A Pilot Study of Thymosin α₁ Therapy for Chronic Hepatitis B Patients

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Abstract

Objective The efficacy of thymosin alpha 1 (Tai) in patients with chronic hepatitis B still requires confirmation. We, therefore, evaluated the efficacy of therapy in patients with chronic hepatitis B.

Methods Sixteen patients were randomly assigned into one of two groups, treated with 0.8 mg of Tai (low dose group; n=8) or 1.6 mg Tai (high dose group; n=8), administered six times weekly for two weeks, followed by twice weekly for another 22 weeks. Responders were defined as patients having clearance of hepatitis B e antigen by radioimmunoassay and negativity of hepatitis B virus (HBV)-DNA by branched DNA signal amplification and normalization of serum alanine aminotransferase (ALT) 24 months after initiation of Tai therapy. Transient acute exacerbation was defined as an increase of more than 300 IU/l in serum ALT level during Tai therapy.

Results The response rate was 37.5% (6/16). Tai therapy had a significant effect when, 1) transient acute exacerbation was present (p=0.0029), 2) the serum HBV-DNA level was <100 Meq/ml prior to the commencement of Tai therapy (p=0.0063). The difference between low and high dose groups was not statistically significant (p=0.608).

Conclusion The results of this trial show that: 1) a 24-week course of Tai could be a worthwhile strategy for chronic hepatitis B patients with a serum HBV-DNA level of less than 100 Meq/ml; and 2) patients with a transient acute exacerbation during Tai therapy generally respond well.

Key words: chronic hepatitis B, thymosin alpha 1, HBV-DNA

Introduction

Chronic hepatitis B is a serious liver disease with significant mortality and morbidity. However, in patients with clearance of hepatitis B virus (HBV) DNA, the prognosis of the disease is generally good (1-4). Regardless of some beneficial drugs, such as interferon (IFN), lamivudine and steroid, there are no absolute treatments in chronic hepatitis B at the present time.

Thymosin alpha 1 (Tai), a 28 amino acid thymic polypeptide hormone, is a chemically synthesized immunomodulator (5). A randomized, controlled study conducted by Chien et al showed that a 26-week course of Tai therapy was effective and safe in patients with chronic hepatitis B compared with placebo or a 52-week course of Tai (6). However, a multicentre, randomized, double-blind and placebo-controlled study of 97 patients did not confirm observations of treatment efficacy (7). Thus, the efficacy of Tai still necessitates confirmation (8, 9). We, therefore, conducted a randomized, clinical trial of Tai in patients with chronic hepatitis B and examined the independent factors that might influence the response to Tai therapy.

Materials and Methods

Patients

Sixteen Japanese patients were enrolled in this trial from April 1997 to December 1999. The patients were randomly assigned to receive one of two dose groups of Tai by the computer generated program designed by a pharmaceutical company (SciClone Japan K.K., Tokyo, Japan). The selection criteria for patients included in the study were as follows: 1) an average ALT increase greater than the upper normal limits (normal serum ALT level is 6-50 IU/l) for longer than 6 months, 2) liver biopsy (taken within 6 months before IFN administration) showing histological features of
chronic active hepatitis, 3) positive hepatitis B surface antigen (HBsAg), and HBV-DNA (b DNA probe signal amplification technology) for more than 6 months, 4) no corticosteroid, immunosuppressant or antiviral agent use within one year, 5) no serum hepatitis C virus antibodies detectable by enzyme immunoassay (EIA) or radioimmunoassay (RIA), and 6) no antinuclear antibodies or antimitochondrial antibodies detectable in the serum by immunofluorescence on rat liver and kidney.

**Drug assignment**
All patients were given subcutaneous injections of Tα1, six times weekly for 2 weeks, followed by Tα1 twice weekly for another 22 weeks. The total amount of Tα1 administered was 46.4 mg in the low dose group (0.8 mg per dose) and 92.8 mg in the high dose group (1.6 mg per dose). This study was approved by the institutional review board of our hospital. The physicians in charge explained the purpose and method of this clinical trial, as well as potential adverse reactions, to each patient, and informed consent for participation was obtained from all subjects.

**Blood tests**
Routine biochemical and hematological tests were made at weekly to monthly intervals during treatment and during a follow-up of more than 6 months after the termination of Tα1 treatment. The remaining serum samples were divided and stored at −80°C until use. HBsAg and HBeAg/HBeAb were determined by EIA (Abbott Diagnostics, Chicago, IL). HBV-DNA was measured by branched DNA signal amplification technology (Chiron Corp., Emeryville, CA), and the results were expressed as 10^6 genomic equivalents (Meq) per milliliter. The lower limit of the assay was 0.7 Meq/ml. Genotyping of HBV was performed by an ELISA using monoclonal antibodies for the genotype specific epitopes in the pre S2 region product (10). Anti-hepatitis C virus antibody was detected by third generation enzyme-linked immunoassay (Ortho Diagnostics Japan, Tokyo, Japan).

**Definition of response after Tα1 therapy and transient acute exacerbation during Tα1 therapy**
Responders were defined as patients who satisfied the following three items 24 months after the initiation of Tα1 treatment: (a) negativity of HBeAg by RIA; (b) undetectability of serum HBV-DNA by branched DNA signal amplification; and (c) normalization of serum ALT. Transient acute exacerbation was defined as an increase of more than 300 IU/l in serum ALT level during Tα1 therapy.

**Patterns of serum ALT before, during, and after Tα1 therapy**
Patterns of serum ALT levels were classified into three types according to the ratio of maximum ALT level (a) to minimum ALT level (b). In the constant type, the a/b ratio was 1–3; in the slight fluctuation type, the a/b ratio was 3–6; and in the severe fluctuation type, the a/b ratio was 6 or more. Patterns of serum ALT levels were assessed every 6 months from 6 months before the initiation of Tα1 therapy to 6 months after the termination of Tα1 therapy.

**Liver histology**
Baseline histological activity (grading) and fibrosis (staging) before Tα1 therapy were scored semi-quantitatively as previously described (11).

**Statistical analysis**
The Fisher's exact test was used for comparison of group frequencies. Moreover, relative to an identified distribution (Ridit) analysis was used for the patterns of serum ALT before, during, and after Tα1 therapy. A p value of <0.05 by the two-tailed test was considered to indicate a significant difference.

**Results**

**Clinical background**
The background characteristics and baseline measurements for each group are summarized in Table 1. There were no significant differences between the two groups in age, sex, body weight, serum ALT, platelet count, HBV-DNA levels, HBV-genotype, HBeAg and the histopathological diagnosis of biopsied liver specimens prior to Tα1 therapy.

**Efficacy of Tα1 therapy**
The response rate was 37.5% (6/16) 24 months after the initiation of Tα1 therapy. Due to Fisher's exact test, Tα1 therapy had a significant effect when, 1) transient acute exacerbation was present (p=0.0029), 2) the serum HBV-DNA level was <100 Meq/ml prior to the commencement of Tα1 therapy (p=0.0063). The difference between the low and high dose group was not statistically significant (p=0.608) (Table 2).

**Patterns of serum ALT before, during, and after Tα1 therapy**
Figure 1 shows the fluctuation pattern of the serum ALT level in patients with a response after Tα1 therapy. Based on the Ridit analysis, the rate of slight and severe fluctuation pattern of serum ALT during Tα1 therapy was significantly high (p<0.05). In contrast, Fig. 2 shows the fluctuation pattern of the serum ALT level in patients without a response after Tα1 therapy. Most patients showed a constant pattern in the serum ALT level before, during, and after Tα1 therapy.

Two patients with acute exacerbation during Tα1 and one patient without acute exacerbation during Tα1 are shown in Figs. 3, 4, and 5, respectively.

Case 1 was a 33-year-old male (Fig. 3). This patient had marked fluctuation of serum ALT before Tα1 therapy. The serum ALT level rose to 983 IU/l one month after the initiation of Tα1. After this acute exacerbation, the ALT level decreased to normal with disappearance of HBeAg (EIA) and HBV-DNA (b DNA probe). Case 2 was a 24-year-old female
**Thymosin α1 Therapy for Chronic Hepatitis B**

**Table 1. Characteristics of Patients before Thymosin α1 Treatment**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0.8 mg (n=8)</th>
<th>1.6 mg (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y.o)</td>
<td>24-45 (34)*</td>
<td>26-48 (33)*</td>
<td>1.0</td>
</tr>
<tr>
<td>(&lt;35/&gt;35)</td>
<td>(4/4)</td>
<td>(5/3)</td>
<td></td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>5/3</td>
<td>7/1</td>
<td>0.569</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>41-69 (64)*</td>
<td>46-74 (61.5)*</td>
<td>1.0</td>
</tr>
<tr>
<td>(&lt;65/&gt;65)</td>
<td>(5/3)</td>
<td>(6/2)</td>
<td></td>
</tr>
<tr>
<td>Liver histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staging (F1/F2 or F3)</td>
<td>6/2</td>
<td>4/4</td>
<td>0.608</td>
</tr>
<tr>
<td>Activity (A1/A2 or A3)</td>
<td>6/2</td>
<td>4/4</td>
<td>0.608</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>35-314 (96)*</td>
<td>36-158 (97)*</td>
<td>1.0</td>
</tr>
<tr>
<td>(&lt;100/&gt;100)</td>
<td>4/4</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>HBV-genotype (A or B/C)</td>
<td>0/8</td>
<td>2/6</td>
<td>0.467</td>
</tr>
<tr>
<td>HBV-DNA (Meq/ml)</td>
<td>7.9-2,662 (49)*</td>
<td>2-787 (226)*</td>
<td>1.0</td>
</tr>
<tr>
<td>(&lt;100/&gt;100)</td>
<td>5/3</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>HBeAg (+/-)</td>
<td>6/2</td>
<td>6/2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Expressed as range (median).

**Table 2. Factors Associated with Response after Thymosin α1 Therapy**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>Responder</th>
<th>Non-responder</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum ALT (IU/l) during Tα1 therapy</td>
<td>&lt;300/≥300</td>
<td>2/4</td>
<td>10/0</td>
<td>0.0029</td>
</tr>
<tr>
<td>HBV-DNA (Meq/ml)</td>
<td>&lt;100/≥100</td>
<td>3/3</td>
<td>6/4</td>
<td>1.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&lt;35/≥35</td>
<td>4/2</td>
<td>8/2</td>
<td>0.604</td>
</tr>
<tr>
<td>Sex</td>
<td>male/ female</td>
<td>4/2</td>
<td>8/2</td>
<td>0.604</td>
</tr>
<tr>
<td>Fibrosis of the liver</td>
<td>F1/F2, F3</td>
<td>3/3</td>
<td>7/3</td>
<td>0.607</td>
</tr>
<tr>
<td>Activity of the liver</td>
<td>A1/A2</td>
<td>4/2</td>
<td>7/3</td>
<td>1.0</td>
</tr>
<tr>
<td>ALT (IU/l) before Tα1 therapy</td>
<td>&lt;100/≥100</td>
<td>3/3</td>
<td>9/1</td>
<td>0.118</td>
</tr>
<tr>
<td>HBe antigen</td>
<td>(+)/(-)</td>
<td>3/3</td>
<td>9/1</td>
<td>0.118</td>
</tr>
<tr>
<td>HBV-genotype</td>
<td>A or B/C</td>
<td>0/6</td>
<td>2/8</td>
<td>0.5</td>
</tr>
<tr>
<td>Method of Tα1 therapy</td>
<td>0.8 mg/1.6 mg</td>
<td>4/2</td>
<td>4/6</td>
<td>0.608</td>
</tr>
<tr>
<td>Tα1 (mg)/body weight (Kg)</td>
<td>&lt;0.025/≥0.025</td>
<td>4/2</td>
<td>4/6</td>
<td>0.608</td>
</tr>
<tr>
<td>History of interferon therapy</td>
<td>(-)/(+)</td>
<td>3/3</td>
<td>3/7</td>
<td>0.607</td>
</tr>
</tbody>
</table>

**Six months before thymosin**

<table>
<thead>
<tr>
<th>ALT</th>
<th>Six months during thymosin</th>
<th>Six months after thymosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALTX</td>
<td>4/6 (66.7%)</td>
<td>0/6 (0%)</td>
</tr>
</tbody>
</table>

**Six months after thymosin**

<table>
<thead>
<tr>
<th>ALT</th>
<th>Six months during thymosin</th>
<th>Six months after thymosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALTX</td>
<td>9/10 (90%)</td>
<td>9/10 (90%)</td>
</tr>
</tbody>
</table>

**Figure 1. The fluctuation pattern of serum ALT level in patients with response after Tα1 therapy.**

**Figure 2. The fluctuation pattern of serum ALT level in patients without response after Tα1 therapy.**

**Internal Medicine Vol. 42, No. 10 (October 2003)**

943
Figure 3. Clinical courses of response case 1 displayed transient acute exacerbation during thymosin α₁ therapy.

Figure 4. Clinical courses of response case 2 displayed transient acute exacerbation during or after thymosin α₁ therapy.

(Fig. 4). The serum ALT level displayed 822 IU/l two months after the initiation of Tα₁. Moreover this patient had marked elevation of serum ALT at six months after end of treatment. After the second marked elevation of serum ALT, the serum level of ALT decreased to within the normal range with disappearance of HBeAg and HBV-DNA. Case 3 was a 35-year-old male (Fig. 5). This patient had a constant pattern of serum ALT before, during, and after Tα₁ therapy. He showed positivity of HBeAg and HBV-DNA 24 months after the initiation of Tα₁ therapy.

Clinical safety
No dose adjustment was necessary in patients given Tα₁. Tα₁ therapy did not reduce the leukocyte count or platelet

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**CASE 1. H.H 33 y.o male**
HBV-genotype: C
Low dose group

**CASE 2. T.Y 24 y.o female**
HBV-genotype: C
Low dose group
Thymosin α₃ Therapy for Chronic Hepatitis B

| eAg (RIA) | + + + + + + + |
| HBV-DNA   | 2,662 2,000 1,900 670 310 |

Thymosin α₃ Therapy for Chronic Hepatitis B

**Figure 5.** Clinical courses of response case 3 displayed constant pattern of serum ALT before, during, and after thymosin α₃ therapy.

The median (range) of leukocyte count was 4,700 (3,200–9,600)/mm³ and the platelet count was 187,000 (125,000–272,000)/mm³ before the initiation of α₃ therapy, whereas the values were 4,400 (3,900–8,200)/mm³ and 187,000 (97,000–217,000)/mm³, respectively, at the termination of the therapy. Other signs and symptoms, such as proteinuria, neurological and psychiatric symptoms, were not observed.

**Discussion**

IFN therapy suppresses chronic HBV infection (12–16), however a sustained response to IFN has proven difficult to attain. In many studies the clearance rate of HBeAg after daily treatment with IFN for 4 weeks was only 15–25% for HBeAg positive patients with chronic hepatitis (17–19). The HBeAg seroconversion rate is at most 30–40% with larger IFN doses administered for 4 to 6 months. Moreover, patients treated with larger IFN doses often drop out of treatment studies due to IFN-related side effects and high-dose IFN is also very expensive. Thus, IFN therapy for HBeAg-positive chronic hepatitis B entails problems with efficacy, cost, and side effects.

Next, lamivudine therapy has recently been approved for treatment of chronic hepatitis B. However, the HBeAg seroconversion rate is at most 20–30% with lamivudine administered for one year. Moreover, prolonged lamivudine therapy sometimes accompanies tyrosine-methionine-aspartate-aspartate amino acid motif of HBV polymerase (YMDD) variant. The YMDD mutant virus often progresses to acute exacerbation in chronic hepatitis B (20, 21). Other treatments such as steroid withdrawal therapy and glycyrrhizin have been reported to suppress chronic hepatitis B. However, there are no absolute treatments in chronic hepatitis B at the present time.

α₃ is a synthetic polypeptide of thymic origin. The structure of α₃ is composed of twenty-eight amino acids and the molecular weight is 3,108. Next α₃ has been shown to modulate T-cell function, to promote endogenous IFN-γ, and interleukin-2 production by human lymphocytes and to increase lymphocyte interleukin-2 receptor expression (6,22). Moreover, α₃ can stimulate natural killer activity (23). Previous studies on α₃ therapy have had conflicting results, some suggesting that α₃ therapy was effective for chronic hepatitis B, whereas others found that α₃ therapy was not statistically effective. In these studies there were differences in the time of determination of response or not. The later the time to determine the therapeutic effect in α₃ therapy, the better the response rate (6).

Thus, we assessed the efficacy of α₃ therapy at 24 months after the initiation of α₃ therapy. Following the results of the present study, α₃ therapy at a dose of 0.8 or 1.6 mg via subcutaneous injection for 24 weeks was effective in chronic hepatitis B patients when, 1) transient acute exacerbation of ALT was present during the 24 week-course of α₃ therapy, 2) serum HBV-DNA level was <100 Meq/ml prior to the initiation of α₃ therapy. All patients with a response after α₃ therapy showed a slight or severe fluctuation pattern of serum ALT level. This fluctuation of serum ALT might be due to the natural course of chronic hepatitis B. However, in patients with a response the degree of serum ALT fluctuation during α₃ therapy was significantly
marked. This finding might indicate that Tα therapy affects the fluctuation of serum ALT. Most patients with a constant pattern of serum ALT during and after Tα therapy showed positivity of HBeAg and HBV-DNA 24 months after the initiation of Tα therapy. Therefore, when patients do not show a fluctuation of serum ALT after the initiation of Tα therapy, we suggest that another therapy should be selected without natural observation.

In the present study, the treatment schedule of high-dose (1.6 mg) Tα was chosen on the basis of previous clinical trials reporting the effectiveness of this dosage in the treatment of chronic hepatitis B (6–9). The treatment schedule of low-dose (0.8 mg) Tα was selected to compare the efficacy based on dosage of Tα. However, we found that the efficacy of low-dose (0.8 mg) Tα was not inferior to that of high-dose (1.6 mg) Tα. With respect to the total dose of Tα, Chien et al reported that the response rate of patients treated with Tα for 26 weeks was not statistically different from that of patients treated for 52 weeks (6). Tα has pharmacological actions with modulate T-cell function to promote endogenous IFN-γ, interleukin-2 production and to stimulate natural killer activity. The present results seem to indicate that the pharmacological actions of low-dose (0.8 mg) Tα, are similar to those of high-dose (1.6 mg) Tα.

With respect to side effects, no patients had a significant decrease in the leukocyte count or platelet count. Four patients showed serum ALT levels of more than 300 IU/l during Tα therapy and two of them had serum ALT levels of greater than 800 IU/l. However, no patient had acute hepatic failure, icterus or edema. Therefore, it is critical to carefully monitor patients treated with Tα therapy for possible acute exacerbation. Taken together, as Tα therapy should a moderate efficacy with few side effects, it would thus be a desirable strategy for chronic hepatitis B patients with a low level of serum HBV-DNA.

In the present study, we did not examine the antibody of human immunodeficiency virus. However, all patients had a normal lymphocyte count and serum γ-globulin level.

The results of the present trial showed that: 1) a 24-week course of Tα could be a worthwhile strategy for chronic hepatitis B patients with a serum HBV-DNA level of less than 100 Meq/ml; and 2) patients with transient acute exacerbation during Tα therapy generally respond well.

References