A Low Viral Load Predicts a Higher Initial Virologic Response to Adefovir in Patients with Lamivudine-Resistant Chronic Hepatitis B

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Background/Aims: Adefovir (ADV) is the preferred drug for treating lamivudine (LAM)-resistant hepatitis B. However, not all patients who face virologic breakthrough during LAM treatment respond to ADV. The aim of this study was to determine the factors associated with efficacy of ADV in LAM-resistant hepatitis B patients. **Methods:** The medical records of 231 patients who received ADV due to LAM-resistance were reviewed. Efficacy was assessed by the initial virologic response (IVR), defined as hepatitis B virus (HBV) DNA not being undetectable by real-time PCR at 6 months of ADV treatment. **Results:** Seventy patients (30%) achieved IVR. While 'add-on' modality, hepatitis B e antigen (HBeAg) negativity, and low baseline HBV DNA levels were associated with IVR in univariate analysis, multivariate analysis revealed HBeAg status and the DNA level to be the significant factors. The probability of IVR achievement increased sharply per each log₁₀ copies/mL decrement in the baseline viral load, which was 133 times in patients who had HBV DNA <10⁵ copies/mL compared with those who had ≥10⁸ copies/mL. **Conclusions:** Factors associated with the IVR were HBeAg negativity and a low baseline viral load. Therefore, when virologic breakthrough with genotypic resistance emerges during LAM therapy, ADV treatment should be considered immediately before further increases in viral load. Additional long-term follow-up data are warranted. (*Gut Liver* 2010;4:530-536)

Key Words: Chronic hepatitis B; Lamivudine; Antiviral drug resistance; Adefovir; Viral load

INTRODUCTION

Despite the high rate of emergence of resistance, as much as 70% after 5 years,¹ lamivudine (LAM) is still widely used for several reasons. LAM has the longest surveillance for safety, a proven efficacy to reduce disease progression, the lowest price, and is sometimes mandatorily prescribed by insurance.² Consequently, the countermeasure against LAM-resistance is a major issue in treatment for chronic hepatitis B (CH-B) these days.

Adefovir dipivoxil (ADV) is a prodrug of adefovir, an acyclic phosphonate nucleotide analogue of adenosine monophosphate. Although ADV demonstrated good efficacy as a first line monotherapy for both of HBeAg-positive and -negative CH-B patients,^{3,4} the major indication of ADV is to treat LAM-resistant CH-B. Recent studies demonstrated that ADV add-on therapy was mostly effective in suppressing viral replication in LAM-resistant hepatitis B patients, and the latest guidelines uniformly advise adding ADV to an obligation or alternative plan.^{1,5,6}

However, not all LAM-resistant hepatitis B patients respond well to ADV treatment, some of them show partial or non-response and sometimes represent resistant mutations such as rtN236T and rtA181V/T.⁷⁻¹⁰ Moreover, it is harder to manage CH-B showing combined resistance against LAM and ADV.

The aim of this study was to identify factor(s) related to the efficacy of ADV treatment for LAM-resistant CH-B patients. The efficacy was preliminarily assessed by virologic suppression at 6 months (initial virologic response)

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as a surrogate marker.

MATERIALS AND METHODS

1. Patients

From January 2006 to February 2008, LAM-resistant CH-B patients with compensated liver disease who received ADV 10 mg a day at Samsung Medical Center were reviewed. Before ADV treatment, all patients had documented LAM-resistance with or without biochemical breakthrough. Whether patients are treated by switching to or adding on ADV was determined on full reviewing of cost and benefit.

A diagnosis of CH-B was made either histologically or clinically. The clinical criteria for CH-B included documented hepatitis B surface antigen (HBsAg) over a 6-month period and HBV DNA $>10^5$ copies/mL with or without elevated serum ALT levels before the initial antiviral therapy. Patients who received ADV combined with LAM temporarily were excluded because incoherent duration of 'overlap' might confound the analysis of data. Patients with any clinical evidence of portal hypertension, such as esophagogastric varices, ascites, hepatic encephalopathy, and imaging features that were suggestive of cirrhosis on sonographic examination or computed tomography were excluded. Patients who had malignancies including hepatocellular carcinoma, history of habitual alcohol ingestion (≥ 30 g per day), co-infection with chronic hepatitis C or human immunodeficiency virus, severe obesity (body mass index ≥ 28 kg/m²), autoimmune disease

such as autoimmune hepatitis, or hepatic infiltrative disease were excluded.

2. Laboratory evaluation

Serum alanine aminotransferase (ALT) was measured with standard automated techniques. Serum HBsAg, antibodies to HBsAg, hepatitis B e antigens (HBeAg), and antibodies to hepatitis B e antigen (anti HBe) were detected by microparticle enzyme immunoassay (AXSYM; Abbott Laboratories, Abbott Park, IL, USA). Serum HBV DNA was assessed by real-time polymerase chain reaction (PCR) assay, the COBAS TaqMan HBV quantitative test (Roche Molecular Systems Inc, Branchburg, NJ, USA), with a lower limit of quantification of 60 copies/mL (12 IU/mL). LAM-associated mutations including rtL180M and rtM204V/I/S were determined using a line probe assay (INNO-LiPA HBV DR v2; Innogenetics NV, Ghent, Belgium).

3. Definition

The LAM-resistance was defined as virologic breakthrough confirmed genotypic resistance. A virologic breakthrough was an HBV DNA increase of more than 1 log₁₀ from nadir during antiviral therapy. The initial virologic response (IVR) was defined as undetectable HBV DNA by real-time PCR at 6 months of ADV treatment. A partial response was defined as a decrease in serum HBV DNA of more than 2 log₁₀ copies/mL but still at a detectable level within 6 months. A non-response was defined as a decrease in serum HBV DNA of less than 2 log₁₀ cop-

Table 1. Characteristics of the 231 Patients

Variables	IVR (n=70)	Non-IVR (n=161)
Age, yr	47.5±11.2 (21-77)	45.8±11.1 (15-79)
Sex, M:F	50:20 (71:28)	113:48 (29:70)
Duration of prior LAM treatment, mo	49±27 (7-142)	37±22 (5-115)
Initial ALT, U/L	136±206 (8-1,371)	149±157 (24-1,458)
Treatment modality, Add-on:Switch	50:20 (71:28)	76:85 (47:52)
HBeAg*, positive:negative	27:35 (38:50)	132:24 (82:14)
Mutation profile		
rtM204I±rtL180M	39 (55.7)	89 (55.3)
rtM204V±rtL180M	29 (41.4)	63 (39.1)
rtM204I+rtM204V+rtL180M	2 (2.9)	9 (5.6)
HBV DNA level, copies/mL		
<10 ⁵	25 (35.7)	2 (1.2)
10 ⁵ -10 ⁶	13 (18.6)	9 (5.6)
10 ⁶ -10 ⁷	14 (20.0)	26 (16.1)
10 ⁷ -10 ⁸	14 (20.0)	51 (31.7)
≥10 ⁸	4 (5.7)	73 (45.3)

Continuous variables are expressed in mean±standard deviation (range) and categorized variables in number (percentage). IVR, initial virologic response; LAM, lamivudine; ALT, alanine aminotransferase; HBV, hepatitis B virus.

*Available in 218 patients.

ies/mL after at least 6 months of therapy.

4. Statistical analysis

All data are expressed as mean±standard deviation with ranges in continuous variables and numbers with percentage in categorized variables, unless otherwise indicated. Comparison between two groups was tested by Mann-Whitney-U test and Pearson's chi-square test. Binary logistic regression analysis was used to estimate adjusted odds ratios for achievement of IVR. Those analyses were performed using SAS software (version 8.2; SAS Institute Inc., Cary, NC, USA). p-values less than 0.05 were considered to be statistically significant.

RESULTS

Data from 231 patients were analyzed. Among them, 70 patients (30.3%) achieved IVR. Baseline characteristics of the patients are presented in Table 1. One hundred five patients were treated by switching and 126 patients by adding on. Differences between modalities of 'switch' and 'add-on' were demonstrated in Table 2.

1. Factors related with IVR

Using univariate analysis, age, sex, and mutation profile were not related to IVR achievement. The treatment modality of 'add-on,' HBeAg negativity, and low HBV DNA level were significantly associated with IVR (Table 3).

The IVR rate was inversely proportional to initial DNA level, which was 92.6% (25/27) in $<10^5$ copies/mL, 59.1% (13/22) in 10^5 - 10^6 copies/mL, 35.0% (14/40) in

10^6 - 10^7 copies/mL, 21.5% (14/65) in 10^7 - 10^8 copies/mL, and 5.2% (4/77) in $\geq 10^8$ copies/mL (Fig. 1).

With multivariate analysis, HBeAg negativity and low DNA level were significantly associated with IVR. The HBeAg-negative patients were more likely to achieve IVR, with an odds ratio of about 6.0. Baseline viral load was closely related to IVR, showing significant p-value. The odds ratio for IVR was 132.8 in patients with less than 10^5 copies/mL, 12.3 in 10^5 - 10^6 copies/mL, 7.2 in 10^6 - 10^7 copies/mL, and 4.7 in 10^7 - 10^8 copies/mL compared to those who had more than 10^8 copies/mL (Table 3).

There were 143 partial responders and 18 non-responders in non-IVR group. Among the 18 non-responders, 17 subjects were HBeAg-positive (Table 4). Non-responders showed relatively higher baseline HBV DNA levels, which were 5.6% (1/18) having $<10^5$ copies/mL, 5.6% (1/18) having 10^5 - 10^6 copies/mL, 27.8% (5/18) having 10^6 - 10^7 copies/mL, 27.8% (5/18) having 10^7 - 10^8 copies/mL, and 33.3% (6/18) having $\geq 10^8$ copies/mL (Fig. 2).

2. Serologic response

Among 159 HBeAg-positive patients, 146 patients were checked for follow-up serologic evaluation. Of them, 28 seroconversions (19.6%) and three lone HBeAg losses (2.1%) were observed at 6 months of ADV treatment. Age, sex, ALT level, treatment modality, profile of mutation, and baseline HBV DNA level did not affect HBeAg seroconversion (Table 5).

Table 2. Comparison of Characteristics according to the Treatment Modalities

Variables	Switch (n=105)	Add-on (n=126)	p-value
Age, yr	45.2±10.6 (15-79)	46.6±11.1 (19-79)	0.303
Sex, M:F	73:32 (69.5:30.5)	90:36 (71.4:28.6)	0.773
Duration of prior LAM treatment, mo	37.1±22 (5-94)	40.1±25 (9-142)	0.260
Initial ALT, U/L	187±216 (28-1,458)	114±119 (8-837)	0.078
HBeAg*, positive:negative	75:28 (71.4:26.7)	84:31 (66.7:24.6)	1.000
Mutation profile			0.081
rtM204I±rtL180M	61 (58.1)	67 (53.2)	
rtM204V±rtL180M	36 (34.3)	56 (44.4)	
rtM204I+rtM204V+rtL180M	8 (7.6)	3 (2.4)	
HBV DNA level, copies/mL			<0.001
$<10^5$	0 (0.0)	27 (21.4)	
10^5 - 10^6	7 (6.7)	15 (11.9)	
10^6 - 10^7	17 (16.2)	23 (18.3)	
10^7 - 10^8	42 (40.0)	23 (35.4)	
$\geq 10^8$	39 (37.1)	38 (30.1)	

Continuous variables are expressed in mean±standard deviation (range) and categorized variables in number (percentage). IVR, initial virologic response; LAM, lamivudine; ALT, alanine aminotransferase; HBV, hepatitis B virus.

*Available in 218 patients.

Table 3. Univariate and Multivariate Analysis of Predictors of Initial Virologic Response

Variable	Univariate analysis					Multivariate analysis				
	B	Exp (B)	p-value	95% CI		B	Exp (B)	p-value	95% CI	
Age, yr	0.014	1.014	0.284	0.989	1.040					
Sex, male	−0.060	0.942	0.849	0.507	1.748					
ALT										
<2×UNL	0.340	1.405	0.238	0.467	4.226					
2-5×UNL	0.773	2.167	0.545	0.805	5.831					
≥5×UNL			Reference					Reference		
Treatment modality, add-on	1.028	2.796	0.001	1.529	5.114	0.347	1.415	0.405	0.624	3.207
HBeAg*, negativity	1.964	7.130	<0.001	3.669	13.853	1.800	6.047	<0.001	2.715	13.472
Mutation profile										
rtM204I±rtL180M	0.679	1.972	0.399	0.407	9.552					
rtM204V±rtL180M	0.728	2.071	0.371	0.421	10.199					
rtM204I+rtM204V+rtL180M			Reference							
HBV DNA level, copies/mL										
<10 ⁵	5.430	228.125	<0.001	39.362	1322.113	4.889	132.831	<0.001	20.869	84.459
10 ⁵ -10 ⁶	3.272	26.361	<0.001	7.061	98.416	2.508	12.277	0.001	2.889	52.176
10 ⁶ -10 ⁷	2.285	9.827	<0.001	2.966	32.560	1.978	7.227	0.002	2.071	25.215
10 ⁷ -10 ⁸	1.611	5.010	0.007	1.559	16.099	1.557	4.747	0.012	1.403	16.063
≥10 ⁸			Reference					Reference		

B, logistic regression coefficient; Exp (B), odds ratio; CI, confidence interval; ALT, alanine aminotransferase; UNL, upper normal limit; HBV, hepatitis B virus.

*Available in 218 patients.

Fig. 1. Initial virologic response (IVR) rate according to baseline hepatitis B virus (HBV) DNA level. The IVR rate is inversely proportion to the baseline viral load when starting adefovir.

DISCUSSION

In this study, we revealed two significant factors related to IVR in ADV treatment for LAM-resistant CH-B patients, which were HBeAg negativity and low baseline viral load.

By multivariate logistic analysis, the odds ratio for IVR

of HBeAg-negative status was 6.0 as compared with HBeAg-positive status. Considering that more than 90% of HBeAg-negative CH-B patients in Korea have core/pre-core mutations,^{11,12} such a favorable response in HBeAg-negative patients seems to be an effect of basal core promoter/precore mutation. Previous clinical studies have shown that stop codon mutations were related to lower baseline viral load, replication rate, and breakthrough on LAM treatment.¹³⁻¹⁵

The adverse impact of high baseline HBV DNA level was already known through studies in LAM-treated populations.¹⁶⁻¹⁸ Likewise, high baseline DNA level (cut-off level of 10⁵-10⁸ copies/mL) caused insufficient viral suppression, and even frequent emergence of resistance in ADV treatment for LAM-resistance CH-B patients.^{8,10,19,20} Those phenomena may have resulted from a high replication rate and subsequent selection of a resistant strain. In this study, a chance to achieve IVR increased sharply by a log₁₀ copies/mL decrement of HBV DNA level. Patients who had HBV DNA <10⁵ copies/mL were even 133 times more likely to achieve IVR than those who had ≥10⁸ copies/mL. To our knowledge, this is the first report to show how much impact baseline HBV DNA levels when starting ADV affect virologic suppression inversely in LAM-resistant CH-B patients.

We used IVR as a surrogate marker for treatment outcome. Despite the small number of patients and varia-

Table 4. Characteristics of Non-responders

Patients	Age/Sex	Baseline ALT, U/L	Treatment modality	HBeAg status	Mutation profile	Baseline HBV DNA, copies/mL	ALT at 6 months, U/L	HBV DNA level at 6 months, copies/mL
1	35/M	30	Add-on	+	rtM204I	2,499,456	30	29,432
2	46/F	93	Switch	+	rtM204V	183,978,300	93	2,203,438
3	49/M	57	Add-on	+	rtM204V	3,824,179	57	48,393
4	61/F	138	Switch	+	rtM204I	2,122,500	138	26,885
5	39/M	81	Switch	+	rtM204I	2,399,274	81	31,979
6	36/M	34	Add-on	+	rtM204I	408,086	34	5,943
7	48/M	232	Switch	+	rtM204I+rtM204V	19,641,049	232	348,373
8	50/M	205	Add-on	+	rtM204V	77,786,795	205	1,598,101
9	49/M	35	Add-on	+	rtM204V	541,849,346	35	12,048,725
10	51/M	35	Add-on	+	rtM204I	57,449	35	1,302
11	52/M	63	Switch	+	rtM204I	2,348,900	63	78,108
12	30/M	108	Switch	+	rtM204I	59,628,100	108	2,489,268
13	54/M	86	Switch	+	rtM204I	23,007,900	86	11,376,600
14	37/M	121	Switch	+	rtM204I	260,982,600	121	464,488,749
15	41/M	137	Switch	+	rtM204I	77,768,400	137	26,812,269
16	43/M	105	Add-on	—	rtM204I	64,973,687	105	> 641,275,000
17	46/M	598	Switch	+	rtM204V	153,669,000	598	2,007,602
18	44/M	100	Switch	+	rtM204I+rtM204V	397,453,690	100	> 641,278,000

ALT, alanine aminotransferase; HBV, hepatitis B virus.

Fig. 2. Baseline viral load according to virologic response at 6 months of adefovir treatment in lamivudine-resistant hepatitis B. Each column of bobbles represents the distribution of the viral load of the initial virologic response (IVR), partial response, and nonresponse groups respectively from left to right. The size of the bobble indicates the percentage of patients corresponding to the hepatitis B virus DNA level in each response group.

ble definitions, recent observational studies showed general agreement in viral suppression at six months correlating with long-term antiviral efficacy in nucleos(t)ide analogue therapy.^{18,21-28} Most of them defined 'virologic response at 6 months' as suppression under 4 log₁₀ copies/mL. However, this definition was set arbitrarily and a lower standard for viral response has been applied as

more sensitive detection methods have evolved.^{24,26,28,29} We defined IVR as suppression under 60 copies/mL by using real-time PCR, which was the lowest limit ever reported, to assess the primary outcome strictly. Interestingly, not a small proportion of patients (30%) achieved IVR. This attempt using lowest cut-off level may be meaningful work in the era of real-time PCR.

Several known predictors for treatment efficacy in CH-B patients were not proven to be significant in this study. First, high ALT level was known as a predictive determinant for treatment efficacy, particularly HBeAg loss.⁶ It was assumed that a high ALT level indicated an active immune response of the host resulting in early clearance of HBeAg. However, the significance of ALT level in viral suppression has been reported to be variable. Our study demonstrated that the viral factors indicating low replicative state were more influential in viral suppression than the host factor represented by ALT level. Second, previous literatures have suggested that ADV add-on was better in preventing occurrence of resistance than monotherapy with or without an overlapping period in LAM-resistant CH-B patients.³⁰ However, multivariate analysis revealed that treatment modality did not affect the IVR achievement, in spite of a significant association in univariate analysis. This result seems to be attributed to uneven distribution of treatment modalities that patients in 'add-on' group were likely to have low viral load (Table 2). In addition, the period of this study might be too short to

Table 5. Univariate Analysis of Predictors of Seroconversion in 146 HBeAg-positive Patients

Variables	Univariate analysis				
	B	Exp (B)	p-value	95% CI	
Age, yr	0.013	1.013	0.490	0.976	1.051
Sex, male	-0.315	0.730	0.511	0.285	1.867
ALT					
<2×UNL	0.154	1.167	0.827	0.293	4.646
2-5×UNL	0.087	1.091	0.893	0.307	3.877
≥5×UNL			Reference		
Treatment modality, add-on	0.061	1.063	0.885	0.465	2.429
Mutation profile					
rtM204I±rtL180M	-0.861	0.423	0.335	0.073	2.432
rtM204V±rtL180M	-0.182	0.833	0.838	0.145	4.785
rtM204I+rtM204V+rtL180M			Reference		
HBV DNA level, copies/mL					
<10 ⁵	-0.981	0.375	0.372	0.044	3.226
10 ⁵ -10 ⁶	0.474	1.607	0.534	0.360	7.166
10 ⁶ -10 ⁷	-0.065	0.938	0.914	0.291	3.017
10 ⁷ -10 ⁸	-0.316	0.729	0.548	0.260	2.043
≥10 ⁸			Reference		

B, logistic regression coefficient; Exp(B), odds ratio; CI, confidence interval; ALT, alanine aminotransferase; UNL, upper normal limit; HBV, hepatitis B virus.

prove significance of treatment modality.

There are several limitations to this study. First, this was a retrospective observational study, in which it is possible that patients who missed follow-up or disagreed with the medical adviser were excluded. Nevertheless, our results are briskly in favor of low HBV DNA level when starting ADV with a considerable number of patients from a single institution. Second, a genotypic analysis for 18 non-responders was not evaluated. Non-response may be caused by slow viral suppression of ADV and/or anti-viral resistance. It is a weak point because the viral kinetics of non-response during the first 6 months could yield valuable information. Third, there was relatively small number of patients with a HBV DNA level less than 10⁵ copies/mL. Unfortunately, ADV treatment was delayed in most patients under the national insurance system in Korea. Lastly, we did not measure actual long-term outcome. This will be overcome by continuing data analysis and through the assessment for the adequacy of surrogate marker.

Biochemical breakthrough usually occurs a few weeks or a few years after virologic breakthrough.^{31,32} This condition can result in hepatitis flares associated with hepatic decompensation. In this study, we demonstrated that HBeAg negativity and baseline viral load when starting ADV were significant predictors for achieving IVR in LAM-resistant CH-B patients. Given that HBeAg negativity is uncorrectable and increasing HBV DNA level is the initial sign of following biochemical breakthrough,

early ADV treatment when HBV DNA level is low is the critical factor to achieve IVR.

In conclusion, if a virologic breakthrough emerges during LAM treatment, early intervention of ADV before further increase of viral load should be considered. Since IVR as a predictor of long term response is not fully evaluated yet, additional long term follow-up data are warranted.

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