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Efficacy of 2 years of entecavir plus adefovir therapy in patients with chronic hepatitis B who had failed on prior nucleos(t)ide analog treatment

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ABSTRACT

Entecavir (ETV) plus adefovir (ADV) combination therapy may be a promising option for chronic hepatitis B (CHB) patients who have failed on prior nucleos(t)ide analog (NA) treatment. However, the long-term efficacy and safety of this combination are not well-defined. In a single-center, retrospective study, 104 patients (mean age 31.7 years; 88.5% male) with HBV DNA >10^3 IU/mL who had received one or multiple prior NAs for ⩾6 months (median 44.5 months) were treated for ⩾24 months with ETV (0.5 mg/day) plus ADV (10 mg/day). Among patients with available samples, 44/90 (48.9%) had drug-resistant mutations. At 2 years, HBV DNA levels were undetectable (<12 IU/mL) in 52/104 (50.0%) patients. The mean HBV DNA level was 2.0 ± 1.2 log 10 IU/mL, and it was decreased by 3.2 ± 2.0 log 10 IU/mL from the pre-combination treatment (V0) value. The 2-year HBeAg loss rate was 14.4% (13/90), HBeAg seroconversion rate was 10.0% (9/90), and ALT normalization rate was 75%. In multivariate analyses, the prior NA treatment duration, the V0 HBV DNA level, and the HBV DNA reduction at 1 year after ETV + ADV therapy were associated with the virological response after 2 years. No patients developed renal impairment, clinical decompensation or new HCC, and no relapses of HCC or deaths occurred. Thus, 2-year rescue therapy with ETV + ADV was effective and well-tolerated in CHB patients who had previously failed on multiple NA treatments. The HBV DNA level just before ETV + ADV combination therapy and the decrease of HBV DNA at 1 year could predict the efficacy of 2 years of ETV + ADV treatment.

1. Introduction

In China, about 93 million individuals have been infected with hepatitis B virus (HBV), and around 30 million have chronic hepatitis B (CHB) (Lu and Zhuang, 2009), some of whom progress to liver failure, decompensated cirrhosis and hepatocellular carcinoma (HCC). As is well-known, antiviral therapy is a key component of the management of HBV-related liver diseases. Clinical evidence suggests that anti-HBV therapy can delay disease progression, improve patients' quality-of-life, and prolong the survival time (Liaw, 2006; Liaw et al., 2004).

At present, two important classes of antiviral drugs are available for treatment of CHB: nucleos(t)ide analogs (NAs) and interferons (IFNs). In China, the available NAs include lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), and telbivudine (LDT), all of which are recommended as first-line anti-HBV therapies (Chinese Society of Hepatology and Chinese Society of Infectious Diseases, 2011). Because of viral persistence in infected hepatocytes, long-term antiviral therapy is needed in the majority of patients with HBV-related liver diseases (Ganem and Prince, 2004). The selection of potent NAs with a high barrier to resistance as first-line therapy such as ETV or tenofovir disoproxil fumarate (TDF) provides the best chance of achieving long-term treatment goals, and should be used wherever possible. However, the selection of treatments with a high barrier to resistance is not always possible in China. Thus, antiviral resistance and suboptimal virological responses have begun to emerge as important challenges for clinicians due to poor patient compliance, the pharmacologic properties of the particular drug(s), and individual genetic variations occurring during NA therapy (Fung and Lok, 2004; Carroué-e-Durantel et al., 2008; Zoulim and Locarnini, 2012).

Abbreviations: ADV, adefovir dipivoxil; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CHB, chronic hepatitis B; CK, creatine kinase; Cr, creatinine; Cys C, cystatin C; ETV, entecavir; GLB, globulin; HAV, hepatitis A virus; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HDV, hepatitis D virus; HEV, hepatitis E virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; IFN, interferon; LAM, lamivudine; LDT, telbivudine (L-deoxythymidine); LLD, lower limit of detection; NA, nucleos(t)ide analog; OR, odds ratio; PCR, polymerase chain reaction; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; V0, value at the initiation of combination therapy.

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With increasing knowledge of drug resistance and wide application of many antiviral therapy regimens, interventions for treatment failure have become an important issue. Theoretically, a combination of ETV and ADV or TDF should be a promising salvage treatment for patients who fail on various NA therapies as there is no cross-resistance between the two drugs. Recently, ETV + ADV combination therapy has been widely used as one of the regimens advocated for managing CHB patients in clinical practice after multi-drug failure (Kim et al., 2012; Yang et al., 2012; Jeon et al., 2012; Cho et al., 2013; Chae et al., 2012; Lim et al., 2012; Xu et al., 2013; Seo et al., 2014); however, the long-term efficacy and safety of this combination regimen are not well defined.

In this study, we retrospectively evaluated the long-term efficacy and safety of ETV + ADV combination therapy, and the factors influencing viral responsiveness in CHB patients who had failed on previous multiple sequential NA therapies, rather than instituting drug verification tests with various strict control standards.

2. Materials and methods

2.1. Study subjects

This single-center, retrospective investigator-initiated cohort study enrolled 158 patients with HBV-related liver diseases (including CHB, cirrhosis or HCC) who were switched to ETV (0.5 mg/day) and ADV (10 mg/day) combination therapy after the failure of sequential NA monotherapy or combination therapy (excluding ETV + ADV) regimens during the period July 2006 to September 2012. Of these, 104 patients who received 2 years of ETV + ADV combination therapy were included in final analysis. The Chinese Clinical Trial Registry Number of the study was ChiCTR-ONC-12002285. It was approved by the Ethical Committee of Southwest Hospital.

Eligible patients included those with HBV-related liver diseases who were serum hepatitis B surface antigen (HBsAg)-positive for at least 24 weeks and who had failed on single or multiple NA therapies (LAM/ADV/ETV/LDT) previously, which was defined as sequential NA therapy for more than 6 months with the persistence of serum HBV DNA levels $>10^3$ IU/mL. Patients were excluded if they were coinfected with other hepatitis viruses (HAV, HCV, HDV, HEV) or human immunodeficiency virus (HIV); had other concurrent autoimmune or metabolic liver diseases; or had a history of alcohol or substance abuse. Of the 158 patients who were initially enrolled, 54 were not included in the analysis as 1 was coinfected with HCV, 6 were lost to follow-up after 1 year of therapy; 3 withdrew or changed drugs for economic reasons after 1 year of therapy; 3 had incomplete data; and 41 received less than 2 years of therapy. The patient recruitment process is summarized in the flowchart displayed in Fig. 1.

2.2. Study procedures

Detailed clinical data were retrieved from patient's medical record. The patients' serum samples, which were collected at the initiation of ETV + ADV combination therapy and after 1 and 2 years and preserved at 80 °C, were tested for various laboratory markers, including alanine and aspartate aminotransferases (ALT, AST), total bilirubin (TBIL), albumin (ALB), globulin (GLB), blood urea nitrogen (BUN), creatinine (Cr), creatine kinase (CK), cystatin C (Cys C), HBV DNA, and quantitative HBsAg and HBeAg.

The primary study endpoints were the proportion of patients whose serum HBV DNA levels were <12 IU/mL and the mean decrease of HBV DNA at 1 and 2 years after initiation of ETV + ADV combination therapy. Secondary endpoints were the cumulative normalization rates of ALT after 1 and 2 years of combination therapy; the HBeAg loss rate and the seroconversion rate of HBeAg-positive patients; the viral breakthrough rate; and changes in serum biochemical markers such as CK, calcium, phosphorus, Cr, BUN, and early indicators of renal dysfunction (Cys C).

For the analysis of factors influencing the efficacy of the ETV + ADV regimen, which was based on serum HBV DNA levels after 2 years of combination therapy, patients were divided into two groups: (1) those who achieved a complete response (HBV DNA <12 IU/mL); and (2) those who had a suboptimal response (HBV DNA $\geqslant$12 IU/mL). In both groups, disease characteristics and biochemical and virological markers were analyzed statistically.

2.3. Laboratory assessments

Serum HBV DNA levels were determined by the COBAS® AmpliPrep/COBAS® TaqMan® HBV test (Roche Molecular Systems, Branchburg, NJ, USA), for which the lower limit of detection (LLD) is 12 IU/mL and lower limit of quantitation (LLQ) is 54 IU/mL. HBV DNA genotype and resistant profiles were identified by a genotype-specific primer pairs PCR system (Chen et al., 2007) and by HBV P region sequencing and phylogenetic analysis. A chemiluminescence system (Roche ELECSYS 2010) was used to detect HBV markers, and a latex-enhanced immunoturbidimetric method (Sichuan Maker Biotechnology Co., Ltd, Chengdu, China) was used to determine the Cys C concentration. Serum biochemical markers such as BUN, Cr, calcium (Ca^{2+}), phosphorus (P^{3+}) and CK were routinely assessed by standard laboratory detection (Sichuan Maker Biotechnology Co., Ltd, Chengdu, China).

2.4. Statistical analysis

All data were analyzed using SPSS 13.0 software. Logarithmic conversion of quantitative HBV DNA values was performed before statistical analysis; the log value was defined as 1.08 when below the LLD value. For analysis of factors influencing efficacy, a multivariate logistic regression model was used; for independent variables selection, the backward stepwise (likelihood ratio) method was used.

To assess the effect of HBV DNA resistant mutation profiles on the efficacy of combination therapy, Fisher's exact test was used. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the 104 patients analyzed are summarized in Table 1. The mean duration of NA treatment prior to ETV + ADV therapy was 44.5 months (range 6–108 months). The frequency of treatment regimen changes was $\geqslant$3 in 44 patients (42.3%); 53 patients (51.0%) had experienced brief discontinuations during previous NA treatments.

At the time of the initial NA treatment, the patients' mean baseline HBV DNA level was 7.2 ± 1.3 log 10 IU/mL and the median ALT level was 91 IU/L (range 18–1458 IU/L). During sequential NA therapy prior to ETV + ADV therapy, ALT levels in 18 patients were persistently normal, 29 had levels 1 to 2 times the upper limit of normal (ULN) fluctuation, and 50 had a peak at least 2 times the ULN fluctuation. In 43 patients, HBV DNA levels did not reach the LLD at any stage. In 7/104 patients, data were not available.

At the beginning of ETV + ADV therapy, the patients' mean serum HBV DNA level (V0) and median ALT level were 5.2 ± 1.9 - log 10 IU/mL and 41.5 IU/L (range 5–1335 IU/L), respectively. 90 patients (86.5%) were HBeAg-positive.