Journal of Viral Hepatitis, 2010, **17,** 171–177 doi:10.1111/j.1365-2893.2009.01161.x

Low efficacy of entecavir therapy in adefovir-refractory hepatitis B patients with prior lamivudine resistance

S. W. Cho,[1] K. H. Koh,[1] J. Y. Cheong,[1] M. H. Lee,[1] S. P. Hong,[2] W. D. Yoo[2] and S.-O. Kim[2]
[1]*Department of Gastroeneterlogy, Ajou University School of Medicine, Suwon, South Korea; and* [2]*GeneMatrix Inc., Yongin, South Korea*

Received January 2009; accepted for publication April 2009

SUMMARY. We determined the virologic response, incidence of entecavir resistance, and evolution of lamivudine and adefovir-resistant mutants during entecavir (ETV) therapy in adefovir-refractory patients with prior lamivudine resistance. Forty adefovir-refractory chronic hepatitis B patients with prior lamivudine resistance who had received entecavir for ≥6 months were included and monitored for virologic response and entecavir resistance. Ten per cent of patients achieved HBV DNA < 50 copies/mL by PCR after 24 weeks of ETV therapy, and an initial virologic response was observed in 12 of 40 patients (30%). Higher pretreatment ALT ($P = 0.039$) and the presence of the rtL180M mutation ($P = 0.038$) were associated with an initial virologic response. During a mean follow-up of 11.4 months, four patients (10%) experienced virologic breakthrough, while ETV-resistant mutants were detected in six patients (15%). YMDD and adefovir-resistant mutants were detected in 57 and 35% of patients at baseline, respectively. At 48 weeks of therapy, 96 and 4% of patients had YMDD and adefovir-resistant mutants, respectively. These data suggest an early development of ETV resistance and low antiviral response during ETV therapy in adefovir-refractory patients with prior lamivudine resistance.

Keywords: adefovir, drug resistance, entecavir, hepatitis B, lamivudine.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is an important health problem throughout the world, and leading frequently to cirrhosis, liver failure, and hepatocellular carcinoma [1,2]. Nucleos(t)ide analogues have been found to suppress HBV replication and to improve biochemical and histological status of hepatitis B patients [3–5]. Prolonged antiviral therapy in patients with chronic HBV infection can prevent progression to cirrhosis and hepatocellular carcinoma [6]; however, it also often results in the emergence of drug-resistant mutants and an ensuing treatment failure [7].

Prolonged lamivudine (LAM) therapy is associated with a high rate of selection for LAM-resistant HBV, at approximately 24 and 70% after 1 and 4 years of therapy, respectively [7]. Mutations in the YMDD catalytic motif in the C domain of HBV polymerase (rtM204V/I) are responsible for LAM resistance [8]. Although adefovir (ADV) has shown to be effective against both wild-type and LAM-resistant HBV [9,10], suboptimal viral response has been frequently observed in LAM-resistant patients [11] and ADV-resistant mutants were found to appear more frequently in LAM-resistant patients than in treatment-naive patients [12]. Combination therapy with ADV and LAM is considerable by international guidelines as the standard of care options for LAM-resistant patients. The selection of the rtN236T or rtA181V/T mutants was associated with ADV resistance [12,13]. Entecavir (ETV) is another drug that displays potent antiviral activity against wild-type HBV [14,15]. LAM-resistant mutants exhibit an intermediate susceptibility to ETV as administration of a high dose of ETV is required to suppress these mutants [16,17]. Although ETV resistance seems to be rare in treatment-naive patients [18,19], it does emerge with a rate of 6, 15 and 51% after 1, 2 and 5 years therapy, respectively, in LAM-resistant patients [17,20]. The emergence of rtT184, rtS202, and rtM250 mutations is associated with viral rebound in LAM-resistant patients [21].

Sequential nucleos(t)ide analogue monotherapies increase the risk of selection of multi-drug resistant strains [11] and the development of multi-drug resistance to LAM and ADV is becoming a common problem. Combination therapy with ETV and tenofovir has been recommended for the treatment

Abbreviations: ADV, adefovir; ALT, alanine aminotransferase; anti-HBeAb, anti-hepatitis B e antibody; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LAM, lamivudine; RFMP, restriction fragment mass polymorphism; RT, reverse transcriptase.

Correspondence: Sung Won Cho, M.D., Department of Gastroenterology, Ajou University School of Medicine, San-5 Wonchon-Dong, Yountong-Ku, Suwon, 442-821, South Korea. E-mail: sung_won cho@hotmail.com

of patients with resistance to LAM and ADV [22]; however, tenofovir has not yet been available for the treatment of chronic HBV infection in many countries, and the ministry of Health, Welfare, and Family Affairs does not reimburse for combination therapy in Korea.

In vitro studies have shown that ETV is effective in suppressing ADV-resistant mutants [23]. Although ETV has been reported to be effective in suppressing HBV DNA levels in two ADV-resistant patients with prior LAM resistance [11], the antiviral effect of ETV in this setting has not been fully investigated. Furthermore, studies on the emergence of ETV resistance in ADV-refractory patients with prior LAM resistance are limited. In the present study, we determined the virologic response and emergence of ETV-resistant mutants in ADV-refractory patients with prior LAM resistance during ETV therapy, and the evolution of LAM and ADV-resistant mutants was observed.

PATIENTS AND METHODS

The study subjects included consecutive 40 ADV-refractory chronic hepatitis B patients with prior LAM resistance. All patients had LAM resistance documented by virologic breakthrough defined as an increase in the level of HBV DNA of at least 1 $\log_{10}$ copies/mL from the lowest point during therapy, genotypic analysis of rtM204 sequences, and LAM was switched to ADV monotherapy. Patients were considered to be ADV refractory if they had an inadequate virologic response with or without documented ADV mutations while on ADV. Inadequate virologic response was defined as an HBV DNA level of more than 4 $\log_{10}$ copies/mL at 24 weeks of treatment. Fifteen patients developed ADV resistance and another twenty-five patients experienced inadequate virologic response to ADV monotherapy. They were switched to ETV monotherapy from ADV. Patients were positive for HBsAg at least 1 year before LAM therapy. None of the patients had co-infections (HCV, HIV) or other concomitant liver disease such as alcoholic liver disease or autoimmune liver disease. All patients had HBV DNA level >5 $\log_{10}$ copies/mL before ETV administration and received 1.0 mg ETV once daily. Biochemistry and HBV DNA levels were tested before and every 3 months during ETV therapy. Serial blood samples were taken before and every 3 months during therapy and stored at −70 °C until used for HBV molecular analyses. This study was approved by the Institutional Review Board of our institution, and all the patients gave their informed consent.

Analysis of virological markers

Routine biochemical tests were performed using standard procedures during therapy. HBsAg, HBeAg, and anti-HBe were tested with a commercial radioimmunoassay kit (Abbott Laboratories, Chicago, IL, USA). HBV DNA was determined quantitatively by branched DNA (bDNA) assay (versant^{TM}3.0; Bayer Healthcare LLC Diagnostic Division, New York, NY, USA), which has a detection limit of 2 000 copies/mL. In samples showing undetectable HBV DNA by bDNA assay, detection of HBV DNA was done by the COBAS TaqMan^{TM} HBV test (TaqMan test; Roche Diagnostics, Branchburg, NJ, USA), which has a detection limit of 50 copies/mL (or 12 IU/mL).

Genotypic analysis

We performed restriction fragment mass polymorphism (RFMP) to detect LAM-resistant mutations (rt180, rt204), Adefovir-resistant mutations (rt181, rt236), and ETV-resistant mutations (rt169, rt184, rt202, rt250 plus rt204) at baseline and every 3 months in all patients during ETV administration, as previously described [12,24]. The genotypic analysis by RFMP was confirmed in some patients by sequencing analysis. This analysis was performed using primers with the sequences 5'-TCC TAC GAC CCC TGC TCG TGT TAC-3' (nucleotide 177–200) and 5'-CTG TAA ATA GAC CTA TTG ATT GGA-3' (nucleotide 959–982). For HBV genotype analysis, PCR was performed using primers BF105 (5'-TCCTGCTGCTATGCCTCATC-3', nucleotide number 411–430) and BR112 (5'-TTCCGTCCACATATCCCA TGAAGTTAAGGGA-3', nucleotide number 895–865) as previously described [24]. Sequence analysis was performed by ABI PRISM 310 Genetic Analyzer (Applied Biosystems, New York, NY, USA) and HBV genotype was assigned by web-based NCBI retroviruses genotyping analysis of the obtained sequences (http://www.ncbi.nlm.nih.gov/projects/genotyping).

Statistical analysis

Statistical testing was performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA). Results are reported as mean ± SD or median (range). HBV DNA levels were logarithmically transformed for analysis. Continuous variables were compared using the independent sample's *t*-test. Categorical data were compared using the Pearson χ^2 test or Fisher's exact test. Factors associated with an initial virologic response were analysed by univariate analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The study population comprised 40 adefovir-refractory patients who had previously shown LAM resistance. The baseline characteristics of the patients studied are shown in Table 1. Thirty-five patients were men and the mean age was 45 ± 10.48 years. Ten patients (25%) had cirrhosis and 36 patients (90%) were positive for HBeAg. Fourteen

Table 1 Baseline characteristics of patients (n = 40)

	Patients
Mean age, years (SD)	45.1 (10.48)
Male (%)	35 (87.5)
HBeAg-positive (%)	36 (90)
Mean ALT, IU/L (SD)	83.75 (155.72)
Mean AST, IU/L (SD)	84.28 (252.83)
HBV DNA, $\log_{10}$ copies/mL(SD)	6.68 (0.93)
Mean duration of ADV prior to ETV, months (SD)	17.13 (7.72)
Mean duration of ETV, months (SD)	11.40 (3.21)
Cirrhosis (%)	10 (25)
HBV genotype C (%)	40 (100)
LAM-resistant mutation	
rtM204I (%)	13 (32.5)
rtM204V (%)	7 (17.5)
rtM204I+rtM204V (%)	3 (7.5)
rt204M (wild) (%)	17 (42.5)
rtL180M (%)	19 (47.5)
rt180L (wild) (%)	21 (52.5)
ADV-resistant mutation	
rtA181T (%)	6 (15)
rtA181V (%)	4 (10)
rtA181T + rtA236T (%)	1 (2.5)
rtA181V + rtA236T (%)	3 (7.5)
rt181A + rt236A (wild) (%)	26 (65)

ADV, adefovir; ETV, entecavir; LAM, lamivudine.

patients (35%) were treated with ETV at the time of virologic breakthrough due to ADV resistance, and the remaining 26 patients were treated due to inadequate response to ADV. The mean duration of ETV therapy was 11.4 ± 3.2 months. At the commencement of ETV therapy, 23 patients (57.5%) had YMDD mutants; seven with rtM204V, 13 with rtM204I, and three with rtM204V/I. Nineteen patients (47.5%) had rtL180M. ADV-resistant mutants were found in 15 patients. All patients were infected with genotype C.

Virologic and biochemical response to entecavir

At the start of ETV therapy, all patients had HBV DNA > 5 $\log_{10}$ copies/mL and 23 patients had elevated ALT levels. ETV reduced HBV DNA levels to undetectable by PCR (<50 copies/mL) in 10 and 12% of patients by week 24 and week 48, respectively, and initial virologic response (IVR) defined as HBV DNA < 4 $\log_{10}$ copies/mL after 6 months of therapy was observed in 12 of 40 patients (30%) (Table 2). Patients who achieved IVR had higher baseline ALT and AST levels (80 *vs* 44 IU/L, $P = 0.039$; 51 *vs* 31 IU/L, $P = 0.036$, respectively) compared to those who did not achieve IVR. The rtL180M mutations were significantly more detected at baseline among patients with IVR (75 *vs* 35%, $P = 0.038$). However, there was no difference in baseline HBV DNA levels, HBeAg positivity, presence of YMDD mutation or ADV-resistant mutation between patients with and without IVR (Table 3). Serum ALT levels were normalized in 13 of 23 patients (56%) with high baseline ALT level at 6 months of therapy. Among 36 HBeAg-positive patients, four (11.1%) achieved HBeAg loss (n = 2) or HBeAg seroconversion (n = 2) during ETV therapy (mean 11.4 months) (Table 2).

Emergence of ETV-resistant mutants

During a mean follow-up of 11.4 ± 3.2 months, four patients (10%) experienced virologic breakthrough. ETV-resistant mutants emerged in six of 40 patients (15%). Among the six patients with ETV-resistant mutants, four had virologic breakthrough and ETV-resistant mutants transiently appeared in two patients. ETV-resistant mutants emerged in one, one and four patients at 6, 9, and 12 months of therapy, respectively. Among the six patients

Table 2 Virologic, serologic and biochemical response to entecavir

	Week 12 (n = 40)	Week 24 (n = 40)	Week 36 (n = 34)	Week 48 (n = 33)	Total
HBV DNA, $\log_{10}$ copies/mL, n (%)					
Undetectable by PCR*		4 (10)		4 (12.1)	
<3.3	1 (2.5)	8 (20)	7 (20.6)	5 (15.2)	
3.3–3.9	5 (12.5)	4 (10)	2 (5.9)	4 (12.1)	
4.0–4.9	8 (20)	7 (17.5)	7 (20.6)	6 (18.2)	
≥ 5.0	26 (65)	21 (52.5)	18 (52.9)	18 (54.5)	
HBeAg seroconversion/loss (n = 36) (%)	0/1	2 /1			2 (5.5)/2 (5.5)
Virologic breakthrough (n = 40) (%)			1	3	4 (10)
Emergence of ETV resistance (n = 40) (%)		1	1	4	6 (15)

ETV, entecavir. *Detection limit of COBAS TaqMan™ assay is <50 copies/mL (or 12 IU/mL).

Table 3 Baseline factors associated with an initial virologic response

	Patients with IVR (n = 12)	Patients without IVR (n = 28)	P-value
Mean age, years (SD)	49 (11) 02	43 (9)	0.102
Male (n = 35)	10	25	0.627
HBV DNA, $\log_{10}$ copies/mL			
<7 (n = 24)	9	15	0.297
≥7 (n = 16)	3	13	
Median ALT, IU/L	80 (12–1007)	44 (19–136)	0.039
Median AST, IU/L	51 (18–1594)	31 (19–61)	0.036
HBeAg-positive (n = 36) (%)	11 (30.5)	25 (69.4)	1.000
Cirrhosis (n = 10) (%)	5 (41.7)	5 (17.9)	0.133
rtM204V/I (n = 23) (%)	7 (58.3)	16 (57.1)	0.738
rtL180M (n = 19) (%)	9 (75)	10 (35.7)	0.038
rtA181T/V, rtA236T (n = 14) (%)	4 (33.3)	10 (35.7)	1.000

IVR, initial virologic response. Initial virological response defined as HBV DNA <4 log10 copies/mL after 6 months of entecavir therapy.

with ETV-resistant mutants, four patients had the rtS202G and two had the T184L mutants (Tables 2 and 4).

Evolution of LAM and ADV-resistant mutants in patients who developed ETV-resistant mutations

Among the six patients with ETV-resistant mutants, four had wild-type YMDD before ETV administration. YMDD mutations were found to emerge at 12 weeks of ETV therapy in all four patients. The rtM204V mutation was detected in three patients, and the rtM204I mutation in one at the time of emergence of ETV-resistant mutants. ADV-resistant mutants (rtA181V/T, rtA236T) were detected in three patients before ETV therapy. ADV-resistant mutants were replaced with wild-type HBV within 24 weeks of therapy in all three patients and were not detected at the time of emergence of ETV-resistant mutants (Table 4).

Evolution of LAM and ADV-resistant mutants during ETV therapy

Among the 40 patients studied, 23 (57.5%) had rtM204V/I and 19 (47.5%) had rtL180M mutants before ETV administration. RFMP analysis of the position rtM204 in patients receiving ETV therapy showed that rtM204V/I mutants were detected in 87.5% (35/40) and 96% (25/26) of patients at 24 and 48 weeks of therapy, respectively. In addition, rtL180M mutants emerged in 62.5% (25.40) and 73.0% (19/26) of patients at 24 and 48 weeks of ETV therapy. These results suggest that ETV therapy selects for LAM-resistant mutants. ADV-resistant mutants (rtA181V/T, rtA236T) were detected in 14 of 40 patients (35%) before ETV administration. ADV-resistant mutants remained positive in five (12.5%) patients at 24 weeks and one patient (3.8%) at 48 weeks of ETV therapy (Table 5).

DISCUSSION

Multi-drug resistance to LAM and ADV is becoming prevalent due to sequential treatment of LAM followed by ADV. ETV displays antiviral activity against both LAM-resistant and ADV-resistant HBV [14,15,23]. A previous study showed that 79% of LAM-resistant patients had undetectable HBV DNA levels by bDNA assay at 24 weeks of ETV therapy and that HBV DNA was undetectable by PCR assay in 26% of patients at 48 weeks [25]. A preliminary study of 12 patients showed ETV administration reduced HBV DNA levels in patients with a limited virological response to adefovir but only 33% of patients achieved HBV DNA levels of less than 3 $\log_{10}$ copies/mL at 24 weeks [26]. These results suggest a low response to ETV in patients with LAM resistance and in those with a limited response to ADV. In our study investigating the efficacy of ETV in ADV-refractory patients with prior LAM resistance, IVR was observed in 30% of patients and HBV DNA levels were undetectable by PCR assay in 10% of patients after 6 months of ETV therapy. These findings demonstrated that the antiviral activity of ETV is low in ADV-refractory patients with LAM resistance.

In this study, high baseline ALT/AST levels were found to be associated with IVR on ETV in ADV-refractory patients with LAM resistance. Previous studies with LAM therapy have shown that baseline ALT is the most important predictor of HBeAg seroconversion [27]. Thus, the results of this study confirmed that nucleos(t)ide analogues are more effective in patients who have high pretreatment ALT levels. Among 40 study subjects who had had YMDD mutations previously, YMDD mutations were detected in 23 subjects (57.5%) before ETV therapy. The presence of YMDD mutations was not associated with IVR on ETV. However, the presence of the rtL180M mutation appeared to be associated with IVR. The role of rtL180M as a predictor of IVR needs to

Table 4 Evolution of ETV, LAM, and ADV-resistant HBV during ETV therapy in six patients who developed ETV-resistant mutant

Patients	Time to resistance (weeks)	HBV DNA level (log_{10} copies/mL) and genotypic resistance				
		Baseline	Week 12	Week 24	Week 36	Week 48
1	24	5.7	5.0	ND†	ND	ND
		rt202S	rt202S	rtS202G>rt202s	rt202S	not detected
		rtM204V	rtM204V	rtM204V	rtM204V	not detected
		rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	not detected
2	36	> 8.0	5.2	ND	5.4	7.9
		rt202S	rt202S	rt202S	rt202S>rtS202G*	rt202S< rtS202G*
		rt204M	rtM204V	rtM204V	rtM204V	rtM204V
		rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A>rtA181T, rt236A
3	48	6.1	5.0	ND	ND	ND
		rt202S	rt202S	rt202S	rt202S	rS202G
		rt204M	rt204M>rtM204I	rt204M>rtM204I	rtM204I	rtM204I
		rtA181T,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A
4	48	>8.0	6.4	6.9	6.8	7.4
		rt184T	rt184T	rt184T	rt184T	rt184T>rtT184L*
		rt204M	rtM204V>rt204M	rtM204V	rtM204V	rtM204V
		rtA181V,rtA236T	rt181A>rtA181V, rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A
5	48	6.3	6.2	6.2	6.0	7.2
		rt184T	rt184T	rt184T	rt184T	rtT184L*
		rtM204V	rtM204V	rtM204V	rtM204V	rtM204V
		rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A
6	48	6.0	4.1	3.6	3.3	7.1
		rt184T,rt202S	rt202S	rt202S	rt202S	rtS202G*>rt202S
		rt204M	rtM204I>rt204M	rtM204I>rt204M	rtM204V	rtM204V
		rt181A>rtA181V, rt236A>rtA236T	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A	rt181A,rt236A

*Combined with virologic breakthrough. †Not detectable, <3.3 log_{10} copies/mL. ETV, entecavir; LAM, lamivudine; ADV, adefovir. Entecavir-resistant mutations are rtS202G, rtT184L, lamivudine-resistant mutations are rtM204V/I, and adefovir-resistant mutations are rtA181T/V, rtA236T.

Table 5 Evolution of lamivudine and adefovir-resistant mutants during entecavir therapy

Genotype	Baseline (n = 40) (%)	Week 12 (n = 40) (%)	Week 24 (n = 40) (%)	Week 36 (n = 35) (%)	Week 48 (n = 26) (%)
YMDD, wild-type	17 (42.5)	5 (12.5)	5 (12.5)	3 (7.5)	1 (2.5)
YIDD, YVDD	23 (57)	35 (87)	35 (87)	32 (91)	25 (96)
rt181A, rt236A	26 (65)	30 (75)	35 (87.5)	33 (94)	25 (96)
rtA181T/V, rtA236T	14 (35)	10 (25)	5 (12)	2 (6)	1 (4)
rt180L	21 (52.5)	12 (30)	15 (37.5)	11 (31)	7 (26.9)
rtL180M	19 (47)	28 (70)	25 (62)	24 (68)	19 (73)

be validated in further studies with larger numbers of patients. We could not investigate the effect of HBV genotypes on the antiviral efficacy of ETV because all study subjects were infected with genotype C in this study.

It had been reported that viral rebounds due to ETV resistance were detected in 10% of LAM-resistant patients after 48 weeks and an additional 9% after 96 weeks [17]. In the present study, virologic rebounds were observed in four

(10%) of 40 ADV refractory patients with prior LAM resistance during a mean follow-up of 11.4 months, suggesting that ETV-resistant mutations develop early during therapy in these patients. We observed emergence of ETV-resistant mutants in six of 40 patients. Among those six patients, four had a rtS202G mutant and two had a T184L mutant.

Virologic rebound occurs in nucleoside-naive patients receiving ETV treatment due to selection of a LAM-associated mutation [18]. We investigated the emergence of LAM-associated mutations during ETV treatment in ADV-refractory patients who had had previous LAM resistance. Fifty-seven per cent of patients had YMDD mutants before ETV treatment, and ETV treatment increased the rate of emergence of YMDD mutants to 96% of patients at 48 weeks. This suggests that YMDD mutants had reappeared in almost all patients within 1 year of ETV therapy. ETV has been shown to be less effective against LAM-resistant mutants than wild-type HBV [25]. Thus, ongoing ETV treatment may confer a selective advantage to YMDD mutants over wild-type HBV in patients infected by a mixture of wild-type and LAM-resistant mutant HBV. Early emergence of YMDD mutants, coupled with suboptimal response to ETV, can increase the risk of selection for ETV-associated mutations in ADV refractory patients with LAM resistance. LAM is not recommended as first-line therapy because LAM induces a high rate of resistance and prior treatment may reduce options for further therapy.

On the other hand, reversion from ADV-resistant mutants to wild-type HBV occurred in nine (64.2%) of 14 patients at 24 weeks of ETV therapy and most patients had reverted wild-type HBV by 48 weeks of therapy. These findings suggest that ETV may suppress ADV resistant mutants more effectively than wild-type HBV and that ETV could be effective for the treatment of those ADV-refractory patients with no prior exposure to LAM.

A study of a small number of patients with dual resistance to both LAM and ADV showed that sequential tenofovir monotherapy suppressed completely HBV DNA only in a minority of the patients [28]. As observed with sequential ETV monotherapy in this study, the antiviral activity of tenofovir might be low in these patients. Combination therapy of tenofovir and emtricitabine was reported to result in a greater reduction in HBV DNA levels than tenofovir monotherapy in patients who failed ADV monotherapy [29]. Thus, combination therapy may be more effective than monotherapy for this setting of patient. An add-on therapy with ETV or a switch to a combination of tenofovir plus emtricitabine or tenofovir plus ETV may be treatment options [22]. We speculate that combination therapy with ADV and ETV may delay the emergence of LAM-resistant HBV and subsequent emergence of ETV-resistant HBV in ADV-refractory patients with prior LAM resistance.

In conclusion, only 30% of ADV-refractory patients with prior LAM resistance achieved IVR on ETV and high pretreatment ALT level and the presence of rtL180M mutation were associated with IVR. We also found that YMDD mutants reappeared in the majority of patients within 1 year of therapy, even in the absence of YMDD mutants before therapy. However, ETV was efficacious in suppressing the replication of ADV-resistant mutants. A suboptimal response to ETV, coupled with early emergence of YMDD mutants, led to early and frequent development of ETV resistance in these patients.

ACKNOWLEDGEMENTS

This study was supported by the grants of the Korean Health 21 R&D project, Ministry of Health, welfare and family Affairs, Republic of Korea (A010383).

REFERENCES

1 Ganem D, Prince AM. Hepatitis B virus infection-natural history and clinical consequences. *N Engl J Med* 2004; 350: 1118–1129.
2 Wright TL, Lau JY. Clinical aspects of hepatitis B virus infection. *Lancet* 1993; 342: 1340–1344.
3 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–68.
4 Dienstag JL, Goldin RD, Heathcote EJ *et al.* Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; 124: 105–117.
5 Leung NWY, Lai CL, Chang TT *et al.* Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001; 33: 1527–1532.
6 Liaw YF, Sung JJY, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–1531.
7 Lai CL, Dienstag J, Schiff E *et al.* Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; 36: 687–696.
8 Melegari M, Scaglioni PP, Wands JR. Hepatitis B virus mutants associated with 3TC and famciclovir administration are replication defective. *Hepatology* 1998; 27: 628–633.
9 Marcellin P, Chang TT, Lim SG *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; 348: 808–816.
10 Schiff ER, Lai CL, Hadziyannis S *et al.* Adefovir dipivoxil therapy for lamivudine-resistant hepatitis B in pre-and post-liver transplantation patients. *Hepatology* 2003; 38: 1419–1427.
11 Fung SK, Chae HB, Fontana RJ *et al.* Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; 44: 283–290.
12 Lee YS, Suh DJ, Lim YS *et al.* Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006; 43: 1385–1391.

13 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; 131: 1743–1751.
14 Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of a BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob Agents Chemother* 1997; 41: 1444–1448.
15 Zoulim F. Entecavir: A new treatment option for chronic hepatitis B. *J Clin Virol* 2006; 36: 8–12.
16 Levine S, Hernandez D, Yamanaka G *et al.* Efficacies of entecavir against lamivudine-resistant hepatitis B virus replication and recombinant polymerase in vitro. *Antimicrob Agents Chemother* 2002; 46: 2525–2532.
17 Tenney DJ, Rose RE, Baldick CJ *et al.* Two-year assessment of entecavir resistance in lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother* 2007; 51: 902–911.
18 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naive patients with hepatitis B. *Hepatology* 2006; 44: 1656–1665.
19 Gish RG, Lok AS, Chang TT *et al.* Entecavir therapy up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007; 133: 1437–1444.
20 Tenney DJ, Pokornowski KA, Rose RE *et al.* Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance. *Hepatol Int* 2008; 2: A88.
21 Tenney DJ, Levine SM, Rose RE *et al.* Clinical emergence of entecavir resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. *Antimicrob Agents Chemother* 2004; 48: 3498–3507.
22 Lok AS, Zoulim F, Locarnini S *et al.* Antiviral drug-resistant HBV: Standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; 46: 254–265.
23 Vileneuve JP, Durantel D, Durantel S *et al.* Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. *J Hepatol* 2003; 39: 1085–1089.
24 Hong SP, Kim NK, Hwang SG *et al.* Detection of hepatitis B virus YMDD variants using mass spectrometric analysis of oligonucleotide fragments. *J Hepatol* 2004; 40: 837–844.
25 Chang TT, Gish RG, Hadziyannis SJ *et al.* A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 2005; 129: 1198–1209.
26 Reijnders JG, de Man RA, Pas SD, Schutten M, Janssen HL. Entecavir: a rescue therapy for chronic hepatitis B patients with a limited virological response to adefovir. *Hepatology* 2007; 46: 660A.
27 Chien R-N, Liaw Y-F, Arkins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. *Hepatology* 1999; 30: 770–774.
28 van Boemmel F, Trojan J, Feucht HH *et al.* Tenofovir shows limited efficacy in treatment of HBV innfections resistant against adefovir (abstract). *Hepatology* 2007; 46: 664A.
29 Tan J, Degertekin B, Wong SN *et al.* Tenofovir monotherapy is effective in hepatitis B patients with antiviral treatment failure to adefovir in the absence of adefovir-resistant mutations. *J Hepatol* 2008; 48: 391–398.