Combined effects of tenofovir and interferon α1b on viral load and levels of peripheral regulatory T cells in chronic hepatitis B subjects

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Abstract

Purpose: To study the combined effects of tenofovir and interferon α1b on viral load and peripheral blood regulatory T cell concentrations of chronic hepatitis B (CHB) subjects.

Methods: Patients with chronic hepatitis B (86 cases) were randomly assigned to two groups: control group and study group. In control subjects, tenofovir was given orally (300 mg/kg bwt/day). In addition to tenofovir, the study group received interferon α1b injection intramuscularly at a dose of 50 μg/kg thrice a week. Liver function, serum hepatitis B viral (HBV) load, and serum levels of peripheral blood regulatory T-lymphocytes were determined. Clinical effectiveness and adverse reactions in both groups were also assessed.

Results: After treatment, total effectiveness was higher in the study group (86.04 %) than in control patients (62.79 %) (p < 0.05). Serum aspartate transaminase (AST), alanine aminotransferase (ALT) and total bilirubin (TBIL) significantly decreased in the study group, relative to control, but HBV DNA-negative, HbeAg-negative and HbsAg-negative cells were markedly higher in patients in the study group (p < 0.05). Moreover, there were higher CD4+T and CD8+T counts, and CD4+T/CD8+T ratio in study subjects than in control subjects (p < 0.05).

Conclusion: The combination of tenofovir with interferon α1b effectively improves liver functions in patients with CHB, reduces viral load, and exerts anti-HBV effect by regulating the levels of peripheral blood T-lymphocytes.

Keywords: Tenofovir, Interferon α1b, Chronic hepatitis B, Viral load, Peripheral blood regulatory T cells

INTRODUCTION

Chronic hepatitis B (CHB) is an infectious disease caused by hepatitis B virus (HBV). The incidence of HBV infections worldwide has been estimated to be about 2 billion, including approximately 400 million patients with CHB which accounts for 40 to 50 % of all cases of hepatitis B infections. The disease leads to serious liver problems such as cirrhosis and hepatic carcinoma, thereby adversely affecting the quality of life of patients [1,2]. Since the
pathogenesis of the disease has not been fully elucidated, the current treatment strategy is simply to improve immunity and the rate of clearance of HBV. Tenofovir is a nucleotide-based antiviral drug which exerts potent therapeutic effects on CHB [3].

Interferon α1b is a recently introduced remedy for CHB, and it directly inhibits viral replication while improving immune function [4]. The present study was carried out to determine the combined effects of tenofovir and interferon α1b on viral load and levels of peripheral regulatory T cells in CHB subjects.

METHODS

Clinical profiles of subjects

Patients with CHB (86 cases) were recruited over a 1-year period for this study and randomly assigned to two groups of 43 patients each: control group and observation group. They consisted of 45 men and 41 women aged 24 to 39 years (mean = 31.25 ± 7.62 years), and the course of disease ranged from 1 to 10 years (mean = 7.92 ± 2.35 years). The mean plasma activity of ALT was 148 U/L, while the clinical scores were mild (58 cases) and severe (28 cases).

The included patients were: (1) subjects who met the 2015 Diagnostic Criteria for Chronic Hepatitis B developed by the Chinese Medical Association [5]; (2) patients aged 18 to 60 years; (3) patients who had clear history of hepatitis B infection and disease course more than 6 months; (4) patients treated with anti-hepatitis B virus and immune-modulators before admission to hospital; (5) patients who were HBV-DNA, HBeAg, and HbsAg positive; and (6) patients who signed written informed consent with their family members.

The patients in the following categories were excluded: (1) patients who had hepatitis V, C, E (HAV, HCV, HEV) and other viral infections; (2) patients who were alcoholics, and had fatty and hereditary metabolic liver disease; (3) patients who had severe heart, liver and kidney dysfunctions, and systemic diseases; (4) patients with allergies, especially those allergic to nucleotides; and (5) pregnant and lactating women. The clinical data such as age, gender, course of disease and clinical classification were comparable between the observation and control subjects (Table 1).

The study received approval from the Ethical Committee of Hunan University of Traditional Chinese Medicine, Hanpu Science and Technology Park, Changsha City, Hunan Province (approval no. 20187309). It was carried out in line with the Helsinki declaration of 1964 which was subjected to amendment in 1996 [6].

Table 1: Baseline data of patients with chronic hepatitis B (CHB)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/ female)</td>
<td>21/22</td>
<td>22/19</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31.23 ± 7.52</td>
<td>31.70 ± 7.36</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>7.68 ± 2.16</td>
<td>7.80 ± 2.31</td>
</tr>
<tr>
<td>Mild CHB</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>Moderate CHB</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>143.24 ± 40.85</td>
<td>151.58 ± 50.54</td>
</tr>
</tbody>
</table>

Treatment regimen

Control group

Tenofovir was orally administered at a dose of 300 mg/kg/day.

Study group

In study patients, interferon α1b injection was also administered intramuscularly at a dose of 50 μg/kg thrice a week. Treatment in both groups lasted 48 weeks.

Collection of blood sample

Following overnight fast, blood (5 mL) was drawn from the vein of all subjects in the morning prior to treatment and 4 weeks post-medication, and subjected to centrifugation for 10 min at 3000 rpm. The resultant serum was taken and kept in a refrigerator at -20 °C, and used for biochemical analyses.

Assessment of clinical effectiveness

Based on the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B" (2015), the clinical effectiveness was classified into three: remarkably effective, effective and ineffective [7].

(a) **Remarkably effective**: In this group, indices of liver function were normalized, and HBV-DNA load was < 1000 cps/mL; (b) **effective**: indices of liver function were significantly improved and HBV-DNA load was ≥ 1000 cps/mL, i.e., ≥ 2000 IU/mL lower than that before treatment; (c) **ineffective**: indices of liver function did not improve, and the HBV-DNA load decreased (< 2000 IU/mL). The total effectiveness (TE) was calculated as in equation 1.

\[
TE(\%) = \frac{AE+I}{N} \times 100\% \quad \ldots \quad (1)
\]
where RE is remarkably effective, E is effective, and T is total number of subjects

**Evaluation of liver function**

The plasma activities of aspartate aminotransferase (AST), ALT, total bilirubin (TBIL) concentration, and albumin (ALB) were determined using enzyme coupling, Jendrassik and Grof, and bromocresol green (BCG) methods, respectively.

**Determination of serum HBV viral load**

The quantitation of HBV-DNA was done using fluorescence qPCR). The levels of HBeAg negative (He) and HbsAg negative (Hs) were calculated as shown in equations 2 and 3, respectively.

\[
He(\%) = \frac{N_{He}}{T} \times 100 \quad \ldots \ldots (2)
\]

\[
Hs(\%) = \frac{N_{Hs}}{T} \times 100 \quad \ldots \ldots (3)
\]

where NHe is number of cases with disappearance of HbeAg, NHs is number of cases with disappearance of HbsAg, and T is total number of cases

**Determination of serum levels of peripheral blood regulatory T cells**

The serum levels of CD4+ T, CD8+ T, and CD4+/CD8+ T were measured by flow cytometry (BeamCyte-1026, Bidake Bio, China).

**Statistical analysis**

The data were analyzed with SPSS19.0 software package. Numerical data are expressed as mean ± standard deviation (SD), and were analyzed using t-test. Count data are expressed as frequency (n (%)), and were analyzed using χ² test. Statistical significance was fixed at \( p < 0.05 \).

**RESULTS**

**Effect of treatment on patients’ clinical symptoms**

After treatment, the improvements in symptoms such as fatigue and liver pain occurred in a significantly shorter time in observation subjects, relative to control (\( p < 0.05 \), Table 2).

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Viral load-related indices

Table 5 shows that treatment led to marked increases in HBV-DNA negative, HbeAg negative and HbsAg negative cells in the observation patients, relative to control subjects ($p < 0.05$).

Table 5: Viral load-related indices of patients (n, %)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>HBV-DNA negative</th>
<th>HbeAg negative</th>
<th>HbsAg negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>43</td>
<td>36 (83.72 %)</td>
<td>32 (74.41 %)</td>
<td>28 (65.11 %)</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>21 (48.83 %)</td>
<td>20 (46.51 %)</td>
<td>18 (41.86 %)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td></td>
<td>5.93</td>
<td>6.34</td>
<td>7.02</td>
</tr>
<tr>
<td>$p$</td>
<td></td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Peripheral blood regulatory T-lymphocytes

As shown in Table 6, post-treatment CD4$^+$ T, CD8$^+$ T, and CD4$^+$ T/CD8$^+$ T were markedly increased in observation patients, when compared to control patients ($p < 0.05$).

Table 6: T-lymphocyte levels in patients before and after treatment (n, %)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>39.75 ± 5.13</td>
<td>66.03 ± 3.24</td>
<td>35.19 ± 1.98</td>
<td>60.29 ± 7.05</td>
</tr>
<tr>
<td>Control</td>
<td>58.53 ± 2.11</td>
<td>2.86</td>
<td>36.05 ± 1.17</td>
<td>49.75 ± 5.29</td>
</tr>
<tr>
<td>$t$</td>
<td>2.34</td>
<td>8.92</td>
<td>2.34</td>
<td>9.34</td>
</tr>
<tr>
<td>$p$</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

DISCUSSION

Immediately following HBV infection, the virus continues to replicate in the host body and persists for a long time. About 15 to 20 % of patients with active HBV replication develop cirrhosis within 5 years. Due to the continuous replication of HBV in hepatocytes, the patient’s immunity is compromised, followed by damage of secondary hepatocytes, leading to liver fibrosis [8]. Studies have shown that patients with CHB have severe T-lymphocyte subset disorder and cellular immune dysfunction [9]. Tenofovir has a broad-spectrum antiviral effect and can inhibit viral replication by inhibiting HBV polymerase. Interferon α1b inhibits HBV replication, improves cellular immunity, enhances the expression of human leukocyte antigen (HLA) in hepatocytes and stimulates circulating peripheral blood T-Lymphocytes [10].

In the present study, the improvement times of symptoms such as fatigue and liver pain were significantly longer in control subjects than in the study subjects, while total effectiveness was significantly higher in the latter. These results suggest that combined treatment with tenofovir and interferon α1b in patients with CHB may have complementary advantages. The activities of AST and ALT, and levels of TBIL were markedly lower in the observation subjects than in control group. These results appear to suggest that combined use of tenofovir and interferon α1b may markedly enhance liver function in CHB subjects, and it is consistent with the results of Luo Yan et al [11].

It is known that HBV-DNA is the most direct and sensitive marker of HBV infection. Its positive expression indicates that HBV has serious infectious and replication abilities. Patients with CHB having high HBV load are at higher predisposition to liver cirrhosis and liver cancer, since they have reduced immunity [12]. In the present study, there were higher HBV-DNA negative, HbeAg negative and HbsAg negative cells in the study patients than in control. This suggests that tenofovir combined with interferon α1b may enhance the inhibition of viral replication and improve the body immunity.

The most probable explanation for this is that following oral administration of tenofovir, it is phosphorylated in the cells to tenofovir diphosphate which is involved in the synthesis of DNA. After gaining entrance into the virus, DNA synthesis is blocked due to the unavailability of the 3-hydroxyl substituent, thereby inhibiting replication of the virus. Interferon α1b binds to cell surface receptors, allowing cells to produce a
variety of antiviral proteins which inhibit intracellular viral replication [13].

The T-lymphocytes are at the heart of cellular immunity, with CD4+ and CD8+ cells as the main representatives. The CD4+ T are the T helper lymphocytes (Th), which play central roles in the immune responses to viruses. The activation and inhibition of CD8+ T rely strictly on CD4+ T, thus representing T-inhibitory lymphocytes (Ts). When the body is infected with HBV, the levels of CD4+ cells are significantly reduced, CD8+ cells are significantly increased, and the ratio of CD4+ T to CD8+ T is significantly reduced, an indication of an immunosuppressed state [14].

In this study, the levels of CD4+ T, CD8+ T, and CD4+ T/CD8+ T were markedly higher in study group subjects than in control subjects, which indicates that combined application of tenofovir and interferon α1b may effectively improve the levels of peripheral blood regulatory T-lymphocyte subsets and enhance immunity. These results are consistent with those of Li et al [15].

Interferon α1b promotes differentiation and maturation of T cells via the stimulation of human peripheral blood T cells, induction of the expressions of interferon β and IL-2, and stimulation of immune function. It may also reduce immune damage by increasing the killing activity of natural killer (NK) cells, thus directly playing the role of an antiviral agent [16].

Limitations of the study

This study was based on a single center. Moreover, the number of subjects used was low study. Thus, the application of the conclusions reached should be done with some caution.

CONCLUSION

The findings of this study show that the combination of tenofovir and interferon α1b effectively improves liver function in patients suffering from CHB, reduces viral load, and exerts anti-HBV effect by regulating the levels of peripheral blood T-lymphocytes.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Jinwen Ge conceived and designed the study, Wanfeng Wu, Chengting Jiang, Cheng Cheng, Yihang Sun, Ning Luo, Jinwen Ge collected and analysed the data, while Wanfeng Wu wrote the manuscript.

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