Background: There is no standard management of chronic hepatitis B (CHB) patients with suboptimal response to nucleoside/nucleotide analogues (NAs). This study aimed to evaluate two different NA combination therapies in patients with suboptimal response to adefovir (ADV).

Methods: In this study, 72 CHB patients with suboptimal response to ADV were assessed, with 37 patients receiving lamivudine plus ADV (group A) and 35 patients receiving telbivudine plus ADV (group B).

Results: Baseline characteristics between two groups were similar. At month 12, rates of biochemical response (BR) and virological response (VR) were similar between groups A and B (17/19 versus 18/20 for BR, \[P=0.269\] and 30/37 versus 31/35 for VR \[P=0.377\]), and cumulative rates of serological response were greater in group B than in group A (10/26 versus 2/28 in hepatitis B e antigen (HBeAg) loss \[P=0.006\] and 7/26 versus 1/28 in HBeAg/antibody (HBeAg) seroconversion \[P=0.022\]). After 12-month treatment, 8.1% (3/37) of patients in group A and 5.7% (2/35) of patients in group B had VR; among patients in group A, two had rtM204V/I and rtL180M and one had rtN236T, whereas the two patients in group B had rtM204I+rtL180M.

Conclusions: Both combination therapies led to a significant decrease in HBV DNA. HBeAg serological outcomes were higher with telbivudine plus ADV combination therapy.

Introduction

Approximately one-quarter of the world’s population, >2 billion people, have been infected with HBV, including 350 million patients with chronic hepatitis B (CHB) [1]. According to a report from the World Health Organization, the majority of CHB patients live in Asia-Pacific, sub-Saharan Africa, and other developing regions; one-third of them reside in China. Recent studies have shown that as many as 20% of CHB patients will progress to life-threatening liver disease including cirrhosis and hepatocellular carcinoma [2].

Entecavir and tenofovir, highly potent nucleoside/nucleotide analogues (NAs) with high barriers to resistance, are recommended as first-line treatment options by the practice guidelines of EASL and AASLD [2,3]. However, tenofovir remains largely unavailable in developing countries; for example, it will not be available in China for the next 2–3 years [4,3]. Currently, less potent agents such as adefovir (ADV) are still widely used for the treatment of CHB in developing countries, because of considerable effectiveness, lower resistance and affordable price [5,6].

As we know, total eradication of HBV is seldom achieved by current treatment [7–9], thus long-term NA oral therapy appears to be necessary in a majority of patients [2,3,5,6]. At present, in addition to drug resistance [10,11], suboptimal response to NAs has also become a new challenge for the management of CHB patients [12–14]. Of CHB patients receiving initial ADV therapy, 37% of hepatitis B e antigen (HBeAg)-negative patients and 87% of HBeAg-positive patients showed suboptimal response at week 48 [15]. Evidence indicated that the clinical relevance of suboptimal response to NA therapy would relate to the high risk of developing resistance to long-term antiviral treatment [2]. Some studies reported that among HBeAg-negative patients with HBV DNA >3 log_{10} copies/ml at week 48, 49% would develop ADV resistance at year 4 [16]. The roadmap for management of CHB patients receiving NA therapy has suggested optimizing adaptation at an
early stage in case of suboptimal suppression of HBV DNA levels [17], so as to effectively suppress viral replication and significantly delay or prevent drug resistance, in particular when NAs with a low barrier to resistance are used [16,18–20].

It has been reported that switch to entecavir monotherapy dosed at 1 mg resulted in a slow reduction of serum HBV DNA in HBeAg-positive patients with suboptimal response to ADV therapy [21]. Indeed, switch to another drug is not currently recommended for patients with suboptimal response to NA therapy [2,3]; by contrast, combination therapy offers a potentially attractive therapeutic option. However, there is no standard optimization strategy for management of suboptimal response to NA therapy at present.

In search of a reasonable optimization strategy, we compared the virological, biochemical and serological responses to the combination of lamivudine (3TC) and ADV with telbivudine (LdT) and ADV in patients who showed suboptimal response to ADV monotherapy.

Methods

Study subjects
A total of 109 adult CHB patients, who had serum HBV DNA levels >3 log10 copies/ml by PCR assay after ≥12 months of initial antiviral treatment with ADV, regardless of HBeAg status and serum alanine aminotransferase (ALT) levels, were screened between December 2007 and January 2010. All patients who fulfilled one of the following criteria were excluded: evidence of ADV-associated resistance (rtA181V or rtN236T), presence of serum antibodies against HCV or HIV, breastfeeding, pregnancy or inadequate contraceptive measures, other acquired or inherited causes of liver disease, coexisting serious medical disease, and advanced liver diseases (including decompensated cirrhosis, severe hepatitis and hepatic carcinoma).

A total of 72 patients were eventually included and analysed in this study (Figure 1). Of these, 37 patients were treated with a combination of 3TC (100 mg/day) and ADV (10 mg/day; 3TC+ADV group) and 35 were treated with LdT (600 mg/day) and ADV (10 mg/day) (LdT+ADV group) according to individual choice, for ≥12 months.

Study design
This was a prospective controlled study (ChiCTR.org identifier ChiCTR-TNC-11001495), aimed to evaluate and compare the efficacy of 3TC+ADV with that of LdT+ADV treatment for patients with suboptimal response to initial ADV monotherapy. All patients were followed-up every 3 months by clinical examination and biochemical and virological assessments.
Adherence to treatment was assessed during each visit to the clinic.

The primary efficacy outcomes were biochemical, virological and serological responses. Secondary efficacy outcomes were antiviral resistance and safety.

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

Serum assays
Routine biochemical tests were performed by standard procedures every 3 months during optimization therapy (Olympus AU5400, Olympus Corporation, Tokyo, Japan), and the upper limit of normal (ULN) for ALT was defined as 55 IU/l for men and 38 IU/l for women. Serological markers, including hepatitis B surface antigen, HBeAg and hepatitis B e antibody (anti-HBe), were tested by electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Indianapolis, IN, USA). Serum HBV DNA levels were quantified using a commercially available real-time PCR assay (Da An Gene Co., Ltd., Guangzhou, China), according to the manufacturer’s instructions. Genotypic resistance to 3TC and ADV were determined by direct sequencing if virological breakthrough was observed.

Definitions
Suboptimal response to ADV was arbitrarily defined as serum HBV DNA>3 log$_{10}$ copies/ml by PCR assays after ≥12 months of ADV treatment, regardless of HBeAg status and serum ALT levels. Biochemical response was defined as normalization of ALT. Virological response was defined as a disappearance of serum HBV DNA or fall to undetectable levels by PCR assay (<3 log$_{10}$ copies/ml). Serological response was defined as either confirmed loss of HBeAg or as confirmed seroconversion of HBeAg to anti-HBe in patients with HBeAg-positive CHB. Virological breakthrough was defined as an increase in serum HBV DNA by 1 log$_{10}$ above nadir on two consecutive occasions ≥1 month apart after achieving an initial response, or 10× the lower limit of detection after achieving undetectable HBV DNA.

Statistical analysis
Quantitative variables were expressed as means, categorical variables were presented as numbers and percentages, and HBV DNA levels were presented as log transformation. Comparisons between groups of quantitative variables were performed using the Student’s t-test, Mann-Whitney U test or analysis of variance, and comparisons of categorical variables were performed using the χ² test or Fisher’s exact test. Statistical analysis was carried using the SPSS software package version 12.0 (SPSS Inc., Chicago, IL, USA). A P-value of <0.05 (two-tailed) was considered to indicate a significant difference.

Results
Baseline characteristics
The baseline characteristics of the 3TC+ADV (n=37) and LdT+ADV (n=35) groups are shown in Table 1, and include 28 HBeAg-positive CHB patients in the 3TC+ADV group and 26 HBeAg-positive CHB patients in the LdT+ADV group. Demographic and laboratory characteristics were similar for the two groups (P>0.05), and mean (sd) serum HBV DNA levels in the 3TC+ADV and LdT+ADV groups were 4.25±0.62 log$_{10}$ copies/ml and 4.07±0.62 log$_{10}$ copies/ml, respectively (P=0.154).

Virological response
As shown in Figure 2A, the mean decrease of serum HBV DNA was similar between two groups during

---

Table 1. Comparison of baseline characteristics of patients in the two groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>3TC+ADV (n=37)</th>
<th>LdT+ADV (n=35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>37</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>33 (21-49)</td>
<td>36 (22-61)</td>
<td>0.108</td>
</tr>
<tr>
<td>Male/female patients, n</td>
<td>19/18</td>
<td>25/10</td>
<td>0.081</td>
</tr>
<tr>
<td>Median body weight, kg (range)</td>
<td>55 (39-81)</td>
<td>56 (46-91)</td>
<td>0.261</td>
</tr>
<tr>
<td>Median duration of disease, years (range)</td>
<td>10 (2-17)</td>
<td>10 (3-20)</td>
<td>0.532</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>18 (48.6)</td>
<td>21 (60)</td>
<td>0.334</td>
</tr>
<tr>
<td>Median duration of previous ADV therapy, months (range)</td>
<td>15 (12-36)</td>
<td>15 (12-30)</td>
<td>0.815</td>
</tr>
<tr>
<td>Alanine aminotransferase, xULN (range)</td>
<td>0.95 (0.36-2.33)</td>
<td>0.8 (0.29-2.53)</td>
<td>0.106</td>
</tr>
<tr>
<td>Normalized, n (%)</td>
<td>12 (32.4)</td>
<td>15 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Elevated, n (%)</td>
<td>25 (67.6)</td>
<td>20 (57.1)</td>
<td>0.901</td>
</tr>
<tr>
<td>HBeAg-positivity, n (%)</td>
<td>28 (75.7)</td>
<td>26 (74.3)</td>
<td>0.892</td>
</tr>
<tr>
<td>Mean HBV DNA level, log$_{10}$ copies/ml (so)</td>
<td>4.25 (0.48)</td>
<td>4.07 (0.62)</td>
<td>0.154</td>
</tr>
<tr>
<td>Compensated cirrhosis, n (%)</td>
<td>3 (8.1)</td>
<td>1 (2.9)</td>
<td>0.331</td>
</tr>
</tbody>
</table>

*From the first diagnosis of chronic hepatitis B to the present. †Family history of hepatitis B. ADV, adefovir; HBeAg, hepatitis B e antigen; LdT, telbivudine; ULN, upper limit of normal; 3TC, lamivudine.
the 12-month combination therapy. Among patients in both groups, there was a trend toward increasing virological response with time since the implementation of combination therapy. A higher proportion of patients in the LdT+ADV group achieved virological response at month 3 (18/35 [51.4%] versus 14/37 [37.8%]; $P=0.246$), month 9 (31/35 [88.6%] versus 30/37 [81.1%]; $P=0.377$) and month 12 (31/35 [88.6%] versus 30/37 [81.1%]; $P=0.377$) than did patients in the 3TC+ADV group, respectively (Figure 2B). However, the difference between two groups was not statistically significant.

Biochemical response

The difference in mean reduction of ALT levels from baseline over 12 months of treatment did not differ significantly between 3TC+ADV and LdT+ADV groups ($0.24 \times \text{ULN}$ versus $0.28 \times \text{ULN}; P=0.784$). As shown in Figure 3, among patients with elevated ALT at baseline, the proportions of patients achieving normalization of ALT at month 12 in the 3TC+ADV and LdT+ADV groups were 76% (19/25) and 90% (18/20), respectively, and the difference was not significant between the two groups ($P=0.269$).

Serological response

Among patients who were HBeAg-positive at baseline, a greater proportion of patients in the LdT+ADV group showed HBeAg loss (10/26 [38.5%] versus 2/28 [7.1%]; $P=0.006$) and HBeAg/anti-HBe seroconversion at month 12 (7/26 [26.9%] versus 1/28 [3.6%]; $P=0.022$) than did patients in the 3TC+ADV group; the differences between the two groups were statistically significant (Figure 4).

Virological breakthrough and resistance

In total, 5 patients experienced virological breakthrough during 12 months of combination therapy, 3 of 37 patients (8.1%) in the 3TC+ADV group and 2 of 35 (5.7%) in the LdT+ADV group. Genotypic analysis was performed on those 5 patients. Among patients in the 3TC+ADV group, two patients had 3TC-resistant
mutations (rtM204V/I and rtL180M) and one patient had an ADV-resistant mutation (rtN236T), whereas the two patients in the LdT+ADV group had an LdT-resistant mutation (rtM204I+rtL180M).

Safety
For the majority of patients, treatment was well tolerated in the 3TC+ADV and LdT+ADV groups. Four patients in the 3TC+ADV group and three patients in the LdT+ADV group had slightly increased serum creatinine but there was no discontinuation due to this adverse event. Among patients in the LdT+ADV group, seven patients had increased serum creatine kinase (CK), which returned to normal after a temporary withdrawal of LdT. Across the two groups, decompensated cirrhosis and hepatocellular carcinoma was not reported.

Discussion
Because of the unavailability of tenofovir in developing countries, ADV is still involved in the treatment of CHB, as well as entecavir (ETV) [5]. However, due to the weak antiviral activity, suboptimal response is particularly common in ADV [15]. Evidence has shown that the persistence of suboptimal response during long-term treatment is associated with the emergence of drug-resistant viral strains, which could result in poorer clinical outcomes [22,23]. At present, management of suboptimal response to NA therapy has recently been of concern [12], and combination therapy rather than switching therapy offers a potentially attractive therapeutic option [21,24].

According to the hepatitis B roadmap and guidelines, in patients with suboptimal response to a drug with a low genetic barrier to resistance, the addition of an appropriate non-cross-resistant second drug should be considered [2]. In patients with suboptimal response to ADV, the choices of combination treatment include 3TC add-on, LdT add-on and ETV add-on [6]. However, data on the comparison of different combination strategies is rare. Previously, our two independent short-term studies on patients with poor response to ADV demonstrated that higher proportions of patients in the LdT+ADV group achieved a virological response at week 24 than did patients in the 3TC+ADV group (66.7% [16/24] versus 35.5% [11/31]) [25,26].

Is it really true that LdT+ADV treatment is superior to 3TC+ADV treatment in reducing the level of HBV DNA of patients with ADV suboptimal response? In the present study, we provided a head-to-head comparison of LdT+ADV with 3TC+ADV in CHB patients with suboptimal response to ADV, and we found that both 3TC+ADV and LdT+ADV treatments produced greater HBV DNA suppression and higher rates of ALT normalization. As compared to our previous study on HBeAg-positive patients with suboptimal response to ADV [25], the virological response rate of patients in the present study was significantly increased, which might be correlated to the lower baseline viral load when another NA was added.
Experience from the management of NA-resistance patients suggested that combination therapy could significantly delay or prevent the emergence of drug resistance [27,28]. In this study, our findings were in agreement with the results of previous studies [29]; of the 72 patients with suboptimal response to ADV, NA-associated mutations were detected in only 6.9% (three patients in the 3TC+ADV group and two patients in the LdT+ADV group), which further indicated that combination therapy also can reduce the occurrence of drug resistance in patients with suboptimal response to NAs. Indeed, the mean duration of ADV monotherapy for those five patients prior to combination therapy was ≥1.5 years; therefore, we speculated that the long-term duration of suboptimal response would correlate with the occurrence of NA-associated mutations. If the time-point of optimization therapy for those five patients could be moved forward, the occurrence of NA-associated mutations might be avoided or delayed.

Previous in vitro studies reported that the presence of the rtA181V mutation would reduce sensitivity to 3TC [30,31]. In the present study, all patients at baseline of combination therapy had no evidence of rtA181V mutation, and this might have contributed to the greater virological decline in 3TC+ADV. For patients with suboptimal response to ADV and evidence of rtA181V mutation, we infer that the combination of LdT+ADV may be superior to 3TC+ADV, but further studies are needed to confirm this.

HBeAg seroconversion is a key goal of antiviral therapy for HBeAg-positive CHB patients, which indicates good prognosis, including lower rates of cirrhosis and slower disease progression [32]. In our study, we reported high rates, with 38.5% and 26.9% of HBeAg-positive patients achieving HBeAg loss and HBeAg/anti-HBe seroconversion, respectively, in the first year of LdT+ADV treatment. Most previous studies suggested that combination therapy did not improve HBeAg seroconversion rates as compared to monotherapy [27,28]. Since we did not have a 3TC or LdT monotherapy group, we could not conclude that combination therapy had an additional advantage in terms of serological response. Considering the lower rates of HBeAg loss and seroconversion in 3TC+ADV, we speculate that the good serological response in the LdT+ADV group may be related to the effect of LdT, because evidence in treatment-naive patients demonstrates that LdT could significantly increase the rate of serological response as compared to other NAs (including 3TC, ADV and ETV) [33,34].

Recently, the safety of combination therapy has been a concern. In our study, both the serum creatinine and CK were monitored. Despite some patients having elevated enzymes, they had no signs or symptoms at all. In consideration of the safety of patients with moderate elevations of CK [35], we stopped LdT treatment temporarily and serum level of CK returned to normal within 1 week. After restoring LdT treatment, no moderate elevations of CK were reported. Though the long-term safety of 3TC+ADV and LdT+ADV in patients with suboptimal response to ADV is still unclear, our findings suggested that short-term combination therapy appeared safe in this setting.

Limitations of this prospective study are the small sample size and a potential bias in treatment assignment due to the study design. However, statistical analysis showed that the demographic and laboratory characteristics between two groups were comparable, which reduced the bias of treatment assignment. To fully clarify the optimization strategy for CHB patients with suboptimal response to ADV, prospective controlled studies of sufficient size and adequate treatment duration are urgently needed.

In conclusion, we have shown here that, in patients with suboptimal response to ADV, both the 3TC+ADV and LdT+ADV combination therapies led to significant decreases in serum HBV DNA. Treatment with a combination of LdT+ADV was associated with a higher rate of serological response at 12 months.

Acknowledgements

This work was supported by the National Science and Technology Major Project of China (2012ZX10002007 and 2008ZX10002-006), and National S&T Major Project for Infectious Diseases Control (2009ZX10004-905). We wish to thank Juan Liao and Cong Liu (Center of Infectious Diseases, West China Hospital of Sichuan University) for their assistance in laboratory data collection and management.

Disclosure statement

The authors declare no competing interests.

References


