.037>.
- Lampertico P, Vigano M, Manenti E, Iavarone M, Lunghi G, Colombo M. 2005. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. *Hepatology* 42:1414–1419. <http://dx.doi.org/10.1002/hep.20939>.
- Reijnders JG, Deterding K, Petersen J, Zoulim F, Santantonio T, Buti M, van Bommel F, Hansen BE, Wedemeyer H, Janssen HL, VIRGIL Surveillance Study Group. 2010. Antiviral effect of entecavir in chronic hepatitis B: influence of prior exposure to nucleos(t)ide analogues. *J Hepatol* 52:493–500. <http://dx.doi.org/10.1016/j.jhep.2010.01.012>.
- Zoulim F, Locarnini S. 2009. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 137:1593–1608; e1591–e1592. <http://dx.doi.org/10.1053/j.gastro.2009.08.063>.
- Lui YY, Tsoi KK, Wong VW, Kao JH, Hou JL, Teo EK, Mohamed R, Piratvisuth T, Han KH, Mihm U, Wong GL, Chan HL. 2010. Cost-effectiveness analysis of roadmap models in chronic hepatitis B using tenofovir as the rescue therapy. *Antivir Ther* 15:145–155. <http://dx.doi.org/10.3851/IMP1496>.
- Lampertico P. 2009. Partial virological response to nucleos(t)ide analogues in naïve patients with chronic hepatitis B: from guidelines to field practice. *J Hepatol* 50:644–647. <http://dx.doi.org/10.1016/j.jhep.2009.01.007>.
- Yang YJ, Shim JH, Kim KM, Lim YS, Lee HC. 2014. Assessment of current criteria for primary nonresponse in chronic hepatitis B patients receiving entecavir therapy. *Hepatology* 59:1303–1310. <http://dx.doi.org/10.1002/hep.26910>.
- Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2013. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 58:98–107. <http://dx.doi.org/10.1002/hep.26180>.
- Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Iloeje U, Beebe S, Kreter B. 2010. 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. <http://dx.doi.org/10.1002/hep.23785>.
- Liu F, Chen L, Yu DM, Deng L, Chen R, Jiang Y, Chen L, Huang SY, Yu JL, Gong QM, Zhang XX. 2011. Evolutionary patterns of hepatitis B virus quasispecies under different selective pressures: correlation with antiviral efficacy. *Gut* 60:1269–1277. <http://dx.doi.org/10.1136/gut.2010.226225>.
- Cho JY, Paik YH, Sohn W, Cho HC, Gwak GY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. 2014. Patients with chronic hepatitis B treated with oral antiviral therapy retain a higher risk for HCC compared with patients with inactive stage disease. *Gut* 63:1943–1950. <http://dx.doi.org/10.1136/gutjnl-2013-306409>.
- Lange CM, Bojunga J, Hofmann WP, Wunder K, Mihm U, Zeuzem S, Sarrazin C. 2009. Severe lactic acidosis during treatment of chronic hepatitis B with entecavir in patients with impaired liver function. *Hepatology* 50:2001–2006. <http://dx.doi.org/10.1002/hep.23346>.
- Grace DM, Snelling P, McKenzie P, Strasser SI. 2013. Tenofovir-associated Fanconi syndrome in patients with chronic hepatitis B monoinfection. *Antivir Ther* 18:945–948. <http://dx.doi.org/10.3851/IMP2649>.
- Fung J, Seto WK, Lai CL, Yuen MF. 2014. Extrahepatic effects of nucleoside and nucleotide analogues in chronic hepatitis B treatment. *J Gastroenterol Hepatol* 29:428–434. <http://dx.doi.org/10.1111/jgh.12499>.