Department of Pharmacy[1], Renmin Hospital, Hubei University of Medicine, Shiyan; Department of Clinical Laboratory[2], Renmin Hospital of Wuhan University, Wuhan PR China

Efficacy of entecavir-based rescue therapy in lamivudine-resistant chronic hepatitis B patients in China: a retrospective study

Wei Li[1], Linhai Wang[1], Yanhong Liu[1], Yan Li[2], Shirong Yu[1,*]

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**Corresponding author: Prof. Shirong Yu, Department of Pharmacy, Renmin Hospital, Hubei University of Medicine, 39 Chaoyang Middle Road, Shiyan 442000, Hubei Province, P.R. China*
shirongyu2010@163.com

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Limited studies have investigated the antiviral efficacy of entecavir (ETV)-based rescue therapy in lamivudine (LAM)-resistant chronic hepatitis B (CHB) patients. We retrospectively analyzed the efficacy of entecavir (ETV) monotherapy versus ETV-tenofovir disoproxil fumarate (TDF) combination therapy in 220 LAM-resistant CHB patients. Among 220 patients, 114 patients were treated with ETV monotherapy and 106 were treated with ETV-TDF combination therapy for at least 24 months. There were no significant differences between the two groups in baseline characteristics. During the follow-up of 24 months, virologic response (VR) occurred in 146 (66.4%) patients (58 patients belonged to the ETV monotherapy group and 88 patients belonged to the ETV-TDF combination group). The VR rates were different between the ETV and ETV-TDF groups (32.5% vs. 57.5% at 6 months, 50.0% vs. 77.4% at 12 months; and 50.9% vs. 83.0% at 24 months, $P<0.001$). In addition, both groups showed no difference in terms of the biochemical and HBeAg response. The rates of viral breakthrough at 6, 12 and 24 months were significantly different between ETV and ETV-TDF groups (2.63%, 4.39% and 9.65% vs. 0.00%, 0.94% and 1.89% at 6, 12 and 24 months, respectively). The ETV-TDF group was superior to the ETV group in achieving a virologic response. Moreover, the ETV-TDF was lower than the ETV group in achieving the initial viral breakthrough and genotypic mutations. Therefore, ETV-TDF combination therapy might be a better regimen than ETV monotherapy in the subgroup of LAM-resistant Chinese patients with CHB.

1. Introduction

Approximately 240 million individuals are chronically infected with hepatitis B virus (HBV) worldwide (Chen et al. 2006), which is associated with the risk of development of liver disease to cirrhosis, and/or hepatocellular carcinoma (HCC) in 15-40% of patients (Fang et al. 2009; Gerlich 2013). Nucleotide analogs (NAs) such as lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), and tenofovir disoproxil fumarate (TDF) are widely used to delay disease progression (Ghany and Doo 2009). NAs target hepatitis B virus (HBV) reverse transcriptase (RT) activity and inhibit viral replication. However, long-term use of NAs lead to frequent development of drug-resistant mutations under the drug selective pressure (Ha et al. 2012).

LAM, the first nucleos(t)ide analog approved for the treatment of CHB, had been used extensively due to its safety and low cost. However, long-term LAM monotherapy inevitably contributes to the development of drug resistance. The LAM resistance emerges in 14-32% of patients after 1 year of treatment, in 38% of patients after 2 years and in 53-76% of patients after 3 years (Iloeje et al. 2006). The most important mutation involves substitution of methionine for valine or isoleucine (rtM204V/I) in the highly conserved tyrosine-methionine-aspartic acid-aspartic acid (YMDD) motif of the reverse transcriptase domain of HBV polymerase (Lai et al. 2003).

Therefore, The next-generation nucleoside ETV applied as rescue therapy is highly effective in terms of suppressing HBV replication, and the prevalence of drug resistance at the 5-year follow-up for naive CHB is only 1.2%. However, the cumulative resistance rate increased to 50% after 5 years when ETV was applied as a sequential therapy for patients with LAM-resistance (Tenney et al. 2009). This sequential therapy using a monorescue strategy in patients with LAM resistance resulted in multidrug resistance (MDR) (Lee et al. 2012, 2009). Global clinical practice guidelines have recognized the problems resulting from failure of first-line therapy with low-genetic barrier drugs, and a ETV plus TDF combination therapy strategy is recommended as a rescue therapy (Sarin et al. 2015; KASL 2016).

Experience with ETV-based rescue therapy is limited in China because this drugs have not been approved or are not affordable for the majority of the population. In addition, very limited data are available on the comparison between the efficacy of ETV monotherapy therapy and ETV/TDF combination therapy in CHB patients with LAM-resistance.

Therefore, the aim of this study was to retrospectively investigate the efficacy of ETV-based rescue therapy in LAM-resistant patients with CHB in China and to compare the incidence of drug resistance among them.

2. Investigations and results

2.1. Baseline characteristics

The baseline characteristics of the two rescue treatment groups of ETV monotherapy and ETV-TDF combination therapy were well balanced before receiving different rescue strategies (Table 1). The

Table 1: Baseline characteristics of LAM-resistant patients with CHB patients

Characteristics	ETV monotherapy group (n=114)	ETV-TDF combination therapy group (n=106)	*P* value
Age (years)	46.0±8.5	44.6±7.2	0.191
Sex (male, %)	86 (75.4)	80 (75.5)	0.996
Serum ALT (IU/mL)	106.3±40.2	101.3±37.1	0.340

Characteristics	ETV monotherapy group (n=114)	ETV-TDF combination therapy group (n=106)	*P* value
Serum total bilirubin (μmol/L)	38.8±10.2	40.2±12.2	0.356
HBeAg-positive (%)	56 (49.1)	54 (50.9)	0.787
Genotype (C/B, N)	80/34	75/31	0.925
Serum HBV DNA (log_{10} IU/mL)	6.14±1.23	6.05±1.20	0.584
LAM-resistant mutations			
rtM204I/V (%)	44 (38.6)	37 (34.9)	0.571
rtM204I/V + (and/or rtL180 M) (%)	70 (61.4)	69 (65.1)	0.571

Abbreviations: ETV, entecavir; TDF, tenofovir disoproxil fumarate; LAM, lamivudine; ALT, alanine transaminase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus

mean baseline characteristics of HBV DNA, genotype, serum total bilirubin, serum albumin and serum alanine transaminase (ALT) for the two study groups were comparable. Additionally, the rate of HBeAg positivity, the pattern of the LAM resistance mutation, age, gender and the duration of prior LAM treatment were also comparable between the two study groups.

2.2. Virological response

Fig. 1A and Table 2 show the changes of HBV DNA levels during the 24 months. Overall, serum HBV DNA levels continuously declined. The reduction in serum HBV DNA levels in the ETV-TDF combination therapy group was greater than in the ETV monotherapy group. The virological response (VR) (HBV DNA<3 log_{10} IU/ml) rates at 6, 12, 24 months was observed in 57.5% , 77.4%, 83.0% of patients receiving ETV-TDF combination therapy as compared to 32.5%, 43.9%, 50.9% of patients receiving ETV monotherapy, respectively. The difference in virological response between the two groups was statistically significant at 6, 12 and 24 months, respectively (*P*=0.010 for month 6, *P*=0.001 for month 12 and *P*=0.001 for month 24).

2.3. Biochemical and HBeAg response

During the treatment period, serum ALT levels both declined in two treatment groups, The proportions of patients with serum ALT normalization did not differ significantly between the ETV monotherapy and ETV-TDF combination therapy groups after 3/6/12 months of rescue treatment (64.9%/67.9%, 71.1%/77.4% and 72.8%/79.2%, *P*>0.05) (Fig. 1B and Table 2). HBeAg loss or HBeAg seroconversion was not observed among the HBeAg positive patients in both groups (Table 2).

2.4. Viral breakthrough and genotypic mutations

Viral breakthrough was experienced in 13 patients, with cumulative rates of 2.63%, 2.73% and 5.45% at 6, 12 and 24 months,

Table2: Virologic, biochemical, and HBeAg responses (probability of virological response, normalization of ALT and HBeAg loss/seroconversion using Chi-square test)

	ETV monotherapy group	ETV-TDF combination therapy group	*P* value
Proportion of virological response, n/N (%)			
Month 6	37 /114 (32.5%)	61/106 (57.5%)	0.001
Month 12	50/114 (43.9%)	82/106 (77.4%)	0.001
Month 24	58/114 (50.9%)	88/106 (83.0%)	0.001
Normalization of ALT, n/N (%)			
Month 6	74/114 (64.9%)	72/106 (67.9%)	0.637
Month 12	81/114 (71.1%)	82/106 (77.4%)	0.286
Month 24	83/114 (72.8%)	84/106 (79.2%)	0.265
HBeAg loss/seroconversion n/N (%)			
Month 6	0/56 (0%)	0/54 (0%)	-
Month 12	0/56 (0%)	0/54 (0%)	-
Month 24	0/56 (0%)	0/54 (0%)	-

Abbreviations: ETV, entecavir; TDF, tenofovir disoproxil fumarate; ALT, alanine transaminase; HBeAg, hepatitis B e antigen

respectively. There was a statistically significant difference in the cumulative rates between the ETV and ETV+TDF groups (2.63%, 4.39% and 9.65% vs. 0.00%, 0.94% and 1.89% at 6, 12 and 24 months, respectively). VBT developed in 11 cases in the ETV group and in 2 cases in the ETV+LAM group. Genotypic mutations at 24 months after the initiation of each antiviral treatment were as follows.

Table 3: Genotypic mutations

	ETV monotherapy group (n=114)	ETV-TDF combination therapy group (n=106)
Pattern of resistant mutations (24 months)		
rtM204I/V	7 (6.14%)	8 (7.55%)
rtL180 M	6 (5.26%)	7 (6.60%)
rtT184G/S/A/V/I/L	6 (5.26%)	2 (1.89%)
rtM250V/I/L	3 (2.63%)	1 (0.94%)
rtS202G	5 (4.39%)	2 (1.89%)
rtM204I/V+ rtL180 M	17 (14.91%)	21 (19.81%)
rtM204I+ rtS202G	5 (4.39%)	2 (1.89%)
rtM204I+rtT184I	6 (5.26%)	3 (2.83%)
Total mutation	55 (48.24%)	46 (43.40%)

Abbreviations: ETV, entecavir; TDF, tenofovir disoproxil fumarate

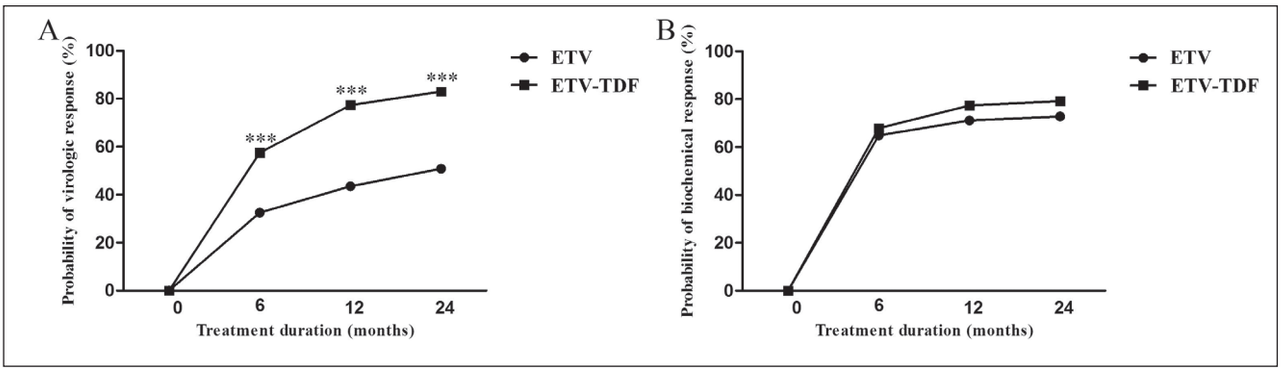

Fig. 1: Flow chart for study methods.

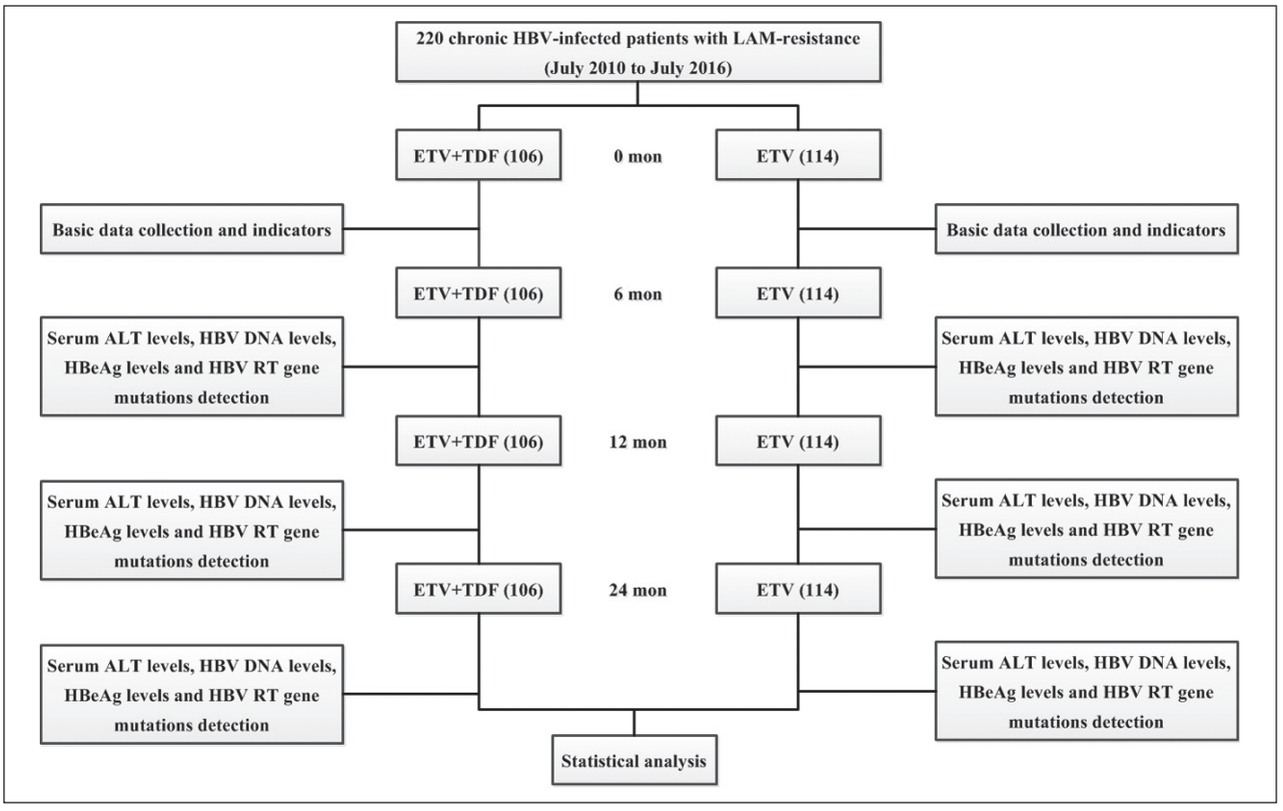

Fig. 2: Virological response rates (A) and Biochemical response rates (B) in patients receiving ETV monotherapy therapy or ETV-TDF combination therapy during the two years of retreatment.

During ETV monotherapy or combination therapy, the levels of LAM-resistant variants (rtM204I/V with or without rtL180M) gradually decreased as treatment progressed, whereas ETV-resistant rtT184G/S/A/V/I/L and rtS202G gradually increased and became dominant in the viral populations, expecially in ETV monotherapy group. In ETV monotherapy group, the most frequently detected mutations of LAM or ETV resistance mutations were rtM204I/V+(and/or rtL180 M) (30/55), rtT184G/S/A/V/I/L (6/55), respectively. In ETV-TDF combination therapy group, rtM204I/V+(and/or rtL180M), rtT184G/S/A/V/I/L were more frequently detected (38/46) (Table 3).

3. Discussion

HBV replication will lead to an increased risk
of cirrhosis and/or HCC in CHB patients (Pallier et al. 2006; Peng et al. 2012). This finding suggests that sustained suppression of HBV replication and prevention of viral breakthrough with anti-HBV therapy can delay disease progression. Therefore, the suppression of the serum HBV DNA level should be considered the ideal therapeutic goal for CHB patients in clinical settings. Current the goal of CHB treatment with NA is to prevent progression of liver disease to cirrhosis and development of hepatocellular carcinoma. However, long-term therapy contributes to the emergence of drug-resistant viral strains which results in increased viral loads, followed by increases in ALT levels and subsequent progression of liver disease (Perrillo et al. 2011). The therapy of drug-resistant patients with one NA, switching to another NA may increase risk of inducing additional resistance as selective pressure, resulting in cross-resistance (Petersen et al. 2012). A European study showed that combination therapy with ETV plus TDF is efficient and safe in patients with viral resistance patterns (Petersen et al. 2012). There are no reports that focus on ETV-based rescue therapy in Chinese LAM-resistant patients. Therefore, we aimed to compare the long-term efficacy between ETV monotherapy and ETV-TDF combination therapy in Chinese LAM-resistant patients with CHB. The efficacy of ETV/TDF rescue therapy has been assessed previously in a number of cohort studies. A retrospective study reported 89% patients achieved virologic suppression in 57 patients (median 3 failed prior therapies) over a median 21 months of ETV/TDF (Wang et al. 2016). Two studies showed that 80% and 86% patients with multidrug-resistant HBV treated with ETV/TDF achieved virologic suppression after 5 and 12 months, respectively (Zhang et al. 2015; Zoulim et al. 2016). Two randomized controlled trials, evaluating ETV/TDF versus TDF rescue therapy in patients with either ETV-resistant or ADV-resistant, reported virologic response rates of 73% and 64% (48 weeks of ETV/TDF), respectively (Zoulim et al. 2007). The present findings confirm and extend these data, demonstrating the efficacy and safety of ETV/TDF rescue therapy through 96 weeks in a multicenter clinical study in patients who had failed on a wide range of NAs.

In our study, we explored and compared retreatment options for chronic hepatitis B patients with LAM-resistance. There was no difference in the proportion of ALT normalization between ETV-TDF combination therapy and ETV monotherapy after 2 year retreatment (79.2% vs 72.8%, P=0.265). Moreover, HBeAg loss or seroconversion was not observed among patients during the entire study period. Although the mechanism was unknown, this result suggests that it is difficult to achieve serologic response in LAM-resistant patients, regardless of rescue therapy (Lim et al. 2012). Additionally, there was a significant difference in virological response between ETV-TDF combination therapy and ETV monotherapy after 2 year retreatment (83.0 % vs 50.9%, P<0.001). This finding indicates that combination therapy does provide an additive suppressive effect in Chinese LAM-resistant patients with CHB. As LAM-resistant patients with CHB is less susceptible to ETV, the probability of achieving an adequate virologic response in ETV monotherapy group is lower than ETV-TDF combination group in LAM-resistant patients with CHB.

We found that ETV-resistant mutations gradually increased and became dominant in the viral populations, expecially in the ETV monotherapy group. rtM204I/V, rtL180M, rtM250V/I/L, rtS202G were detected after an 24-month period of ETV therapy, which is consistent with a previous study that observed that ETV-resistant variants preceded by LAM-resistant variants (Deng et al. 2013). The resistant variants rtM204V and rtS202G co-existed in the viral population and were co-localized in the same viral strain, while virological breakthrough occurred at 24-month. 2 patients occurred Virologic breakthrough occurred in two patients of the ETV-TDF combination group and in 11 patients of the ETV monotherapy group. The present results were consistent with a previous report, which indicated that a new resistant variant, rtS202G, emerged within the backgrounds of rtM204V, and was accompanied by virological breakthrough (Lee et al. 2013). Those findings gave us another hint that the combination of ETV plus TDF was associated with lower antiviral resistance compared with ETV monotherapy as LAM-resistant patients who were switched to ETV monotherapy due to potential cross-resistance (Zoulim and Locarnini 2009).

In summary, our data demonstrate that ETV-TDF combination therapy is a more effective therapy than ETV monotherapy for patients with LAM-resistant. The ETV-TDF group was superior to the ETV group in achieving a virologic response. Moreover, ETV-TDF was lower than ETV group in achieving the initial viral breakthrough and genotypic mutations. The combination of ETV/TDF seems an attractive option for patients with LAM-resistance. However, additional studies comparing ETV monotherapy with ETV-TDF combination therapy that include a larger number of patients and prospective follow-up are needed to determine the optimal rescue treatment option for patients with LAM-resistance and ADV combination therapy for CHB patients with LAM-resistance.

In conclusion, ETV-TDF combination therapy might be a better regimen than ETV monotherapy in the subgroup of LAM-resistant Chinese patients with CHB.

4. Experimental

4.1. Patient characteristics

The electronic medical records of patients with CHB who had developed LAM resistance were reviewed. A total of 220 patients with CHB from July 2010 to July 2016 who had developed LAM resistance under monotherapy with LAM and subsequently took nucleos(t)ide analogs as rescue strategy for at least 24 months in our hospital. The rescue therapy regimens for all of patients were chosen based on attending physicians' own discretion and then were divided into two rescue treatment groups. Among these 220 patients, 114 patients were treated with ETV monotherapy (ETV 1 mg/day) and 106 were treated with ETV plus TDF combination therapy (TDF 300 mg/day and ETV 1 mg/day). Patients with the following characteristics were excluded: 1) patients had discontinued HBV therapy lasting for more than 7 days before initiation of study treatment, 2) coinfection of chronic hepatitis C virus (HCV) or human immunodeficiency virus (HIV), 3) other liver diseases such as autoimmune hepatitis, alcoholic liver disease, or metabolic liver disease, 4) a history of cytotoxic chemotherapy or organ transplantation. Patients were monitored by clinical examination and laboratory measurements assessment at least every 3 to 4 months during the antiviral therapy. Informed written consent for the analysis was obtained from each patient. The study was approved by the ethics committee of Renmin Hospital of Wuhan University. A flow chart for study methods is shown in Fig. 2.

4.2. Biochemical and serological markers and quantification of HBV DNA

Biochemistry was performed using standard laboratory procedures. The viral markers, including hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), HBeAg, and antibody to HBeAg (anti-HBe) were measured using commercial chemiluminescence immunoassay kits on an ARCHITECT i-20000SR automatic chemiluminescence immunoassay analyzer (Abbott Laboratories, Chicago, IL, USA). Serum HBV DNA level was determined by a popular real-time quantitative PCR (qPCR) kit (Abbott Laboratories, Chicago, IL, USA), according to the manufacturer′s instructions, with a with a lower limit of detection of of 20 IU/mL.

4.3. HBV genotype and genotypic resistance analysis

Direct PCR-based DNA sequencing was performed using the Big Dye Terminator version 3.1 Ready Reaction cycle sequencing kit and the ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) for identifying genotypic resistance. The major genotypes of HBV were identified by S-gene sequences encompassing the RT domain of HBV. HBV genotype was determined by molecular evolutionary analysis of the viral sequences using the MEGA4 software as previously reported (Lim et al. 2016).

4.4. Statistical analysis

Quantitative variables were expressed as mean±standard deviation, and categorical variables were presented as counts and percentages, and serum HBV DNA levels were logarithmically transformed for analysis. The Student's t test was used for continuous variables and chi-squared test for categorical variables. *P*-values of less than 0.05 were considered significant. Data were analyzed using SPSS, version 20.0 (SPSS, Inc., Chicago, IL, USA).

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Conflicts of interest: None declared.

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