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Research article

Compare with safety and efficacy of entecavir and adefovir dipivoxil combination therapy and tenofovir disoproxil fumarate monotherapy for chronic hepatitis B patient with adefovir-resistant

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Abstract: *Objective:* To compare the 2-year efficacy and safety of combination therapy with entecavir (ETV) and adefovir dipivoxil (ADV) to that of tenofovir disoproxil fumarate (TDF) monotherapy in treatment of patients with adefovir drug-resistant chronic hepatitis B. *Methods***:** HBeAg-positive CHB patients (n = 100) with adefovir-resistance (rtA181T/V and/or rtN236T) were enrolled. Patients were treated with either ETV 0.5 mg plus ADV 10 mg per day (n = 52) or TDF 300 mg per day (n = 48) for 48 weeks. Tests for liver and kidney function, Serum Phosphorus, HBV serum markers, HBV DNA load and ultrasonography of liver were performed every 3 months. Student's t-test and χ^2 test were used to compare the efficacy, side effects in the two groups. *Results:* Fifty-two patients in ETV + ADV group and forty-eight patients in TDF group were followed-up for 96 weeks. HBV DNA undetectable rate were 76.9% versus 81.3% (P = 0.631) at week 48, and 92.3% versus 95.8% (P = 0.679) at week 96 in ETV + ADV combination therapy and TDF monotherapy group respectively. Serum ALT normalized rate were 84.6% versus 87.5% (P = 0.777) at week 48, and 92.3% versus 95.8% (P = 0.679) at week 96 in ETV+ADV combination therapy and TDF monotherapy group respectively. But the level of serum Phosphorus was significantly lower in ETV + ADV combination therapy group compare with

TDF monotherapy group (1.13 ± 0.15 versus 1.22 ± 0.16, P = 0.004) at week 96. *Conclusion:* Both ETV + ADV combination therapy and TDF monotherapy provided effective treatments in chronic hepatitis B with adefovir-resistant. However, it was associated with poor serological responses up to week 96. The long term treatment of hepatitis B with ETV (0.5 mg/day) combination of ADV (10 mg/day) can potentially cause hypophosphatemia and renal impairment, so regular monitoring of serum phosphate, serum creatinine and evaluation of eGFR is needed.

Keywords: chronic hepatitis B; adefovir-resistant; adefovir dipivoxil; entecavir; tenofovir disoproxil fumarate; combination therapy; hypophosphatemia

1. Introduction

Chronic hepatitis B (CHB) is a challenging disease that can induce severe liver diseases including liver failure, cirrhosis, and primary hepatocellular carcinoma. The World Health Organization estimates that approximately 400 million people globally are actively infected with hepatitis B virus (HBV) [1]. CHB treatment prevents progression of liver injury, fibrosis, and carcinoma through the inhibition of virus replication or virus elimination [2,3]. However, drug-resistant HBV mutants selected by long-term antiviral treatments such as adefovir dipivoxil (ADV) reduce the efficacy of clinical therapies [4,5]. Therapy options include nucleotide analogues and interferon alpha. Interferon alpha is an effective treatment for CHB, but its adverse effects and low response rate account for 20–40% of the whole interferon alpha therapy [6]. Nucleotide analogues are the primary treatment for CHB, however, the long half-life of covalently closed circular (ccc) DNA makes long-term treatment necessary. When patients are treated with nucleotide analogues, the risk of drug resistance increases.

Five nucleotide analogues are available in China. Lamivudine (LAM) is safe and effective for the treatment of CHB, which has the highest incidence of resistant mutations compared with other nucleotide analogues [7]. Adefovir dipivoxil (ADV) is likewise effective with a relatively lower drug resistance rate and no cross-resistance compared with other nucleoside analogues. Telbivudine is effective and has a relatively higher seroconversion rate. Second-generation nucleoside analogues (NUCs) such as Entecavir (ETV) and tenofovir disoproxil fumarate (TDF), with high genetic barrier, have potent anti-HBV effects and still keep very low rate of resistance [8,9]. In China there are many patients with adefovir dipivoxil monotherapy before the listing of entecavir. Virologic breakthrough for the patients with adefovir resistance was higher in the adefovir group than in the lamivudine plus adefovir group. In current practice guidelines, TDF and ETV are recommended for the first-line treatment of CHB, especially in patients with drug resistance for virus mutation. A combination of NUCs such as ETV plus ADV combination therapy as well as TDF monotherapy are two potential options to prevent the development of multi-drug resistance due to viral quasispecies complexity. Both ETV + ADV combination therapy and TDF monotherapy provided effective viral suppression in chronic hepatitis B, but TDF monotherapy provided better kidney safety [9,10].

The purpose of this study was to compare the effects over 96 weeks of combination therapy using ETV and ADV to that of TDF monotherapy in patients with chronic hepatitis B with ADV resistance. This study will provide researchers and practitioners with more information regarding two strategies for treating CHB mutation patients.

2. Methods

2.1. Patient selection

A total of 100 CHB patients with detectable serum HBV DNA levels ($\geq 1.0 \times 10^3$ IU/mL) and genotypically confirmed resistance mutations to ADV (rtA181V/T and/or rtN236T mutation) who sought treatment at our hospital (Hangzhou, China) from January 2015 to December 2016 were included in this study. These patients were seropositive for the hepatitis B e antigen-positive (HBeAg), and had alanine aminotransferase (ALT) levels 2 times higher than the normal level (UNL). Patients with hepatitis delta virus, hepatitis C virus, or HIV coinfection were all excluded from this study. Similarly, patients with hepatocellular carcinoma or diabetes were also excluded. Besides, patients with liver cirrhosis were not included, who were confirmed based on medical history, examination, radiological signs of cirrhosis. Patients with chronic hepatitis B (CHB) and compensated laboratory findings, hepatic cirrhosis were diagnosed according to the guideline of prevention and treatment for chronic hepatitis B proposed by the Chinese Medical Association Chinese Society of Hepatology and Chinese Society of Infectious Diseases. All patients enrolled in this study were given informed consent and were aware of the regular procedures. The protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine.

2.2. Study design

The study was designed as a prospective case-control. The patients were randomly assigned to the monotherapy group (48 patients) or combination therapy group (52 patients). Baseline data were compared between the 2 groups to ensure comparability. All patients were provided detailed study information and provided informed consent before receiving antiviral treatment. Patients in the combination therapy group were prescribed ETV and ADV (0.5 and 10 mg per day, respectively) while the monotherapy group received TDF (300 mg per day).

2.3. Observation and follow-up

Follow-ups of the groups were performed at treatment initiation and during the weekends following weeks 12, 24, 36, 48, 60, 72, 84 and 96 to test for biochemical function, HBV serum markers, HBV DNA load, and to perform liver ultrasonography. Real-time fluorescent PCR assays were performed to detect HBV DNA levels (7300; Applied Biosystems, Inc., Carlsbad, CA, USA). The lower limit of DNA detection used in this study was 1000 IU/mL. HBV DNA values > 1000 IU/mL were considered to show positivity for HBV. An Architect C8000 automated biochemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA) was used to detect biochemical indices.

2.4. Statistical analysis

SPSS for Windows, Version 16.0 (Chicago, SPSS Inc.) was used for data analysis. Measurements are presented as mean ± standard deviation (SD) and comparisons were made using

Student's t-test. Proportions are presented as percentage (%) and rate comparisons were performed using the chi-square test.

3. Results

3.1. Baseline characteristics

Table 1 shows baselines for clinical and laboratory characteristics at the start of treatment. Hundred patients were included in either the ETV + ADV combination group (n = 52) or the TDF monotherapy group (n = 48). In the combination treatment group, 41 patients were men (41/52) and the mean age was 33.87 ± 7.90 years (range: 22–50). Baseline glomerular filtration rate (GFR) was 110.78 ± 8.71 mL/min (range: 85.51–128.41), serum phosphate was 1.21 ± 0.12 mmol/L (range: 0.96–1.67), and serum creatinine was 64.87 ± 8.65 μmol/L (range: 49–91). The mean HBV DNA level was 8.17 ± 1.63 $\log_{10}$ IU/mL (range: 4.24–8.95). The mean ALT level was 355.92 ± 198.95 U/L (range: 100–766). In the monotherapy group, 36 patients were men (36/48) with a mean age of 33.81 ± 9.01 years (range: 22–56). Baseline GFR was 109.54 ± 11.38 mL/min (range: 90.83–147.04), serum phosphate was 1.22 ± 0.16 mmol/L (range: 0.87–1.66), and serum creatinine was 66.29 ± 9.06 μmol/L (range: 46–87). The mean level of HBV DNA was 8.07 ± 1.67 $\log_{10}$ IU/mL (range: 4.52–8.65) and the mean ALT level was 369.15 ± 191.85 U/L (range: 113–803). There were no significant differences in baseline characteristics between the 2 groups.

Table 1. Baseline characteristics of patients.

Variables	ETV+ADV combination group (n = 52)	TDF monotherapy group (n = 48)	P value
Age (yr)	33.87 ± 7.90	33.81 ± 9.01	0.974
Gender (male/female)	41/11	36/12	0.812
ALT (IU ml-1)	355.92 ± 198.95	369.15 ± 191.85	0.736
GFR (ml/min)	110.78 ± 8.71	109.54 ± 11.38	0.541
Cr (umol/l)	64.87 ± 8.65	66.29 ± 9.06	0.423
P (mmol/l)	1.21 ± 0.12	1.22 ± 0.16	0.734
HBV DNA (log(10) IU/mL)	8.17 ± 1.63	8.07 ± 1.67	0.722

ALT: Alanine transaminase; GFR: Glomerular filtration rate; Cr: Creatinine; P: Serum phosphate.

3.2. Virological effects

Serum levels of HBV DNA of the ETV+ADV combination group and the TDF monotherapy group were assayed at baseline and at weeks 12, 24, 36, 48, 72 and 96. Of the 52 patients in the ETV+ADV combination group, 40 and 48 patients achieved undetectable HBV DNA levels (HBV DNA < 1000 IU/mL) by weeks 48 and 96, respectively. The corresponding virological responses were 76.9 and 92.3%, respectively. Of the 48 patients in the TDF monotherapy group, 39 and 46 patients achieved undetectable HBV DNA levels by weeks 48 and 96, respectively. Their corresponding virological responses were 81.3 and 95.8% respectively. No statistically significant differences were found between the 2 groups (P = 0.631 and 0.679 at weeks 48 and 96, respectively) (Figure 1 and Table 2).

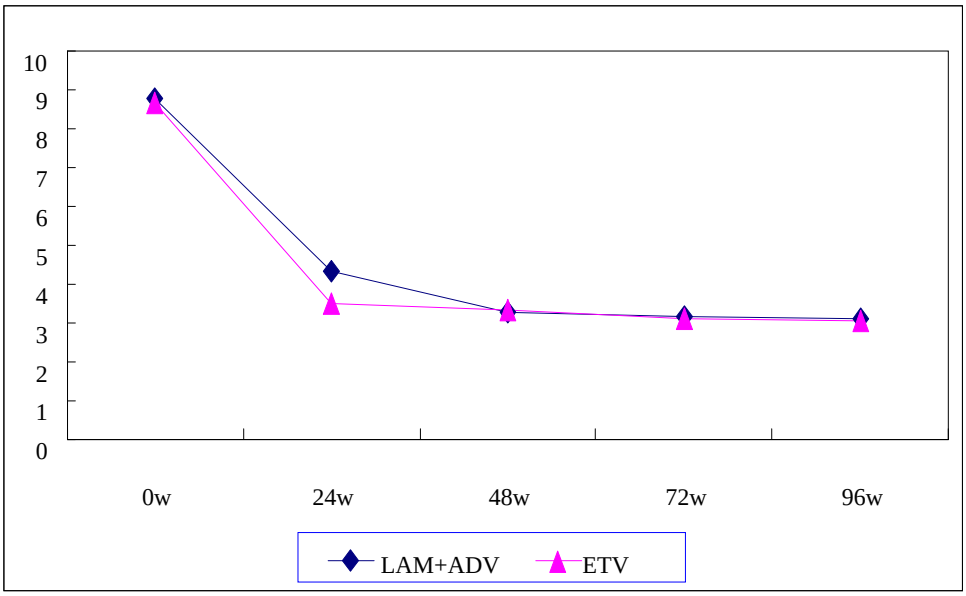

Figure 1. Mean values for HBV DNA over 96 weeks of treatment in ETV + ADV combination group and TDF monotherapy group.

Table 2. Virologic and biochemical response and adverse events in total patients.

Characteristics	ETV + ADV combination group (n = 52)	TDF monotherapy group (n = 48)	P value
HBV DNA undetectable (<1000 IU/mL)	-	-	-
After 48 weeks, N (%)	40/52 (76.9%)	39/48 (81.3%)	0.631
After 96 weeks, N (%)	48/52 (92.3%)	46/48 (95.8%)	0.679
ALT normal (＜40 U/L)	-	-	-
After 48 weeks, N (%)	44/52 (84.6%)	42/48 (87.5%)	0.777
After 96 weeks, N (%)	48/52 (92.3%)	45/48 (95.8%)	0.679
HBeAg negative and seroconversion	-	-	-
HBeAg negative after 96 weeks, N (%)	15/52 (28.8%)	13/48 (27.1%)	0.719
HBeAg seroconversion after 96 weeks, N (%)	6/52 (11.5%)	6/48 (12.5%)	0.782
GFR (ml/min)	-	-	-
After 96 weeks,	105.47 ± 13.30	108.35 ± 10.18	0.230
Cr (umol/l)	-	-	-
After 96 weeks	69.15 ± 16.08	66.21 ± 7.98	0.255
P (mmol/l)	-	-	-
After 96 weeks	1.13 ± 0.15	1.22 ± 0.16	0.004

ALT: Alanine transaminase; GFR: Glomerular filtration rate; Cr: Creatinine; P: Serum phosphate.

3.3. Biochemical responses

Of the 52 patients who received ETV + ADV combination therapy, 44 and 48 patients achieved ALT normalization (ALT < 40 IU/L) by weeks 48 and 96, respectively. Corresponding biochemical response rates were 84.6 and 92.3%. Of the 48 patients who received TDF

monotherapy, 42 and 46 patients achieved ALT normalization by weeks 48 and 96, respectively. The corresponding biochemical response rates for the monotherapy group were 87.5 and 95.8%, respectively (Table 2). These differences were not statistically significant (P = 0.777 and 0.679 at weeks 48 and 96, respectively) (Table 2).

3.4. Serological responses

Of the 100 patients who were HBeAg positive at baseline, 15/52 (28.8%) in the ETV + ADV combination therapy group and 13/48 (27.1%) in the TDF monotherapy group were negative for the antigen at 96 weeks. The number of HBeAg seroconversions was 6/52 (11.5%) and 6/48 (12.5%) in the ETV + ADV combination therapy and the TDF monotherapy groups, respectively. There were no statistical differences between HBeAg negativity and seroconversion (P = 0.719 and 0.782 at week 96, respectively) (Table 2).

3.5. Virological breakthrough and drug resistance

We detected serum HBV DNA in 5 patients (ETV + ADV combination group: 4; TDF monotherapy group: 1) who had not achieved undetectable HBV DNA levels after 96 weeks. No additional resistance substitution was detected.

3.6. Adverse events

In this study, a total of 100 patients were enrolled after applying the inclusion and exclusion criteria. All patients completed the treatment and all follow-ups. Adverse events in the ETV + ADV combination group by week 96 included the following: GFR of 2 patients was lower than 90 mL/min (67.25 and 77.61 mL/min, respectively); the creatinine level of 1 patient was 142 μmol/L, which is higher than the UNL; and serum phosphate was 0.65 mmol/L, which is lower than the normal lower limit. These 2 patients had no physical symptoms or other obvious complaints. After 96 weeks of treatment, there were statistically significant differences in the serum phosphorus levels (1.13 ± 0.15 mmol/L versus 1.22 ± 0.16 mmol/L, P = 0.004) between the ETV + ADV combination and TDF monotherapy groups (Table 2).

4. Discussion

A consensus on the benefits of antiviral therapy for CHB patients has previously been reached and reported [10]. Receiving long-term treatment with nucleotide analogues gradually increases the risk of drug resistance. This increasing resistance risk has become an important factor affecting clinical decisions. Preventing HBV antiviral drug resistance to nucleotide analogues and engaging in appropriate management when resistance occurs have become a major focus in the management of CHB [11,12]. Either combination treatment or monotherapy using agents with a high genetic barrier is recommended for retreatment of CHB with ADV resistance.

This study showed that two different treatment strategies, ETV+ADV combination therapy and TDF monotherapy, were effective treatments for Chinese CHB HBeAg postive patients with ADV resistance. Most patients in both strategies showed effective viral suppression and no additional

emergence in detectable HBV resistance mutations. After completing the 96-week treatment, 4 patients and 2 patients had not achieved undetectable levels of HBV DNA (<1000 IU/mL) in the ETV + ADV combination therapy and TDF monotherapy groups, respectively. The corresponding virological responses were 92.3 and 95.8%, respectively, and there were no statistically significant differences between the two groups. In the ETV + ADV combination therapy group, although 4 patients did not achieve undetectable levels of HBV DNA, 2 still showed normalization of ALT. In the TDF monotherapy group, one of the 2 patients who did not achieve undetectable HBV DNA levels also showed normalization of ALT levels.

After 96 weeks of treatment, there were 4 and 1 patients who did not show ALT normalization (ALT < 40 IU/L) in the ETV + ADV combination therapy group and TDF monotherapy group, respectively. Two of the 4 patients who did not achieve ALT normalization, however, had undetectable levels of HBV DNA. The 2 patients with slightly elevated blood lipids were also diagnosed with fatty liver by B-scan ultrasonography. It is possible that fatty liver disease was a contributing factor for the 2 patients who not achieve ALT normalization.

In this study, although most patients achieved and maintained a virologic response, the rates of HBeAg seroconversion by week 96 were 11.5 and 12.5% in the ETV + ADV combination therapy group and TDF group, respectively. Total HBeAg seroconversion rate was only 12% at 96 weeks, which is a striking contrast to previous reports in treatment naive CHB patients [13,14]. This finding suggests that the influence of host immune factors in achieving serological responses and indicate that further treatment is inevitable in patients with drug-resistant HBV variants.

Renal dysfunction and hypophosphatemia associated with long-term use of ADV have been documented in recent years [15,16]. In our study, 2 patients in the ETV + ADV combination group had a GFR (glomerular filtration rate) lower than 90 mL/min (67.25 mL/min and 77.61 mL/min, respectively). One of the patients had a creatinine level of 142 μmol/L, a value that is higher than the upper limit of the normal range. The serum phosphate level of this patient was 0.65 mmol/L, which is lower than the lower limit of normal. Previous studies have shown that patients who are older, or who have baseline renal insufficiency, hypertension, and/or diabetes mellitus, or hepatocellular carcinoma are more likely to be diagnosed with renal dysfunction and hypophosphatemia [17–19]. However, in our study, these 2 patients had no underlying diseases. Two patients with GFR values lower than 90 mL/min in the ETV + ADV combination group were later changed to entecavir single drug treatment with no further deterioration of GFR. The serum phosphate level of the patient with hypophosphatemia was normal after phosphorus supplementation.

In conclusion, our results show that both ETV + ADV combination therapy and TDF monotherapy are effective treatments for Chinese CHB patients with ADV-resistant HBV mutants. Besides, virologic breakthrough rate was rare, and no emergence of additional resistance mutation. Nonetheless, the HBeAg seroconversion rate was very low up to week 96, which suggests the necessity of continuous treatment to maintain viral suppression in these patients. Besides, long-term ETV + ADV combination therapy can cause renal impairment and hypophosphate. In the present study, the duration of observation was too short, further studies by long-term observation are needed to obtain reliable data. We recommend regular monitoring of serum phosphate and serum creatinine levels as well as evaluation of estimated GFR in patients treated with ADV. If a reduction of GFR or hypophosphatemia is diagnosed, the ADV dose should be reduced or replaced by an alternative antiviral agent.

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All authors discussed the results and implications and commented on the manuscript at all stages.

Conflict of interest

All authors declare that they have no conflict of interest in relation to this scientific work.

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