European Review for Medical and Pharmacological Sciences 2013; 17: 636-643

Two years efficiency of lamivudine and adefovir dipivoxil combined therapy in chronic hepatitis B patients

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Abstract. – BACKGROUND: Lamivudine (LAM) and adefovir (ADV) are widely used in most Asian countries, though monotherapy is associated with the occurrence of resistance.

AIM: To evaluate the efficiency of LAM and ADV combined treatment of chronic hepatitis B patients with compensated cirrhosis.

PATIENTS AND METHODS: 206 eligible Chinese patients were randomly assigned in a 1:1 ratio to receive either LAM or ADV for the first 24 weeks. According to virologic response at 24 weeks, the patients either continued to monotherapy or switched to combined therapy for 48 weeks. After 48 weeks, all patients received LAM and ADV combined therapy for 96 weeks.

RESULTS: Serum HBV DNA levels significantly decreased in patients with ADV or LAM monotherapy and continuously reduced after the combined therapy. Serum ALT normalized rate were 88.24% and 81.37% at week 48, and 95.74% and 87.36% at week 96 in ADV and LAM group respectively, comparing to 60.78% and 56.73% in ADV and LAM groups at baseline. The accumulated virological breakthrough rate at week 48 and 96 was significantly higher in LAM group.

CONCLUSIONS: Both combination strategies were resulted in the long term virological, biochemical improvement in Chinese chronic hepatitis B patients with compensated cirrhosis.

Key Words:
Adefovior, Chronic hepatitis B, Cirrhosis, Combined therapy, Lamivudine.

Abbreviations

CHB = Chronic hepatitis B
HBV = hepatitis B virus
LAM = lamivudine
ADV = adefovir
ALT = alanine aminotransferase
ULN = upper limit of normal
VBT = virological breakthrough
YMDD = tyrosine-methionine-aspartate-aspartate
HBsAg = hepatitis B surface antigen
ITT = intention to treat population
GR = gene resistance
FPG = fasting plasma glucose
HCC = hepatocellular carcinoma
NUCs = nucleoside analogues

Introduction

Chronic hepatitis B (CHB) is a challenging liver disease which can induce several liver diseases including fulminant hepatitis, severe chronic liver disease, cirrhosis, and primary hepatocellular carcinoma. Estimated 400 million patients are actively infected with hepatitis B virus (HBV) all over the world[1]. The treatment purpose is to prevent or reverse the progression of liver injury and fibrosis through inhibition of virus replication or elimination of virus.

Interferon alpha treatment has been proved effective for CHB. While its adverse effects, such as influenza-like symptoms, anorexia, depression, and its low response rate of 20% to 40%, as well as decreased response rate of 10% for patients infected with precore-mutant strains of HBV, inspires people to develop other therapeutic agents.

Lamivudine (LAM), a cytosine analogue, and adefovir dipivoxil (ADV), an adenosine monophosphate analogue, inhibit HBV replication through suppression of HBV DNA polymerase. Both agents improve liver histology, re-

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duce fibrosis, enhance HBeAg seroconversion, normalize alanine aminotransferase (ALT) levels, and improve clinical efficacy for patients with advanced liver disease after 1 year's therapy[2-6]. As LAM is commonly used in most part of Asia due to its low cost, LAM resistance would still be a problem in the near future. Although high-dose entecavir remains active against HBV with LAM and ADV resistance, approximately 50% of patients well develop resistance to entecavir in 5 years in the presence of LAM resistance.

As a nucleoside reverse transcriptase inhibitor, LAM has been demonstrated to be effective for CHB therapy. While long term treatment is associated with frequent appearance of LAM-resistant hepatitis B mutants. The mutant rate increases from 16% to 32% after 1 year, to 38%, 53%, 66% and 69% after 2, 3, 4, and 5 years of treatment [7-10].

Statistics shows that LAM therapy in patients with LAM-resistant HBV leads to the wild-type and LAM-resistant HBV coexistence[11]. The tyrosine-methionine-aspartate-aspartate (YMDD) mutants cause the aggravation of liver disease. During continuing LAM therapy, hepatitis flare, with an elevated serum ALT which is over five times as the upper limit of normal (ULN), occurs frequently, and severe hepatitis with hepatic decompensation or even fatality may occur, especially in patients with advanced liver disease[12-14].

ADV, a pro-drug, is converted to adefovir phosphate which inhibits viral polymerases at a low concentration in plasma. When ADV incorporated into viral DNA, it inhibits viral polymerases and blocks viral replication. HBV is associated with a lower frequency of resistance to ADV than LAM. ADV has been shown to inhibit wild-type and LAM-resistant HBV strains[15]. ADV monotherapy was also reported increasing the risk of emergence of ADV-resistant mutants in patients resistant to LAM, with the rates of 21% at 15-18 months and 22% at 24 months after ADV monotherapy[16,17]. On the other hand, combination therapy of ADV and LAM was reported to reduce the rate of ADV-resistant mutations in patients resistant to LAM[16,17]. A previous open label study in HBeAg negative patients resistant to LAM demonstrated that combination therapy did not result in the development of ADV resistance over a period of 3 years, and that the undetectable rate of serum HBV-DNA was higher than ADV monotherapy[16]. However, some patients resistant to LAM show poor response to LAM plus ADV combination therapy.

This study was to evaluate the long-term efficiency and safety of LAM and ADV combination therapy in the subgroup of patients with advanced liver fibrosis or cirrhosis.

Patients and Methods

Patients

Patients enrolled in the study were 18 to 65 years, with serum hepatitis B surface antigen (HBsAg) presentation for at least 6 months, positive or negative for HBeAg, and all of them were clinical progressive liver fibrosis or compensated cirrhosis. 75 patients had a liver biopsy showing an Ishak fibrosis score of at least 4 (where O indicates no fibrosis and 6 indicates cirrhosis) at baseline and other 131 patients had clinical records of cirrhosis (with imaging or laboratory evidences).

Patients were excluded if they were as follows: the performance of liver decompensation, an elevated serum ALT level 10 times as ULN, autoimmune hepatitis, coinfection with hepatitis C or D viruses or human immunodeficiency virus, an increased level of serum creatinine, a hemoglobin level less than 8 g per deciliter, a white-cell count below 1,500 per milliliter, a platelet count of 50,000 per milliliter, treatment with any investigational drug within the 30 days before the study began, any treatment with LAM previously, with allergy history on nucleoside analogues (NUCs), and cancer, pregnant, alcoholism and serious physical and mental illness were also excluded.

Clinical Study Design

This was a prospective, multicenter, randomized, open-label study. Patients were randomly assigned in a 1:1 ratio, to receive either LAM 100 mg or ADV 10 mg for first 24 weeks. Patients with partial virological response (decline from baseline but $\geq$ 2000 IU ml^{-1}) at w24 and w36 or with virologic breakthrough (VBT) were required to add LAM or ADV (if the patients were treated with LAM initial, the ADV was added, otherwise, the LAM was added). Other patients were treated as originally planned until 48 weeks. And all patients were switched to received LAM and ADV combination therapy (in LAM group, ADV was added, and in ADV, LAM was added) from 48 weeks to 96 weeks.

Patients were assessed at baseline in 2 weeks. And they were followed up the clinical evidence of hepatic decompensation or other complications every 12 weeks. They were recorded about

adverse events, decompensated complications, and combination therapy and drug accountability. Blood test, urine test, prothrombin time, and biochemistry profiles were evaluated. Serum alpha-fetoprotein tests, and liver ultrasonography were performed every 24 weeks. HBV seromarkers were assayed with Abbott reagent at baseline and every 24 weeks. In case of seroconversion, the samples should be both HBeAb positive and HBeAg negative for at least one month.

Serum samples were collected at baseline and at weeks 12, 24, 36, 48, 72 and 96, and stored at –40°C. HBV-DNA was assayed by Amplicor Taqmen assay kits.

The study was approved by the local Ethics Committees of the respective institutions, and patients gave written informed consent before screening.

End Points

The primary end point was the HBV-DNA response, defined as the proportion of patients with undetectable HBV (HBV-DNA levels below 60 IU ml^{-1} by Amplicor TMPCR) and HBV-DNA response ratio (HBV-DNA levels below 200 IU ml^{-1}) after 48 or 96 weeks treatment. The clinical end point was the normalized rate of ALT, total bilirubin, total serum albumin, prothrombin time and the development of complications of liver decompensation such as ascites, esophageal variceal bleeding and the development of hepatocellular carcinoma.

Safety

All adverse events, regardless of their possible association with diseases or treatment, were recorded.

Statistical Analysis

All data were analyzed by WuXi AppTec Clinical Research and Regulatory Services Co. Ltd. Clinical analysis of patients was according to intention to treat population (ITT) principle. The patients with standards treatment were analyzed. And half-way loss or withdrawal was invalid. Any medication patients received were as the actual patient population for safety analysis. The numerical data of the patients were presented as mean ± standard deviation (SD) while categorical variables were presented together with frequency and percentages. The intergroup differences of numerical variables were investigated using Student's *t* test and Mann-Whitney u test, while the differences of categorical values were investigated using the chi-square test. A critical level of $p < 0.05$ was considered to indicate statistical significance. All analyses were performed by the statistical software program SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

206 patients enrolled in the study from 8 hospitals in China, were randomly assigned for treatment. 102 patients were firstly assigned to receive ADV monotherapy (ADV group), and 104 to receive LAM monotherapy (LAM group) before 48 weeks. All patients were assigned to receive ADV and LAM combination after 48 weeks. In ADV group, male patients were 79, and in LAM group male patients were 84. And all patients were well matched in terms of age, laboratory results at baseline (Table I).

Study end Points

HBV-DNA Analysis

Serum HBV-DNA of ADV and LAM groups were assayed at baseline and at weeks 12, 24, 36, 48, 72, and 96 by Amplicor Taqmen assay kits

Table I. Baseline characteristics of patients.

	ADV group (n = 102)	LAM group (n = 104)	*p* value
Age (yr)	44 ± 9.54	44.90 ± 10.03	0.7147
Gender (male/female)	80/22	84/20	1
ALT (IU ml^{-1})	72.76 ± 61.80	72.59 ± 46.37	0.5381
HBV DNA (log(10) ml^{-1})	6.23 ± 1.23	6.13 ± 1.09	0.5948
HBeAg (positive/negative)	64/38	59/45	0.3789
Serum albumin (g dl^{-1})	43.05 ± 5.38	43.31 ± 4.51	0.9896
Platelets ($\times 10^9$ L^{-1})	114.19 ± 60.47	117.09 ± 55.62	0.4825
Prothrombin time(s)	13.96 ± 2.02	13.72 ± 2.49	0.2567
Bilirumbin (mg dl^{-1})	20.37 ± 10.00	19.30 ± 8.33	0.4492

(Roche Molecular Systems, Branchburg, NJ, USA). he proportions of ADV and LAM groups with undetectable HBV (HBV-DNA levels below 60 IU ml^{-1} by Amplicor TMPCR) were 44%, 80.85% with initial ADV group and 63.73%, 69.47% with initial LAM group after 48 and 96 weeks treatment, and HBV-DNA response ratios (HBVDNA levels below 200 IU ml^{-1}) were 53.23%, 85.11% with initial ADV and 67.65%, 75.79% with initial LAM group after 48 or 96 weeks treatment (Figure 1 and Table II). Serum HBV DNA levels significantly decreased in patients with ADV and LAM monotherapy after 24 weeks. When patients were treated with ADV and LAM in combination after 48 weeks, serum HBV DNA levels were continuously reduced. Serum HBV DNA levels of treatment with ADV monotherapy decreased slower than LAM group before 48 weeks, but were lower than LAM group after 24 weeks of combination therapy.

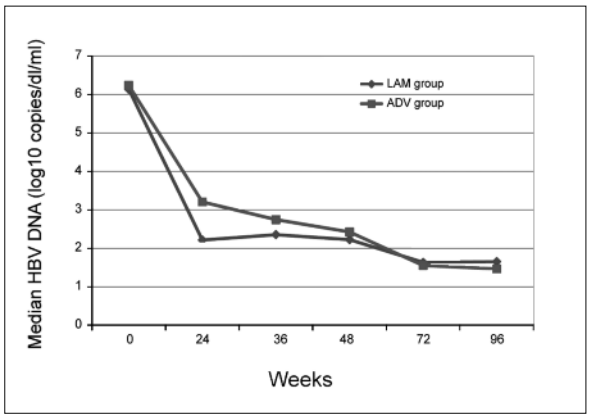

Figure 1. Median values for HBV DNA over 96 weeks of treatment in ADV group and LAM group.

HBeAg Seroconversion and other Biochemical Response

At the recommendation of the data, of the total 123 patients who were HBeAg positive at baseline, 18 (28.12%) in ADV group and 11 (18.64%) in LAM group had achieved HBeAg negative at 96 weeks. And the number of HBeAg seroconversion was 9 (14.06%) and 8 (13.56%) respectively in ADV group and LAM group. Serum ALT levels decreased over 48 weeks in patients receiving ADV and LAM monotherapy separately. And more patients had normalized serum ALT levels with ADV and LAM combination after 96 weeks. They were 88.00% (88/100) and 81.37% (83/102) after 48 weeks, and were 95.74% (90/94) and 87.36% (83/95) after 96 weeks in ADV group and LAM group when compared to 60.78% (62/102) and 56.73% (59/104) in ADV and LAM at baseline ($p < 0.001$).

Total serum albumin increased compared with baseline from 24 weeks ($p < 0.00001$). The increased margin of ADV group was 2.46 g L^{-1}, 3.37 g L^{-1} and 3.22 g L^{-1} after 24, 48 and 96 weeks respectively, and it was 2.43 g L^{-1}, 3.09 g L^{-1} and 4.32 g L^{-1} after 24, 48 and 96 weeks re-

Table II. Virologic and biochemical response in total patients.

	ADV group (n = 102)	LAM group (n = 104)
HBV undetactable (< 60 IU)		
After 48 weeks, N (%)	44/100 (44)	65/102 (63.73)
After 96 weeks, N (%)	76/94 (80.85)	66/95 (69.47)
HBV response (< 200 IU)		
After 48 weeks, N (%)	53/100 (53.23)	69/102 (67.65)
After 96 weeks, N (%)	80/94 (85.11)	72/95 (75.79)
HBV change from baseline		
After 48 weeks	-3.82	-3.89
After 96 weeks	-4.78	-4.41
HBeAg negative and seroconversion		
HBeAg negative after 96 weeks, N (%)	18/64 (28.12)	11/59 (18.64)
HBeAg seroconversion after 96 weeks, N (%)	9/64 (14.06)	8/59 (13.56)
Fraction of normal ALT normal (≤ 1.0 × ULN)		
0 week, N (%)	62/102 (60.78)	59/104 (56.73)
After 48 weeks, N (%)	88/100 (88.24)	83/102 (81.37)
After 96 weeks, N (%)	90/94 (95.74)	83/95 (87.36)
ALT × ULN change from baseline		
After 48 weeks	32.18	22.10
After 96 weeks	38.05	34.36

spectively in LAM group. There were no significant differences between the two groups. Total serum bilirubin, prothrombin time and blood platelet count were neither not significantly changed (Figure 2).

VBT Status

VBT was defined as any increase in serum HBV DNA by > 1 log10 from nadir or redetection of serum HBV DNA at levels 10-fold the lower limit of detection of the HBV DNA assay after having an undetectable result. The accumulative number happened in ADV group was 2 (2.02%), 3 (3.06%), 5 (5.0%) and 8 (8.5%) after 24, 36, 48 and 96 weeks, and it was 5 (5.0%), 16 (16%), 21 (20.58%) and 24 (25%) in LAM group (Table III). But there were no ALT changes and other clinical symptoms. The rate of VBT in LAM group was higher than in ADV group ($p = 0.02$), but dropped after 48 weeks as well (Table III).

Genotypic Mutations

We detected serum HBV DNA genes in 14 patients (LAM group: 11; ADV group: 3) whom appeared VBT during treatment and simultaneously still presented partial response after 96 weeks. In the 14 patients, 5 had gene resistance (GR) in baseline, 5 were remain wild type, only 4 (28.57%) appeared M204V/I mutation toward drug resistance during therapy; there were 3 cases of LAM and 1 case of ADV in patients with VBT companioned genetic drug resistance (Table IV)

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HBV-Related Cirrhosis Complication

There were complications of cirrhosis during therapy. Five had liver ascites (ADV: 2, LAM: 3) and two experienced variceal bleeding (ADV: 1, LAM: 1). All happened within 48 weeks. And one had mild hepatic encephalopathy (ADV group) after taking excess meats at 24 week. Hepatocellular carcinoma had been diagnosed in 8 patients (ADV: 4, LAM: 4).

Safety and Adverse Events

In the present study, 206 patients were enrolled in accord with inclusion criteria. Among them, 189 patients completed both 96-week treatment and the follow-up, 9 were not measured with serum standards after 96 weeks and subsequently restored follow-up, 2 went abroad, 1 died of esophageal varices hemorrhage, 3 turned to the surgery after liver cancer occurred, and 2 were loss of visit.

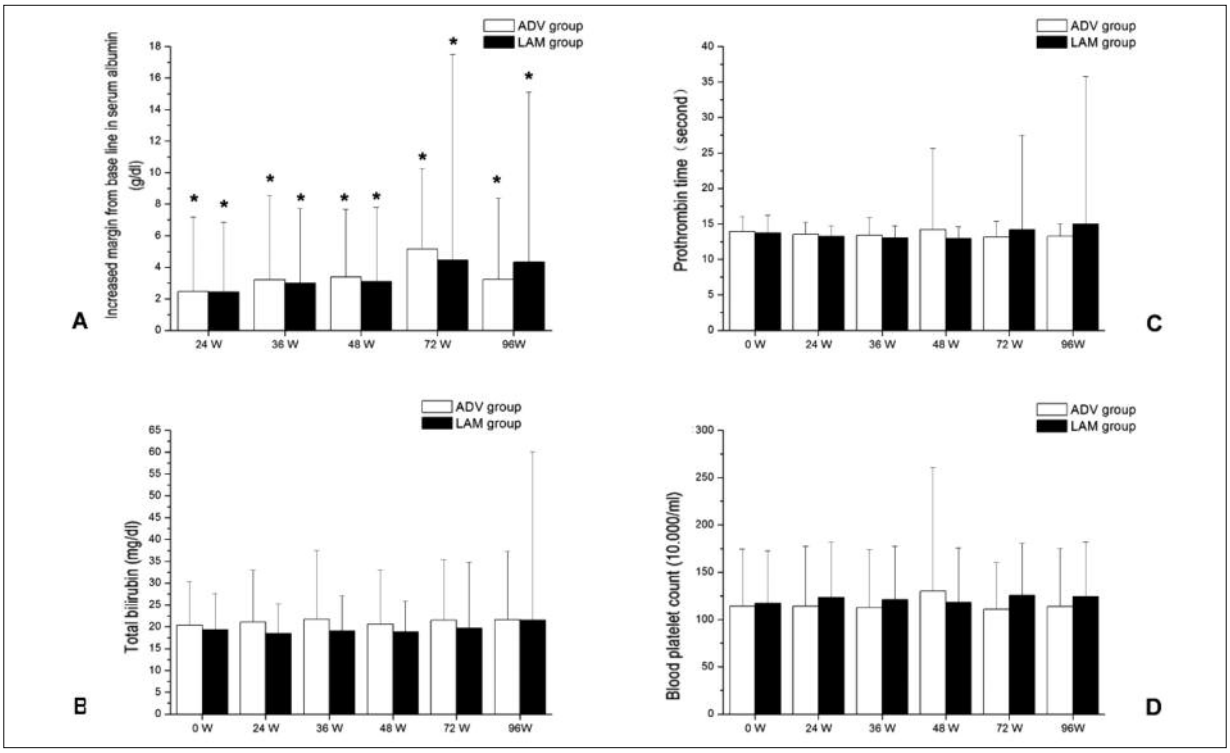

Figure 2. ***A,*** Increased margin from baseline in serum albumin of ADV and LAM groups. *Compared with baseline: $p < 0.00001$. ***B***, Total serum bilirubin (mg ml^{-1}). ***C,*** Prothrombin time (second). ***D,*** Blood platelet count (10,000 ml^{-1}).

Table III. The statistics of VBT in ADV and LAM groups.

Group	Time (weeks)	Number change of cases	Total number of cases	n (%)	*p* value
ADV	24	2	2	99 (2.02)	
	36	1	3	98 (3.06)	
	48	2	5	100 (5.0)	0.02*
	96	3	8	94 (8.5)	0.02*
LAM	24	5	5	100 (5)	
	36	11	16	100 (16)	
	48	5	21	102 (20.58)	
	96	3	24	95 (25)	
Total	24	7	7		
	36	12	19		
	48	7	26		
	96	6	32		

*Compared with LAM group.

On adverse events and reactions during therapy, there were 3 (1.4%) nausea and 2 (0.9%) elevated fasting plasma glucose (FPG) after the first year. There were 4 (1.9%) elevated blood pressure and 1 (0.49%) dizziness after the second year.

Discussion

This study showed that the two different strategies of ADV and LAM combined therapy were effective and safe for Chinese CHB patients with compensated cirrhosis. Serum HNV DNA level significantly decreased in patients with ADV or LAM monotherapy and continuously reduced after combined therapy. Initial ADV combined therapy may have benefit in decreasing VBT over initial LAM combined therapy.

Table IV. Serum HBV resistance testing and baseline resistance genes comparison in patients with poor virological response after 96 weeks.

No. (group)	Sequencing (0 week)	Sequencing (96 weeks)
128 (LAM)	A181V	M204M/V
108 (LAM)	Wild strain	Wild strain
113 (LAM)	Wild type	M204I
120 (ADV)	L180M, M204V	L180M, M204V
073 (LAM)	Wild type	M204
163 (LAM)	Wild strain	Wild strain
164 (LAM)	N236T	N236T
167 (LAM)	M204I	L180L/M
168 (ADV)	Wild type	M204M/I
312 (LAM)	Wild strain	Wild type
221 (ADV)	Wild strain	Wild type
253 (LAM)	Wild strain	Wild type
351 (LAM)	Wild type	L180M, M204V
369 (LAM)	A181T	A181T, M250V

The proportion of patients to achieve HBeAg seroconversion after 12 months of LAM treatment has been reported range between 16% and 18% in Western and Asian studies[18-20]. Our study did not appear superior to these reports in HBeAg loss and seroconversion. Serial studies have shown that pre-treatment high serum ALT and low serum HBV-DNA level are independently associated with the increased rate of HBeAg loss and seroconversion in treatment with LAM. More patients in our studies have normal level of ALT at baseline. Meanwhile, biochemical responses were observed. The liver function was improved after combined therapy even if the patient's ALT was normal at baseline, and the improvement continued over the next 2 years.

Previous papers have reported that LAM treatment increased albumin level 0.185 dl^{-1} per year and decreased Child-Pugh score in 69% of patients; nucleoside analog treatment, in the case LAM plus ADV, was the most beneficial and significant for cirrhosis[21,22]. Some of the patients with LAM monotherapy developed hepatic failure and died after the emergence of VBT. In the present study, no such cases appeared. The increased albumin were 3.22 g L^{-1} and 4.32 g L^{-1} after 96 weeks in ADV and LAM group. It indicated that the LAM-ADV combination may prevent the occurrence in hepatic deterioration after VBT when the patients were treated by LAM monotherapy. Also in the present study, 8 cases occurred complication of cirrhosis bur all happened within 48 weeks and never fined in next 48 weeks. We experienced 8 cases of development of hepatocellular carcinoma (HCC). The occurrence of HCC was probably be-

cause the patients were in an advanced stage of liver disease. The risk of HCC increases with the progression of liver disease, male sex, high viral load and positivity of HBeAg and HBV genotypes C or B. LAM plus ADV may reduce the risk of hepatocarcinogenesis by decreasing the viral load and by liver disease progression to cirrhosis.

Phase-III clinical trials of NUCs in NUC-naive patients revealed that 0%-87.5% of patients with VBT were confirmed to be GR [22]. In phase-III trial of telbivudine versus LAM, 32 of 680 (4.7%) and 99 of 687 (14.4%) patients who received telbivudine and LAM respectively, experienced VBT after 1 year of treatment, but only 28 (87.5%) and 75 (75.8%) patients with VBT were confirmed to be GR. The present study also suggests that not all VBTs are related to antiviral drug resistance. The possible reasons to the discrepancy between the rates of VBT and GR include: poor adherence to medications, failure to detect drug-resistant mutations due to insensitive assays, and failure to recognize new mutations associated with the antiviral drug resistance[23].

Nucleoside analogues can sustainably inhibit HBV replication, slower disease progression and prevent long-term cirrhosis complications such as HCC. But selective drug-resistant HBV mutants (such as YMDD) caused by long-term treatment with antiviral drugs will reduce the efficacy of clinical therapy as reported in LAM treatment[21,24,25]. The most effective strategy for avoiding the emergency of drug resistance was to inhibit viral replication rapidly. New generations of nucleoside analogues have been available to reduce drug-resistance. Some reports have shown that when HBeAg positive patients were treated with Entecavir for 4 years, more than 90% of HBV DNA was undetectable, and the drug-resistant was underneath 1%. Unfortunately, the seroconversion ratio was not better than LAM, and HBsAg negative was just 5% after 2 years therapy[26].

Another treatment strategy suggests that combination therapy is likely to improve clinical efficacy for the emergence of drug-resistant HBV strains, and this benefit has been verified by longer-term studies. This strategy may be more important for patients with cirrhosis, patients with more advanced CHB including those to take transplantation, patients with immunosuppression (post-liver transplantation and HIV/HBV co-infection), and patients with HBeAg-negative CHB[27-29]. The patients in the present study suffered hepatitis B related cirrhosis and need long-term treatment and careful monitoring of drug resistance and relapse. Combined therapy should be the potential strategy to prevent the emergence of drug resistance. In the present study, ADV or LAM was used initially and then another nucleoside analogue for combination therapy was added according to virological response after 24-48 weeks. This strategy inhibited the replication of hepatitis B virus rapidly, reduced the appearance of drug-resistant mutants, decreased the complication of cirrhosis, slowed down the progress of disease, and improved the liver reserve function such as improvement of serum albumin.

In conclusion, the two different combination strategies were of well compliance and resulted in the long-term virologic, biochemical improvement, with substantially prevented disease progression in Chinese CHB patients with compensated cirrhosis. Comparing ADV and LAM groups, taking LAM first could inhibit viruses rapidly, but there was no superiority after 48 weeks. Taking defovir dipivoxil first had less VBT. All these might bring us a good protocol to reduce VBT and biochemical breakout, and prevent further destruction of liver cirrhosis.

Conclusions

Both combination strategies were resulted in the long term virological, biochemical improvement in Chinese chronic hepatitis B patients with compensated cirrhosis.

Acknowledgements

The study was supported by key medical specialties fund (05II011 2-1) of Shanghai Municipal Health Bureau. The drugs medicated were provided by the Glaxo SmithKline (China) Investment Co. Ltd.

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