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ScienceDirect
Antiviral Research 75 (2007) 146–151
Antiviral Research
www.elsevier.com/locate/antiviral

Adefovir dipivoxil treatment of lamivudine-resistant chronic hepatitis B

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Received 21 November 2006; accepted 21 February 2007

Abstract

Adefovir dipivoxil (ADV)-resistant mutations have been identified in treating hepatitis B virus (HBV) infection. This study aimed to analyze the response, the incidence of ADV resistance and the virologic characteristics of ADV therapy. A total of 29 CHB patients with confirmed lamivudine (LAM)-resistant HBV were treated with ADV for more than 52 weeks. Serum HBV DNA, HBV genotypes and sequences of HBV polymerase reverse-transcriptase domain were determined. Rates for the biochemical response, HBeAg loss, HBeAg seroconversion and virologic response (<200 copies/mL of HBV DNA) were 82.8, 23.5, 11.8, and 48.3%, respectively, at week 52 of treatment. Lower pre-treatment mean HBV DNA level was the only significant factor associated with negative HBV DNA after ADV therapy. Six (20.7%) patients had clearance of LAM-resistant YMDD variants with replacement by the wild type HBV at week 52. The rtN236T, rtA181V/T and rtI233V were not identified before ADV therapy and the genotypic mutation of rtN236T was detected in one (3.4%) patient. In conclusion, the 52-week ADV treatment for patients with LAM-resistant HBV variants significantly achieved normalization of ALT levels, reduced serum HBV DNA levels and induced HBeAg loss and seroconversion. The emergence of ADV-resistant mutations seemed rare at weeks 52.

Keywords: Adefovir dipivoxil; Lamivudine; Resistance; CHB; HBV

1. Introduction

Chronic hepatitis B virus (HBV) infection is a major heath problem worldwide affecting more than 400 million people (Lai et al., 2004). A wide spectrum of clinical manifestations, including an asymptomatic carrier state, chronic hepatitis, cirrhosis, and hepatocellular carcinoma has been noted (Chen, 1993; Lee, 1997). One of the most important and urgent issues is the effective treatment for chronic hepatitis B (CHB) to prevent disease progression, major complications, and mortality, aside from prevention with hepatitis B vaccination.

Lamivudine (LAM) is the first nucleoside analogue to treat CHB by inhibiting viral DNA replication, improving liver function, and inducing histologic improvement of fibrosis (Lai et al., 1998; Dienstag et al., 2003; Di Marco et al., 2005). One of its major limitations is the emergence of drug-resistant mutations from substitutions at M204I/V within the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV polymerase gene. These LAM-resistant YMDD variants develop in approximately 70% of patients after 4–5 years of treatment (Lok et al., 2003; Locarnini, 2003; Lai et al., 2003) and can lead to exacerbation of hepatitis or even hepatic failure, which needs further management (Chayama et al., 1998; Lok et al., 2001; Di Marco et al., 2005).

Adefovir dipivoxil (ADV) is a nucleotide analogue that has been shown to effectively reduce serum HBV-DNA levels and improve serum alanine aminotransferase (ALT) levels as well as liver histology (Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003). ADV also has potent anti-viral activity to effectively treat CHB patients with LAM-resistant HBV (Perrillo et al., 2000, 2004; Westland et al., 2005). However, ADV-resistant HBV has likewise occurred,

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doi:10.1016/j.antiviral.2007.02.003

although less frequently. Compared to LAM therapy, there is a cumulative incidence of 0–17% after 1–4 years of therapy for treatment-naïve patients (Yang et al., 2002; Angus et al., 2003; Westland et al., 2005; Locarnini et al., 2005). The important mutations that have been confirmed to confer resistance include rtN236T (substitution of asparagine by threonine) and rtA181V/T (substitution of alanine by valine or threonine) (Angus et al., 2003; Villeneuve et al., 2003). Although such mutations result in only minor reductions in sensitivity to ADV (with decrease sensitivity to ADV by 2–13-fold) (Locarnini et al., 2004; Yang et al., 2004), biochemical and virological rebound, as well as hepatic decompensation, have been reported, which indicate the need to monitor and manage the drug resistance (Angus et al., 2003; Villeneuve et al., 2003; Fung et al., 2005).

Among patients with emerging LAM-resistant HBV with ADV therapy, a higher probability of ADV-resistant HBV was found than what has been previously reported in clinical trials of nucleoside- or nucleotide-naïve CHB patients (Fung et al., 2005; Yeon et al., 2006). A novel rtI233V (substitution of isoleucine by valine) HBV mutation has recently been shown to confer primary resistance to ADV (Schildgen et al., 2006; Chang and Lai, 2006).

The present study aimed to analyze the response to ADV in Taiwanese CHB patients with emerging LAM-resistant HBV and to elucidate the incidence of ADV resistance. The virologic characteristics after ADV therapy and the associated clinical factors were also determined.

2. Materials and methods

2.1. Patients

Between December 2002 and June 2005, 29 CHB patients (24 males, aged range: 22–65 years; mean 47 ± 12 years) with confirmed LAM-resistant HBV at the Liver Clinic of the Kaohsiung Medical University Hospital in Kaohsiung, Taiwan were enrolled in the present study. All of the patients had received LAM therapy for at least 6 months for CHB. LAM-resistant YMDD motif mutations were confirmed by polymerase chain reaction (PCR) and direct sequencing during LAM therapy prior to enrollment.

After the emergence of LAM-resistant YMDD variants, all of the 29 patients were treated with ADV 10 mg once daily orally for more than 52 weeks. The duration of ADV therapy ranged from 52 to 161 weeks. No concomitant hepatitis C, hepatitis D, or human immunodeficiency virus infection, autoimmune or metabolic liver disease, or history of alcohol abuse were noted in any of the patients.

Cirrhosis was diagnosed by histologic findings in previous biopsies or by evidence of imaging features suggestive of cirrhosis, along with the presence of thrombocytopenia, esophageal varices, ascites, or encephalopathy (Liaw et al., 1988). Informed consent was obtained from each patient. Sera collected from the patients were kept at $-70\,^{\circ}C$ until use. The study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the hospital Ethical Committee.

2.2. Laboratory tests

Routine complete blood count and a biochemical study, including ALT, bilirubin, and creatinine tests, were performed for each patient. Hepatitis Be antigen (HBeAg), antibody to HBeAg (anti-HBe), and anti-delta were analyzed by radioimmunoassay [RIA] (General Biological Cooperation, Taiwan). Anti-HCV was detected using a third-generation, commercially available enzyme-linked immunosorbent assay kit (Abbott, North Chicago, IL). Serum HBV DNA levels before ADV therapy were determined by a standardized automated quantitative PCR assay (Cobas Amplicor HBV Monitor; Roche Diagnostics; detection limit 200 copies/mL) (Dai et al., 2004). The identification of HBV genotypes was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the surface gene of HBV, as previously described (Mizokami et al., 1999).

2.3. Definition

Biochemical response was defined as a normalization of ALT level during continued treatment. Virological response and breakthrough were defined as negative HBV DNA results by PCR assay (<200 copies/mL) and an increase in the serum HBV DNA levels of $\geq 1 \log_{10}$ copies/mL during treatment in patients with initially adequate viral suppression (Lok and McMahon, 2004), respectively. Genotypic mutation was defined as the presence of rtA181V/T, rtN236T or rtI233V mutations deduced from sequencing analysis. ADV therapy was discontinued if a patient suffered from nephrotoxicity, which was defined as an increase in serum creatinine level ≥ 0.5 mg/dL.

2.4. Amplification and sequencing of polymerase reverse-transcriptase domain

HBV DNA sequencing was performed after extraction of HBV DNA from serum samples from patients who received ADV for 52 weeks and had HBV DNA levels more than 200 copies/mL. DNA extraction from serum sample is performed by a viral DNA extraction kit (Qiagene). In order to obtain the sequence of the reverse transcriptase domains B, C, and D of polymerase gene of the HBV DNA, the HBV DNA was subjected to amplification by a semi-nest polymerase chain reaction. A final volume of 50 μL mixture for one PCR reaction containing 200 μmol/L 2′-deoxynucleoside triphosphates (dATP, dCTP, dTTP, dGTP), 25 pmol of each primer, and 2.5 U of Taq polymerase (Roche) was prepared.

The first PCR reaction was performed by using two specific primers: HBVRTF1, 5′-AGACTCGTGGTGGACTTCTCT-3′ (nucleotides 252–272) and HBVRTR, 5′-ATGAGCTTTG CTCCAGACC-3′ (nucleotides 1309–1291). The second PCR reaction was performed by using two specific primers: HBVRTR and HBVRTF2, 5′-GGATGTGTCTGCGGCGTTT-3′ (nucleotides 377–395). The amplification reactions were performed as follows: an initial incubation at 94 °C for 5 min and 40 cycles of 94 °C/30 s, 55 °C/15 s, 72 °C/60 s, one last extension step at 72 °C for 7 min, and storage at 4 °C. The PCR

product, a 933 bp sequence, was identified by electrophoresis on a 3% agarose gel. Each PCR product was sequenced using a sequencing primer HBVRTF2 with a 3100 Automatic Sequencer (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical analysis

The values are expressed as means ± S.D. and group means were compared using the Student's *t*-test. Frequency was compared between groups using the chi-square test with Yate's correction or Fisher's exact test. A nominal value of 100 copies/mL was assigned to samples that were undetectable for serum HBV-DNA levels (<200 copies/mL) by PCR for further statistical analysis. Stepwise logistic regression was used where appropriate. All of the statistical tests were 2-tailed and a *P*-value of <0.05 was considered statistically significant. All procedures were performed using the SPSS for Windows Version 12 (SPSS Inc., Chicago, IL).

3. Results

3.1. Demographic characteristics of patients

Of the 29 patients, 17 (57.7%) were HBeAg-positive and 12 (42.3%) were HBeAg-negative/anti-HBeAg-positive. The HBV genotype distribution was as follows: genotype B in 14 (48.3%) and genotype C in 15 (51.7%) patients. The distribution of pre-treatment LAM-resistant YMDD variants was: rtM204I:18 (62.1%) and rtM204V:11 (37.9%). The mean ALT and HBV DNA levels were 326.2 ± 413.4 IU/L and 7.68 ± 1.11 $\log_{10}$ copies/mL, respectively. Cirrhosis was diagnosed in 12 (41.4%) patients. The mean bilirubin level was 2.19 ± 2.43 mg/dL with 8 (27.6%) having bilirubin levels ≥2.0 mg/dL (range: 2.0–9.4 mg/dL).

3.2. Responses to ADV therapy

For LAM-resistant CHB patients, the responses to ADV treatment at week 52 are shown in Fig. 1. After ADV therapy, rates

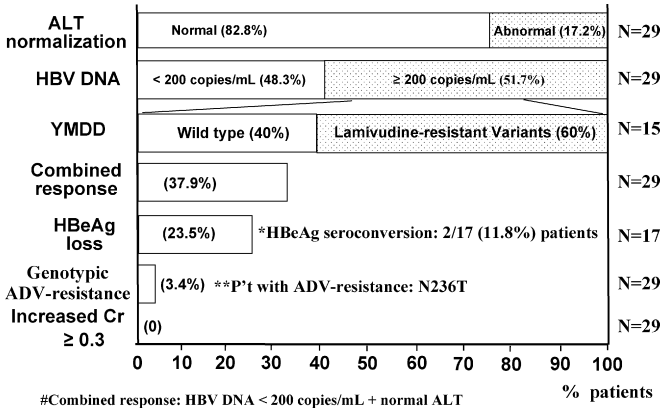

Fig. 1. Adefovir dipivoxil (ADV) 52-week therapy for 29 chronic hepatitis B (CHB) patients with lamivudine (LAM)-resistant tyrosine-methionine-aspartate-aspartate (YMDD) motif variants of the hepatitis B virus polymerase gene.

Table 1
Comparison of demographic and clinical features between chronic hepatitis B patients with negative and positive HBV DNA (52th week post-therapy)

	HBV DNA[a] at week 52		*P*-value
	Negative (*n* = 14)	Positive (*n* = 15)	
Age (year)[b]	46.6 ± 11.7	47.1 ± 11.9	0.912
Sex (M/F)	11/3	13/2	0.651
ALT[c] (IU/L)[b]	349.9 ± 324.7	304.1 ± 492.8	0.772
Pre-treatment YMDD mutant			0.812
rtM204I, *n* (%)	9 (64.3)	9 (60.0)	
rtM204V, *n* (%)	5 (35.7)	6 (40.0)	
Cirrhosis, *n* (%)	6 (42.9)	6 (40.0)	0.876
Positive for HBeAg, *n* (%)	8 (57.1)	9 (60.0)	0.876
HBV genotype			0.191
B, *n* (%)	5 (35.7)	9 (60.0)	
C, *n* (%)	9 (64.3)	6 (40.0)	
HBV DNA level[a] ($\log_{10}$ copies/mL)[b]	7.09 ± 1.24	8.22 ± 0.62	0.004

[a] Quantitative PCR assay with detection limit 200 copies/mL.
[b] Data expressed as mean ± standard deviation.
[c] ALT: alanine aminotransferase.

for the biochemical response, HBeAg loss and HBeAg seroconversion were 82.8, 23.5, and 11.8%, respectively. By week 52, 14 (48.3%) patients had virological response with undetectable viral loads (<200 copies/mL of HBV DNA) and the median decrease from baseline in serum HBV DNA by PCR assay was 4.75 $\log_{10}$ copies/mL. A combined response (with normal ALT and HBV DNA <200 copies/mL) was noted in 37.9% of patients.

Comparison by univariate analysis of clinical factors between patients with positive and negative HBV DNA at week 52 after ADV therapy is shown in Table 1. Patients with negative HBV DNA had significantly lower pre-treatment mean levels of HBV DNA than those with positive HBV DNA (7.09 ± 1.24 $\log_{10}$ copies/mL versus 8.22 ± 0.62 $\log_{10}$ copies/mL, $P = 0.004$). Other factors were similar between patients with negative and positive HBV DNA at week 52.

In stepwise logistic regression analysis, lower pre-treatment mean levels of HBV DNA was the only significant factor associated with negative HBV DNA after ADV therapy for 52 weeks [Odds ratio (OR): 4.468, 95% confidence interval (CI): 1.168–17.101, $P = 0.029$].

3.3. Virology after ADV therapy

Six (20.7%) of the 29 patients on ADV therapy had a clearance of LAM-resistant YMDD variants with replacement by YMDD wild type HBV at week 52. On the other hands, 30% (6/20) with clearance of LAM-resistant YMDD variants had a re-appearance of YMDD wild type HBV, while 40% (6/15) with positive HBV DNA at week 52 had clearance of LAM-resistant YMDD variants but also with replacement by YMDD wild type HBV. The other 9 (60%) patients had persistent YMDD variants [3 (33.3%) with rtM204I and 6 (66.7%) with rtM204V].

Comparison of clinical factors between patients with and those without replacement by YMDD wild type HBV at

Table 2
Comparison of demographic and clinical features between patients with and without re-appearance of YMDD wild type HBV (52th week post-therapy)

	Re-appearance of YMDD wild type HBV at week 52		*P*-value
	Yes ($n=6$)	No ($n=23$)	
Age (year)[a]	49.7 ± 9.8	46.2 ± 12.2	0.552
Sex (M/F)	4/2	20/3	0.269
ALT[b] (IU/L)[a]	371.7 ± 532.8	314.4 ± 390.3	0.768
Pre-treatment YMDD mutant			0.646
rtM204I, *n* (%)	3 (50.0)	15 (65.2)	
rtM204V, *n* (%)	3 (50.0)	8 (34.8)	
Cirrhosis, *n* (%)	3 (50.0)	9 (39.1)	0.669
Positive for HBeAg, *n* (%)	4 (66.7)	13 (56.5)	1.0
HBV genotype			1.0
B, *n* (%)	4 (66.7)	13 (56.5)	
C, *n* (%)	2 (33.3)	10 (43.5)	
HBV DNA level[c] ($\log_{10}$ copies/mL)[a]	8.43 ± 0.49	7.48 ± 1.15	0.064

[a] Data expressed as mean ± standard deviation.
[b] ALT: alanine aminotransferase.
[c] Quantitative PCR assay with detection limit 200 copies/mL.

week 52 after ADV therapy is shown in Table 2. By univariate analysis, patients with re-appearance of YMDD wild type HBV had higher pre-treatment mean levels of HBV DNA than those without (8.43 ± 0.49 $\log_{10}$ copies/mL versus 7.48 ± 1.15 $\log_{10}$ copies/mL, $P=0.064$). There were no differences in other parameters.

In stepwise logistic regression analysis, higher pre-treatment mean levels of HBV DNA was a significant factor associated with the re-appearance of the YMDD wild type HBV after ADV therapy for 52 weeks (OR: 16.992, 95% CI: 1.130–255.5, $P=0.041$).

3.4. Serum bilirubin and creatinine levels and ADV therapy

Pre-treatment mean bilirubin level of all patients was 2.19 ± 2.43 mg/dL with 8 (27.6%) having bilirubin levels ≥2.0 mg/dL (range: 2.0–9.4 mg/dL). With a mean bilirubin level of 0.82 ± 0.33 mg/dL and a mean decrease from baseline in bilirubin level of 1.3 mg/dL, all of the patients had bilirubin levels <1.2 mg/dL except one (bilirubin level: 1.9 mg/dL) by week 52. After the 52-week ADV therapy, all of the patients had serum creatinine levels of <1.2 mg/dL and none had increases from base line of 0.3 mg/dL.

3.5. ADV resistance and clinical courses

Neither the rtN236T nor the rtA181V/T was detected in any of the HBV isolates from CHB patients with LAM-resistant HBV prior to ADV therapy. After ADV treatment for 52 weeks, genotypic mutation of rtN236T was detected in 1 patient (3.4%). This 61-year-old female was diagnosed with cirrhosis, with genotype B HBV infection, negative HBeAg, and the rtM204V + rtL180M before ADV therapy. She had virologic breakthrough (<200 copies/mL at month 9 to 1.07×10^4 copies/mL at month 12). The HBV DNA became negative again at month 15 and she had normal ALT levels since treatment months 3–15 after initiating ADV therapy. All of the patients carried the HBV variant with an isoleucine at position 233 of the reverse-transcriptase domain and no recently identified ADV-resistant HBV variant with substitution of isoleucine by valine (rtI233V) was detected.

4. Discussion

ADV has been approved for hepatitis B therapy with potent anti-viral effects against both the wild type and LAM-resistant HBV (Perrillo et al., 2000, 2004; Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003; Westland et al., 2005). In the present study, ADV was used for more than 52 weeks to treat CHB patients with a confirmed emergence of LAM-resistant mutants in Taiwan. The biochemical response rate of 82.8% seemed better than previous results of 48–72% for HBeAg-positive and HBeAg-negative CHB patients without prior therapy for more than 12 weeks (Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003). The median decrease of HBV DNA level in the present study (4.75 $\log_{10}$ copies/mL), which was similar to 4.3 and 4.5 $\log_{10}$ copies/mL reported recently (Werle et al., 2004; Liu et al., 2006), and virologic response rate of 48.3% (<200 copies/mL) also suggested an effective suppression of HBV replication by ADV on LAM-resistant mutants.

In HBeAg-positive patients with LAM-resistant mutations, the HBeAg loss and HBeAg loss seroconversion rates (26.6 and 13.3%, respectively) were similar to reports by Marcellin et al. (23.5 and 11.8%, respectively) (Adefovir Dipivoxil 437 Study Group, 2003). It is noteworthy that patients with LAM-resistant mutations and elevated bilirubin levels also markedly improved after ADV 52-week therapy in our patients. These results show that ADV is effective for the control of LAM-resistant HBV mutations among Taiwanese CHB patients.

A higher pre-treatment mean level of HBV DNA was noted to be a predictive determinant for positive HBV DNA at 52 week of ADV therapy for patients with LAM-resistance HBV. Since the PCR assay with a lower limit of detection of 200 copies is used in the present study, patients with higher levels of HBV DNA will not achieve virologic response after 52 weeks of treatment with ADV, even if there is a rapid suppression of viral replication (Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003). Patients with high HBV-DNA titers have a higher risk of developing virologic breakthrough during LAM therapy and YMDD mutant viruses might be re-overtaken by the wild type after cessation of therapy (Chayama et al., 1998). Clearance of LAM-resistant YMDD variants with replacement by the wild type HBV is observed among 20.7% (6/29) of our patients. Furthermore, 6 (30%) of 20 patients who were cleared of LAM-resistant mutations had a re-appearance of the YMDD wild type HBV, and 6 (40%) of 15 positive-HBV DNA patients at week 52 had clearance of

LAM-resistant YMDD variants with replacement by the wild type HBV.

Liu et al. (2006) have reported that 16.7% of patients regained the YMDD wild type HBV by real-time PCR quantification method. The median decrease of HBV DNA level in this and previous studies (Werle et al., 2004; Liu et al., 2006) with 10 mg ADV therapy for LAM-resistance HBV seems higher than 3.52 and 3.9 $\log_{10}$ copies/mL in the YMDD wild type strain reported previously (Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003). These finding imply different sensitivity between LAM-resistant and wild type YMDD variants to ADV therapy. The higher pre-treatment mean level of HBV DNA is a significant factor associated with the re-appearance of the YMDD wild type HBV after ADV therapy for 52 weeks. Whether the sensitivity to ADV between different YMDD variants exist requires further studies.

The emergence of drug-resistant mutations is a critical problem that reduces the therapeutic effects and may lead to biochemical and virologic rebound. The high frequency of LAM-resistant mutations, occurring in approximately 70% of patients after 4–5 years of treatment (Lok et al., 2003; Locarnini, 2003; Lai et al., 2003) and possibly leading to severe complications (Di Marco et al., 2005; Chayama et al., 1998; Lok et al., 2001) make it urgent and necessary to treat patients with other nucleoside or nucleotide analogues. The lower cumulative incidences of ADV-resistance for CHB therapy have been reported at 0–17% after 1–4 years of therapy for treatment-naïve patients compared to LAM therapy (Westland et al., 2005; Yang et al., 2002; Angus et al., 2003; Locarnini et al., 2005). The mutations including rtN236T (substitution of asparagine by threonine) and rtA181V/T (substitution of alanine by valine or threonine) have been confirmed to confer resistance to ADV. Yeon et al. (2006) reported that the incidence of ADV resistance was 6.4% in isolates from patients who were infected with LMV-resistant mutations. Nevertheless, Fung et al. (2005) and Liu et al. (2006) did not identify genotypic resistance to ADV at 48 weeks in 11 and 30 CHB patients with confirmed LAM-resistant HBV, respectively.

In the present study among Taiwanese CHB patients who had prior LAM treatment and had confirmed LAM-resistance HBV subsequently, we found that the incidence of ADV resistance was 3.4%. The suboptimal response to ADV, demonstrated by an inadequate initial reduction in HBV DNA compared to baseline, has been reported as the major determinant for development of ADV-resistance in long-term studies (Locarnini et al., 2005; Fung et al., 2005; Adefovir Dipivoxil 438 Study Group, 2005).

The small number of patients in the present study is the major limitation, making it difficult to obtain the predictive determinants of ADV-resistance in Taiwanese patients with LAM-resistant HBV. Since biochemical and virological rebounds are the major concerns, and hepatic decompensation have been reported in patients with the emergence of ADV-resistant HBV (Angus et al., 2003; Villeneuve et al., 2003; Fung et al., 2005), further close and long term monitoring in these patients are mandatory even though the emergence of ADV-resistance was not associated with biological breakthrough or clinical deterioration.

Schildgen et al. (2006) and Chang and Lai (2006) have identified a novel ADV-resistant HBV variant rtI233V, with a valine instead of isoleucine at position 233 of the reverse-transcriptase domain, which is sensitive to tenofovir or entecavir. In recent reports, the rtA181V/T has been found in patients with LAM-resistant HBV without ADV therapy (Marzano et al., 2006) and the rtA181T does not to confer in vitro ADV resistance (Qi et al., 2006). In the present study sequencing the HBV polymerase gene, the rtI233V and the rtA181V/T variants were not detected before and after the 52-week ADV therapy for LAM-resistant HBV. Further studies seem necessary in Taiwanese patients to elucidate the clinical role of these HBV variants.

An increase from baseline of 0.5 mg/dL in serum creatinine levels have been seen in 8 and 0% of treatment-naïve patients with the administration ADV for 48 weeks at 30 and 10 mg/day, respectively (Adefovir Dipivoxil 438 Study Group, 2003; Adefovir Dipivoxil 437 Study Group, 2003). Nephrotoxicity has not observed and no patient suffered from increases from base line of 0.3 mg/dL in serum creatinine levels with 52-week 10 mg/day ADV therapy. Since long-term ADV therapy may result in nephrotoxicity (Adefovir Dipivoxil 438 Study Group, 2005), further large-scale and long-term studies are needed to confirm these observations.

In conclusion, the study demonstrated that 52 weeks of ADV treatment for patients with the variety of LAM-resistant mutations significantly achieved normalization of ALT levels, reduced serum HBV DNA levels, and induced HBeAg loss and seroconversion. The rare presence of ADV-resistant mutations and avoidance of nephrotoxicity during therapy are important advantages. Nevertheless, confirmation of long-term effects and safety of ADV therapy for these patients requires further studies in Taiwan.

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