Sequential treatment with lamivudine and interferon-\(\alpha\) monotherapies in hepatitis B e antigen-negative Chinese patients and its suppression of lamivudine-resistant mutations

Ming Shi\(^1\), Rong Sheng Wang\(^3\), Hua Zhang\(^3\), Yu Fen Zhu\(^3\), Bei Han\(^3\), Yong Zhang\(^2\), Li Ji Jin\(^1\), Zhi-Jun Yang\(^4\) and Yong Ping Xu\(^1\)*

\(^1\)Department of Biotechnology, Dalian University of Technology, Dalian 116023, Liaoning Province, China; \(^2\)No. 6 Hospital of Dalian, Dalian 116001, Liaoning Province, China; \(^3\)Jiangsu Oil Field Hospital, Shaobo, Jiangdu 225261, Jiangsu Province, China; \(^4\)Management School, Shanghai Jiaotong University, Shanghai, China

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Objectives: To assess the efficacy of sequential treatment with lamivudine and interferon-\(\alpha\) monotherapies in Chinese patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B.

Methods: One hundred and sixty-two patients with HBeAg-negative chronic hepatitis B were included in this study. Ninety-eight were treated with lamivudine alone (100 mg per day) for 48 weeks (group B). Sixty-four were treated with lamivudine alone (100 mg per day) for 20 weeks, then combined with interferon-\(\alpha\)-2b (5 million units three times per week) for 4 weeks and then treated for another 24 weeks with interferon-\(\alpha\)-2b alone (5 million units three times per week) (group A). All patients were followed for an additional 24 weeks.

Results: After 48 weeks of treatment, the percentage of patients with normalization of alanine aminotransferase (ALT) levels or hepatitis B virus (HBV) DNA levels below 1000 copies/mL was not significantly different between the lamivudine monotherapy group (55.10% and 55.10%, respectively) and the sequential treatment group (59.36% and 56.25%, respectively). The percentage of patients with normalized ALT levels was significantly higher in group A (53%) than in group B (36%) at week 72 (\(P<0.05\)). The percentage of patients with lamivudine-resistant mutations was significantly higher with lamivudine monotherapy (22.45%) than with sequential therapy (\(P<0.05\)).

Conclusions: Sequential treatment of chronic hepatitis B with lamivudine and interferon-\(\alpha\) monotherapies is as effective as lamivudine-alone treatment in Chinese patients. However, sequential treatment can significantly suppress the emergence of lamivudine-resistant mutations.

Keywords: chronic hepatitis B, hepatitis B virus, HBV, YMDD

Introduction

It is estimated that 350 million individuals are chronically infected with hepatitis B virus (HBV).\(^1\) Carriers of HBV are at increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC).\(^2\) The long-term aim in the treatment of these patients is to prevent or at least to decrease the risk of the development of cirrhosis and HCC. However, this long-term aim is unpractical for clinical use. In clinical trials with follow-up of 1 year or less after therapy, more realistic short-term objectives are usually used. The most widely used short-term objectives are normalization of serum alanine aminotransferase (ALT) levels and reduction of serum HBV DNA levels,\(^3,4\) which can reflect biochemical improvement of the liver and suppression of the virus replication.

The agents currently used or under investigation for the treatment of chronic hepatitis B can be broadly divided into two main groups: immunomodulators and nucleoside analogues.\(^5\) Interferon-\(\alpha\) is the best known of the immunomodulators and lamivudine is the most extensively studied nucleoside analogue. Interferon is effective in white patients but the results in Asian patients are disappointing.\(^6-8\) Lamivudine is effective in normalization of serum ALT levels, loss of hepatitis e antigen (HBeAg) and reduction of serum HBV DNA.\(^8-11\) However, long-term
lamivudine therapy will lead to the emergence of a mutant YMDD (tyrosine, methionine, aspartate and aspartate) motif of the HBV polymerase gene. With the emergence of these mutant virus strains, there is increasing resistance to the therapeutic effects of lamivudine. In several studies, the combination of lamivudine and interferon-α has been used to overcome this emergence of resistance to therapy and this strategy can reduce or delay the emergence of the YMDD variants. Sequential treatment with lamivudine and interferon-α can induce a sustained virological response in patients with chronic hepatitis B not responding to interferon-α alone without the selection of drug-resistant mutants. Sequential combination therapy can induce sustained virological response in a subgroup of HBeAg-negative/virus-DNA-positive chronic hepatitis B. Compared with combination therapy, sequential treatment will be cost-effective, which is important in low-income countries such as China. However, limited by a small number of studies, the effectiveness of sequential treatment with lamivudine and interferon-α monotherapies in patients with chronic hepatitis B needs further exploration.

In the present study, we evaluated the efficacy of sequential treatment with lamivudine and interferon-α in HBeAg-negative Chinese patients with chronic hepatitis B. A sensitive TaqMan-based real-time PCR method was used to investigate lamivudine-resistant mutations during this sequential treatment.

### Patients and methods

#### Patients

This study was performed between June 2002 and July 2004. A total of 162 patients who were untreated with antiviral agents were included in the sequential treatment study. All the 162 patients were over 16 years of age, positive for hepatitis B surface antigen (HBsAg) for at least 6 months, negative for HBeAg and positive for hepatitis B e antibody (anti-HBe), and had HBV DNA levels of more than 100 000 copies/mL and serum ALT levels greater than 1.5 times but less than 10 times the normal range according to recommendations of Chinese Experts Committee for Clinical Use of Lamivudine. Exclusion criteria included co-infection with hepatitis A, C, D and E virus or HIV; decompensated liver diseases or HCC, a history of alcohol or drug abuse within 1 year before entry, other possible causes of chronic liver damage, and previous treatment of chronic hepatitis B.

#### Study design and treatment protocols

The patients were randomly divided into two groups in a ratio of 2:3. Group A (n = 64) for sequential treatment and group B (n = 98) for treatment with lamivudine alone. The study was conducted according to the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients gave their informed consent.

Patients in group A received lamivudine (100 mg/day) (Glaxo Wellcome, Suzhou, China) for 20 weeks, followed by interferon-α-2b (5 million units three times per week) (Schering-Plough, Shanghai, China) plus lamivudine (100 mg/day) for 4 weeks, and they were then treated for another 24 weeks with interferon-α-2b alone (5 million units three times per week). Patients in group B received 100 mg of lamivudine once daily for 48 weeks. All patients were followed up after the end treatment for 24 weeks.

### Detection of YMDD mutations

YMDD mutations were detected at baseline and every 12 weeks thereafter using a TaqMan-based real-time PCR assay established by us. In this assay, three different forward primers that would selectively amplify YMDD (wild-type), YVDD (rtM204V) and YIDD (rtM204I) variants and a common reverse primer to a highly conserved sequence within the polymerase open reading frame were used to discriminate different mutants. The amplicon was detected and quantified by using real-time PCR and a TaqMan probe that annealed to a highly conserved region between the forward and reverse primers. A universal forward primer was used in the control amplification of all kinds of variants. The amplification was performed by incubating the reaction mixture at 50°C for 2 min, followed by 5 min at 95°C and 40 cycles of PCR amplification (94°C for 20 s and 53°C for 30 s) on a ABI 7000 sequence detector (Applied Biosystems, Foster City, CA, USA).

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#### Assays for ALT, HBV markers and HBV DNA

ALT levels, HBsAg, hepatitis B surface antibody (anti-HBs), HBeAg, anti-HBe and hepatitis B core antibody (anti-HBc) were measured using commercially available kits at baseline and every 30 days thereafter. HBV DNA levels were tested using real-time PCR (Fosun Diagnostics, Shanghai, China) on an ABI 7000 sequence detector (Applied Biosystems, Foster City, CA, USA). All reagents used in this study were approved by the State Food and Drug Administration of China for in vitro diagnostic use.

#### Efficacy measures

Efficacy analyses included all randomized patients enrolled in this study. The study had two primary measures of efficacy assessed after 24 weeks of follow-up: the normalization of ALT levels and the suppression of HBV DNA levels to below 1000 copies/mL. Secondary efficacy measures assessed after 24 weeks of follow-up included the proportion of patients with HBsAg loss or HBsAg seroconversion (defined by the loss of HBsAg and the presence of anti-HBs).

#### Statistical analysis

The significance of a difference between two groups was determined using the χ² test. The level of significance was set at P < 0.05.

### Results

#### Baseline characteristics of patients

The baseline characteristics of patients before treatment are given in Table 1. Ninety-eight patients were randomly assigned to receive lamivudine and 64 to receive sequential treatment with lamivudine and interferon-α monotherapies. All the patients were Chinese and well matched in terms of age, weight and laboratory results at baseline. However, the percentage of males is lower in group A than in group B.

#### Biochemical response

At week 24, serum ALT normalized in 28 of 64 (44%) patients in group A and 72 of 98 patients (73%) in group B. However, at week 48, 38 of 64 (59%) patients in group A and only 54 of 98 (55%) patients in group B had a sustained biochemical response.
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Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sequential treatment group (group A, n = 64)</th>
<th>Lamivudine monotherapy (group B, n = 98)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>38 (60%)</td>
<td>78 (80%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>mean ± SD</td>
<td>37 ± 10.4</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>21–56</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>mean ± SD</td>
<td>62 ± 7.5</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>50–77</td>
<td></td>
</tr>
<tr>
<td>HBV DNA (log copies/mL)</td>
<td>mean ± SD</td>
<td>6.73 ± 1.16</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>6.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>5.01–9.01</td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>mean ± SD</td>
<td>135.59 ± 90.81</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>median</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>60–282.00</td>
<td></td>
</tr>
<tr>
<td>ALT &gt;1.5 times ULN</td>
<td>64 (100%)</td>
<td>98 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

The percentage of patients with normalized ALT levels in group B (73%) was significantly higher than in group A (44%, P < 0.05) at week 24 but there was no significant difference at week 48. After 24 weeks of follow-up (week 72), the percentage of patients with normalized ALT levels was significantly higher in group A (53%) than in group B (36%).

Virological response
At week 24, the percentage of patients with HBV DNA levels below 1000 copies/mL was 81% in group A and 78% in group B compared with 56% in group A and 55% in group B at week 48. There were no significant differences in suppression of serum HBV DNA between groups A and B at week 24 and week 48. At week 72, suppression of HBV DNA levels to below 1000 copies/mL occurred in nine patients (14%) from group A and 18 patients (18%) from group B (Table 2).

HBsAg response
At week 72, HBsAg loss or seroconversion was not identified in either group A or group B.

Lamivudine-resistant mutations
No patients had evidence of lamivudine-resistant mutations at baseline and YMDD mutants were monitored in all patients every 12 weeks thereafter. Only two patients (3.13%) were found with the YIDD variant at week 24 and no patients had evidence of YMDD mutations at week 48 among 64 patients in group A. The two patients who had YIDD variants at week 24 had normalized ALT and undetectable HBV DNA at week 48 and week 72. In contrast, YMDD mutations were found in six patients (four YIDD variants and two YVDD variants, 6.12%) at week 24 and in 22 patients (12 YIDD variants, eight YVDD variants and two with a mixture of YIDD and YVDD variants, 22.45%) at week 48 from group B (P < 0.05). All the 22 patients who had YMDD mutations at week 48 had rebounds of serum HBV DNA and 18 (82%) had rebounds of ALT levels.

Adverse events
Lamivudine was well tolerated and no adverse symptoms were identified during treatment. During the course of interferon, six patients had serious adverse events including pyrexia, fatigue, myalgia and headache. Depression was not observed in this study. Mean neutrophil and platelet counts were reduced during treatment but returned to baseline levels shortly after treatment was stopped. All the patients completed their treatments.

Discussion
Interferon-α monotherapy has little long-term benefit in the treatment of Chinese patients with chronic hepatitis B.7,8,24 This is probably due to immunological tolerance to HBV induced by exposure to the virus in early life.24 Sequential treatment with lamivudine and interferon-α monotherapies was effective in patients with chronic hepatitis B not responding to interferon alone.21 The main reason might be reduction in HBV DNA and recovery of T-cell reactivity against HBV induced by lamivudine improving the efficacy of interferon-α.21,25 In the present study, we found that sequential treatment starting with lamivudine monotherapy followed by interferon monotherapy resulted in an effective response in HBeAg-negative Chinese patients with chronic hepatitis B. In addition, lamivudine-resistant mutations were suppressed during sequential treatment. YMDD mutations were found in none of the 64 patients with sequential treatment but in 22 of 98 patients (22.45%) with lamivudine monotherapy at the end of treatment.

Long-term use of lamivudine induced the emergence of YMDD mutations, which was occasionally associated with severe flares of hepatitis.26,27 A previous study showed that sequential treatment with lamivudine and interferon-α monotherapies induced a sustained virological response in non-responders to...
interferon-α alone, without the selection of lamivudine-resistant mutations.21 Combination and sequential combination therapies of chronic hepatitis B with lamivudine and interferon-α-2a were effective and also delayed the selection of lamivudine-resistant variants.4,30–32 A recent study indicated that a three-phase sequential treatment with lamivudine and interferon in young patients with chronic hepatitis B reduced HBV DNA serum levels but did not prevent the emergence of lamivudine-resistant mutations.28 In our study, 56% of patients with sequential treatment with lamivudine and interferon and 55% of patients with lamivudine monotherapy had negative serum HBV DNA by real-time PCR at the end of therapy. No lamivudine-resistant mutations were selected in the sequential treatment group but 22.45% of patients with YMDD mutations were found in the lamivudine-alone group. These findings were consistent with combination treatment studies.4,18,29 The sequential monotherapy appears to be effective and may also suppress lamivudine-resistant mutations.

Two patients in the sequential treatment group were detected with the YIDD variant at week 24 but had normalized ALT and undetectable serum HBV DNA at week 48 and week 72. The mutants emerged 12–24 weeks after the start of lamivudine treatment and disappeared in 12 weeks after interferon-α treatment. This suggests that interferon-α may be effective in some lamivudine-resistant patients. ALT levels were higher in group A than in group B at week 24. This could be attributed to flare-ups of ALT in 21 patients in group A when interferon-α was added. Among these 21 patients, 6 exhibited a sustained biochemical and virological response at week 72. We cannot conclude that ALT flare-ups during the initial interferon-α treatment might have a prolonged response. However, sequential treatment resulted in a higher biochemical response rate at week 72. This might be due to the significant effects of interferon-α on normalization of serum ALT concentrations.3

The major limit of this study is the absence of histological response data. In other studies, patients with prolonged virological responses experienced histological improvements.21 There was a significant association between improved histological activities and either a prolonged biochemical or virological response.4 The percentage of males is higher in group B than in group A, which may cause bias in the results concerning treatment efficacy. In addition, the sustained response rate was low in this study due to the relative short treatment period. A longer treatment period needs to be evaluated in future studies.

Compared with combination therapy, sequential therapy is much more cost-effective. In China, interferon-α is much cheaper. A 24 week treatment costs about 3000 CNY, whereas a 48 week lamivudine treatment costs about 6000 CNY. China has the greatest burden of hepatitis B in the world. Owing to limited income, most Chinese people will benefit from low-cost therapeutic regimens. To date, the State Food and Drug Administration of China has approved three treatments for chronic HBV infection in China. They are interferon-α, lamivudine, adefovir, peginterferon-α-2a and entecavir, which are effective agents for treatment of chronic hepatitis B.4,30–32 Differences in drug administration, duration and efficacy of the treatment, frequency of drug resistance, and cost do exist for these drugs.3,33 Although entecavir has a better outcome in the absence of pre-existing lamivudine-resistant mutants,4,32 lamivudine will be preserved in China because of its much lower price. Our results showed that sequential treatment with lamivudine and interferon-α monotherapies in HBeAg-negative Chinese patients with chronic hepatitis B was as effective as single lamivudine treatment but can significantly suppress the emergence of YMDD mutations. However, sequential treatments and the timing of administration for chronic hepatitis B with different drugs still need further controlled trials.

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Transparency declarations

Dr Z.-J. Y. had been a staff member of Fosun Diagnostics.

References

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