Abstract. - BACKGROUND: Either combination treatment or monotherapy using agents with a high genetic barrier are recommended for the retreatment of chronic hepatitis B (CHB) patients. In search of reasonable treatment, we compared the efficacy and safety of lamivudine (LAM) plus adefovir (ADV) and entecavir (ETV) alone for retreatment of patients with viral relapse after cessation of ADV.

PATIENTS AND METHODS: This is a prospective controlled study, and CHB patients with HBV DNA levels more than 4 log copies/mL were enrolled. All patients were treated with either LAM plus ADV (n=30) or ETV (n=25) for 48 weeks.

RESULTS: After 12-months treatment, the biochemical response (BR) rates were 96.7% and 84.0%, the virological response (VR) rates were 96.7% and 68% in the LAM plus ADV and ETV groups respectively. Between two groups, the difference in BR was not significant, but in VR was statistically significant (p = 0.097 for BR, and p = 0.003 for VR). Eleven patients receiving LAM plus ADV had HBeAg seroconversion, as compared with 1 in patients receiving ETV alone (36.7% versus 4%, p = 0.003). During 12-months retreatment, 1 patients receiving ETV alone had virological breakthrough and detected ETV-resistance strains, while no LAM- or ADV-associated resistance strains were detected in patients receiving LAM plus ADV. All patients receiving LAM plus ADV were well tolerated, and no serious side effects were reported.

CONCLUSIONS: LAM plus ADV combination therapy is effective in retreatment of CHB patients with viral relapse after cessation of ADV, but further studies are needed to obtain long term results.

Key Words: Chronic hepatitis B, Viral relapse, Retreatment, Combination therapy, Monotherapy, Lamivudine plus adefovir, Entecavir.

Introduction

Estimated more than 350 million individuals worldwide are chronically infected with hepatitis B virus (HBV), and chronic hepatitis B (CHB) can progress to end-stage complications including cirrhosis, hepatocellular carcinoma (HCC), and liver failure. Antiviral therapy is widely applied in CHB to minimize the liver damage and progression of disease. At present, nucleos(t)ide analogs approved for use in the treatment of CHB include lamivudine (LAM), adefovir (ADV), entecavir (ETV), telbivudine and tenofovir, and they have been considered to have a good effect in suppressing virus replication with few side effects. As we know, nucleos(t)ide analogs interfere with the elongation of viral DNA chains through competitive inhibition with the viral polymerase, so total eradication of the HBV is seldom achieved by current agents. Evidence had showed that long-term durability of viral response is difficult, and viral relapse would occur frequently in majority of CHB patients if antiviral treatment was terminated too early.

Some investigators had reported that viral relapse was common in CHB patients after cessation of nucleos(t)ide analogs and the relapse rates would be high to 70%. A wealth of clinical experience from us also indicated that the situation of viral relapse after drug withdrawal could not be ignored. Due to the poverty, low level of medical technology, and availability of antiviral agents, many CHB patients in Asia cannot afford their reasonable long-term medication, and they always terminated their treatment just when reaching virological response, irrespective of the occurrence of hepatitis B e antigen (HBeAg) or hepatitis B surface antigen (HBsAg) seroconversion. In China, ADV was widely used in treatment of CHB, and viral relapse was common among those patients who received ADV. How to retreat those CHB patients with viral relapse after cessation of ADV has become an urgent clinical problem that we have to face. Unfortunately, there is no consensus on the retreatment of viral relapse.
without resistance after cessation of ADV treatment. Either combination treatment or monotherapy using agents with a high genetic barrier and no cross-resistance are recommended for the retreatment of chronic hepatitis B (CHB) patients.

The purpose of this study was to compare the efficacy and safety of lamivudine (LAM) plus ADV with that of ETV alone in retreatment of CHB patients with viral relapse after cessation of previous ADV treatment.

Patients and Methods

Patients

Outpatients from No. 416 Hospital of Nuclear Industry Ministry, with viral relapse after cessation of ADV treatment were screened, of whom included in this study should also meet the following criteria: serum hepatitis B e antigen (HBeAg) positive; serum HBV DNA load above 4 log copies/mL; alanine aminotransferase (ALT) levels between 2 and 10 times the upper normal level. Patients who fulfilled one of the following criteria should be excluded: presence of serum antibodies against hepatitis C virus (HCV), or human immunodeficiency virus (HIV); breast-feeding, pregnancy or inadequate contraceptive measures; other acquired or inherited causes of liver diseases; coexisting serious medical diseases; advanced liver diseases (including decompensated cirrhosis with ascites, severe hepatitis, and hepatic carcinoma).

Study Design

The study was designed as a prospective case-control study. The patients were distributed to LAM plus ADV combination therapy group and ETV monotherapy group according to patients’ choice. Baseline data were compared between two groups to ensure comparability. Patients in the combination therapy group were prescribed LAM 100 mg and ADV 10 mg per day (GlaxoSmithKline, Shanghai, China) while the monotherapy group received ETV (Bristol-Myers Squibb, Shanghai, China) 0.5 mg per day.

Patients were followed up in Outpatient Clinic of Hospital. Clinical data were collected at baseline, and every 3 months after retreatment. The primary efficacy outcomes were ALT normalization rate, HBV DNA undetectable rate, and seroconversion of HBeAg. Second efficacy outcome was antiviral resistance.

This study was conducted in accordance with the 1975 Declaration of Helsinki.

Serum Assay Methodology

HBeAg and serum HBV DNA were measured with the use of Enzyme-Linked Immunosorbent Assay (InTec Technology Co., Ltd., Xiamen, China) and real-time PCR-based quantification assays (DA AN gene Co., Ltd., Guangzhou, China) according to the manufacturer’s instructions, respectively. Serum ALT and creatinine (Cr) were measured using automatic biochemistry analyzer (Olympus AU400, Tokyo, Japan) according to standard laboratory procedures. LAM-, ADV-, and ETV-associated mutations were assessed for patients with HBV DNA no less than 3 log copies/mL after 12-month antiviral treatment via direct sequencing.

Definition

Biochemical response (BR) was defined as a decrease in serum ALT to within the normal range. Virological response (VR) was defined as a decrease in serum HBV DNA to undetectable levels by PCR assays (<3 log copies/mL). HBeAg response was defined as a loss or seroconversion of HBeAg in patients who were initially HBeAg positive. Virological breakthrough was defined as an increase in serum HBV DNA by 1 log10 (10-fold) above nadir, or to detectable level (≥3 log copies/mL) after achieving virological response during retreatment. Viral relapse was defined as an increase in serum HBV DNA to detectable level after achieving virological response.

Statistical Analysis

Quantitative variables were expressed as mean and standard deviation, and categorical variables were presented as counts and percentages, and HBV DNA levels were presented as log transformation. Comparisons between groups of quantitative and qualitative variables were performed using the Student’s t test and Chi-square test (or Fisher’s exact test), respectively. A p-value of less than 0.05 (two-tailed) was considered to indicate a significant difference. All statistical analyses were performed using the SPSS software package version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the Study Patients

As described in Figure 1, a total of 55 HBeAg-positive CHB patients were analyzed in the study, which comprised of 30 patients in LAM plus ADV combination group and 25 patients in
ETV monotherapy group. The demographic and disease parameters were well matched at baseline of retreatment between two groups, which were described in detail in Table I.

**Biochemical Response**

Of the 30 patients receiving LAM plus ADV, there were 14, 20, 28, and 29 patients who achieved ALT normalization by months 3, 6, 9, and 12, respectively. Corresponding BR rates were 46.7%, 66.7%, 93.3%, and 96.7%. Of the 25 patients receiving ETV, there were 9, 14, 21, and 21 patients who achieved ALT normalization by months 3, 6, 9, and 12, respectively, and corresponding BR rates were 36.0%, 56.0%, 84.0%, and 84.0%. The BR rates of ETV were higher than those in the LAM+ADV group by months 3, 6, 9 and 12, respectively, but these differences were not statistically significant ($p = 0.425, 0.418, 0.268, 0.097$ for months 3, 6, 9, and 12, respectively).

**Virological Response**

The reduction in serum HBV DNA levels from baseline of retreatment during the first year of observation period was greater in LAM plus ADV than in ETV alone retreatment. Of the 30 patients in LAM plus ADV group, there were 19, 25, 27 and 29 patients who achieved undetectable HBV DNA by months 3, 6, 9, and 12 respectively, and the corresponding VRs were 63.3%, 83.3%, 90.0%, and 96.7%. Of the 25 patients in the ETV group, 10, 13, 16, and 17 patients achieved undetectable HBV DNA by months 3, 6, 9, and 12, respectively, and the corresponding VRs were 40.0%, 52.0%, 64.0%, and 68.0%. The VR rates of LAM plus ADV combination group were higher than those in the ETV monotherapy group by months 3, 6, 9 and 12, respectively, and these differences were statistically significant with the extension of treatment ($p = 0.084, 0.012, 0.020, 0.003$ for months 3, 6, 9, and 12, respectively).

![Flow chart of patients selected in this study.](image)
**HBeAg Response**

More patients receiving LAM plus ADV retreatment had HBeAg seroconversion as compared to patients receiving ETV alone retreatment at month 12, and the difference in HBeAg seroconversion between two groups was significant (11/30 versus 1/25, \( p = 0.003 \)). Additionally, significant more patient receiving LAM plus ADV obtained HBeAg loss as compared to patients receiving ETV alone at month 12 (14/30 versus 2/25, \( p = 0.002 \)).

**Breakthrough and Resistance**

Viral breakthrough was found in 1 patients receiving ETV alone retreatment during the 1-year observation period, and the mutations of rtM204V/I+rtL180M+rtM250V were found in this patients at month 12 of retreatment. In LAM plus ADV retreatment patients, no viral breakthrough were found and no LAM or ADV associated mutations were detected.

**Safety**

Majority of patients were well tolerated in two retreatment groups. Among patients receiving LAM plus ADV, no patient had serum creatinine increasing. ALT flares during the first year of treatment were in 6 patients with ETV alone and 3 patients with LAM plus ADV. In LAM plus ADV combination group, ALT flares in two patients were associated with toil and in one patient was associated with HBV DNA flares; while in ETV alone retreatment group, all the ALT flares were associated with HBV DNA flares. Between two groups, none hepatocellular carcinoma was reported.

**Discussion**

Although ADV is not recommended as first-line therapy for CHB in United States and European countries\(^6,13\), it is still widely used in most Asian countries due to cost constraints\(^10\). Recently, evidence suggests that induction of HBeAg seroconversion by nucleos(t)ide analogues is temporary in most CHB patients\(^14\). Thus, it is necessary for us to concern about the re-treatment issues of patients with viral relapse after discontinuation of ADV monotherapy. Unfortunately, the retreatment options for viral relapse are still controversial. Currently, a variety of retreatment strategies have been used, including back to their original agent, switch to another agent without cross-resistance, or two agents in combination. Theoretically, if there were no evidence of antiviral resistance, it seemed reasonable to switch to agent without cross-resistance for retreatment. However, this therapeutic strategy for rescue failure treatment was recently questioned by clinicians.

In this work, we explored and compared retreatment options for chronic hepatitis B patients with viral relapse after cessation of ADV. After 12 months retreatment, the proportion of undetectable HBV DNA was achieved in 93.3% of patients receiving LAM plus ADV as compared to 80% of patients receiving ETV alone. Moreover, higher proportion of ALT normalization was also reached in LAM plus ADV retreatment group. Several other studies also had reported that the combination of LAM and ADV could lead to effective viral suppression in most cases after development of viral breakthrough due to LAM or ADV monotherapy\(^12,15\); and patients receiving LAM plus ADV combination therapy have a lower risk of developing genotypic resistance to ADV\(^15\). In our study, patients receiving combination therapy of LAM and ADV were well tolerated, and no viral breakthrough was reported and no LAM- or ADV-associated resistant strains were detected. In contrast, among 25 patients receiving ETV alone, 1 patient experienced a viral breakthrough and ETV-associated resistant strains were detected. Those findings suggested that the combination of LAM plus ADV

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LAM plus ADV (N = 30)</th>
<th>ETV (N = 25)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex –no. (%)</td>
<td>22 (73.3%)</td>
<td>19 (76%)</td>
<td>0.821</td>
</tr>
<tr>
<td>Age-yr*</td>
<td>37.9 ± 8.9</td>
<td>34.6 ± 7.5</td>
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<tr>
<td>ALT-IU/L*</td>
<td>129.4 ± 22.8</td>
<td>132.9 ± 31.3</td>
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<td>HBV DNA-log10 copies/ml*</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>0.407</td>
</tr>
<tr>
<td>Previous ADV therapy-yr*</td>
<td>3.3 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>0.421</td>
</tr>
</tbody>
</table>

\*Expressed as mean ± Standard deviation.
was superior to ETV alone in retreatment of patients with failure therapy of ADV. In fact, the combination of agents without cross-resistance had been put forward by authoritative guidelines in management of CHB with poor response to antiviral therapy, and data from many clinical observational trials also suggested combination therapy would bring more benefits to nucleos(t)ide analogs refractory CHB patients.

At present, majority of clinical investigations on LAM plus ADV combination therapy were for HBeAg-negative patients, and few studies on HBeAg-positive patients reported the combination of these agents had no synergistic on HBeAg/Anti-HBe seroconversion. But in present study, among 30 patients receiving combination therapy of LAM plus ADV, we delighted to found that 14 patients obtained HBeAg loss and 11 patients obtained HBeAg seroconversion, which were significant higher to that of patients receiving ETV alone. However, because of the limited sample size, we currently cannot conclude that the combination of ADV plus LAM could increase the rate of HBeAg/Anti-HBe seroconversion as compared to ETV alone.

As we know, the primary end point for HBeAg-positive CHB is HBeAg/Anti-HBe seroconversion, so the follow-up of 12 months is too short. In fact, all our patients are still receiving antiviral therapy and further study is necessary to confirm current results. With the gradual extension of treatment course, not only effect but also cost should be evaluated. In present study, the cost-effectiveness analysis was not done because of relative short-term therapy. So it is valuable to carry on further long-term study to compare cost-effectiveness ratio of the two different treatment strategies.

Conclusions

CHB patients with viral relapse after cessation of ADV retreated by LAM plus ADV exhibited significantly greater virological and serological responses compared with ETV alone. These findings indicated that combination of LAM plus ADV may be a good option for the retreatment of CHB patients with viral relapse after cessation of ADV.

Acknowledgements

We thank all patients who participated in this study.

Conflict of Interest

None declared.

References