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Thymosin α_1 treatment of chronic hepatitis B: results of a phase III multicentre, randomized, double-blind and placebo-controlled study

M. G. Mutchnick,[1] K. L. Lindsay,[2] E. R. Schiff,[3] G. D. Cummings,[1] H. D. Appelman,[4] R. R. Peleman,[1] M. Silva,[3] K. C. Roach,[3] F. Simmons,[2] S. Milstein,[2] S. C. Gordon[1] and M. N. Ehrinpreis[1]

[1]*Division of Gastroenterology, Department of Medicine, Wayne State University School of Medicine, Detroit, MI,* [2]*Division of Gastrointestinal and Liver Diseases, Department of Medicine, University of Southern California, Los Angeles, CA,* [3]*Center for Liver Diseases, University of Miami School of Medicine and the Veterans Affairs Medical Center, Miami, FL and* [4]*Department of Pathology, University of Michigan, Ann Arbor, MI, USA*

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SUMMARY. Previous clinical trials have suggested that thymosin α_1 ($T\alpha_1$), an immunomodulatory peptide, may be effective in the treatment of chronic hepatitis B (CHB). The aim of this study was to determine the efficacy of $T\alpha_1$ in a multicentre, placebo-controlled and double-blind study of 97 patients with serum hepatitis B virus (HBV) DNA- and hepatitis B e antigen (HBeAg)-positive CHB. Patients who had been hepatitis B surface antigen (HBsAg) positive for at least 12 months entered a 3-month screening period prior to randomization. Forty-nine patients received $T\alpha_1$ (1.6 mg) and 48 patients received placebo, twice weekly for 6 months, and were followed-up for an additional 6 months. At inclusion, both groups were comparable for age, gender, histological grading, and aminotransferase and HBV DNA levels. A complete response to treatment, defined as a sustained serum HBV DNA-negative status (two negative results at least 3 months apart) during the 12-month study, with negative HBV DNA and HBeAg values at month 12, was seen in seven (14%) patients given $T\alpha_1$ and in two (4%) patients treated with placebo ($P = 0.084$). Five (10%) patients given $T\alpha_1$ and four (8%) patients given placebo exhibited a delayed response (defined as sustained serum HBV DNA negativity achieved after the 12-month study period with negative HBV DNA and HBeAg values at the last assessment). A total of 12 (25%) patients given $T\alpha_1$ and six (13%) patients given placebo showed a sustained loss of HBV DNA with a negative HBeAg value during or following the 12-month study period ($P < 0.11$). These results do not confirm observations of treatment efficacy reported in other clinical studies.

Keywords: chronic hepatitis B, thymosin, treatment.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is associated with potentially serious sequelae, including cirrhosis, liver failure and hepatocellular carcinoma (HCC). The problem is not inconsequential as newly acquired HBV infection occurs in 200 000–300 000 individuals annually in the USA with ≈ 5% of adults developing chronic HBV infection, which is characterized by persistence of serum hepatitis B surface antigen (HBsAg) and HBV DNA in both serum and liver [1,2]. The treatment of chronic hepatitis B is directed towards inhibition of viral replication followed by elimination of the virus. Histological improvement in the hepatic inflam-

Abbreviations: ALT, alanine aminotransferase; ANA, antinuclear antibody; CR, complete response; DR, delayed response; HAI, histological activity index; HBeAb, antibody to hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; IFN-α, interferon-α; IFN-α2b, interferon-α2b; IR, incomplete response; NR, non-responder; SC, subcutaneously; $T\alpha_1$, thymosin α_1; THF-γ_2, thymic humoral factor γ_2.

Correspondence: Dr Milton G. Mutchnick, Division of Gastroenterology, Harper Hospital, 3990 John R., Detroit, MI 48201, USA.

matory reaction with normalization of aminotransferases are additional goals of successful therapy.

Treatment of chronic hepatitis B with interferon-α (IFN-α) is associated with a sustained response rate of 30–40% [3]. Chronic HBV infection is believed to result, in part, from an inadequate host immune response to the virus [4,5]. Thus, immunomodulatory drugs, including thymic humoral factor γ_2 (THF-γ_2) and thymosin α_1 (Tα_1) have been tested in clinical trials both as single-drug therapy and in combination with IFN [6–11].

Tα_1 is a 28 amino acid peptide that modulates the maturation of T cells and influences immunoregulatory T-cell function [12]. In previous small studies, Tα_1 treatment was associated with cessation of viral replication as well as clinical and biochemical improvement in patients with either hepatitis B e antigen (HBeAg)- or hepatitis B e antibody (HBeAb)-positive, serum HBV DNA-positive chronic hepatitis B [7,13].

In this phase III, multicentre, randomized, double-blind and placebo-controlled study, we evaluated the efficacy of Tα_1 monotherapy in the treatment of chronic hepatitis B.

PATIENTS AND METHODS

Patients

Patients enrolled in the study met the following entry criteria: 18–75 years of age; the presence of serum HBsAg for at least 1 year; elevated serum alanine aminotransferase (ALT) on three occasions, at least 1 month apart, with an average value of ≥ 1.3 times the upper limit of normal; the presence of serum HBeAg and HBV DNA documented on three occasions, at least 1 month apart, within a period of 4 months before randomization; and liver biopsy features consistent with chronic hepatitis. The liver biopsy must have been performed within 1 year prior to screening for entry to the study. Additional requirements included: a haemoglobin value of ≥ 10 g dl^{-1}, a platelet count of ≥ 70 000 mm^{-3}, a white cell count of ≥ 3000 mm^{-3}, a polymorphonuclear count of ≥ 1500 mm^{-3} and normal renal function with normal serum creatinine levels. Candidates were required to have compensated liver disease with a prothrombin time less than 4 s, prolonged over control values, a serum albumin of ≥ 3.0 g dl^{-1}, a total bilirubin of ≤ 4 mg dl^{-1} and no history of hepatic encephalopathy or bleeding oesophageal varices. Exclusion criteria were as follows: a history of corticosteroid treatment within 6 months of entry; previous therapy with IFN within 1 year of inclusion; presence of antibody to human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis D virus (HDV); a history of malignancy, other than curatively treated skin cancer or surgically cured *in situ* carcinoma of the cervix; evidence of other forms of liver disease; or a history of intravenous drug abuse within the previous 5 years. Patients with other significant medical or psychiatric problems were also excluded as were patients with a progressive decrease (i.e. two consecutive samples each being less than 50% of the preceding value) in serum HBV DNA during screening, and women not agreeing to practice birth control for the 12-month duration of the study.

Two hundred and forty-five patients were entered into a 3-month screening period with 146 subsequently excluded for the following reasons: 37% were serum HBV DNA negative; 20% had low ALT levels; 10% were HBeAg negative; 3% were positive for antibody to HCV (anti-HCV); 13% were non-compliant; 8% were being screened when the study was closed; and 10% were excluded for miscellaneous causes. Signed informed consent, approved by the institutional review boards at each centre, was obtained from each patient.

Treatment protocol

In this prospective, double-blind, randomized and placebo-controlled study, 99 patients were stratified into two groups according to serum HBV DNA levels above or below 200 pg ml^{-1}, as measured at the final screening visit prior to entry. The two groups were sequentially randomized in pairs to receive either Tα_1, 1.6 mg subcutaneously (s.c.; Alpha One Biomedicals, Bethesda, MD), or placebo (mannitol, $NaPO_4$) s.c., twice weekly for 6 months. Patients were instructed on self-administration of the study drug. One patient from each treatment arm was randomized but did not receive the study drug and was therefore removed from the data analysis. In the initial study period, patients were seen at 2 weeks, 4 weeks and monthly thereafter until study completion at 12 months. Following the initial study period, patients were enrolled for long-term follow-up when they were seen at non-regular intervals.

At each visit, patients were examined and blood samples were taken for biochemical (on each visit) and

haematological (months 1, 3, 6 and 12) analyses, and for evaluation of HBsAg (months 3, 6 and 12), HBeAg (months 3, 6 and 12) and HBV DNA (months 1, 3, 6, 9 and 12). At a long-term follow-up visit, blood samples were taken for analysis of HBV DNA, HBeAg and ALT levels. Patients were monitored for compliance with the protocol by injection vial counts, telephone communications and attendance at scheduled out-patient clinic appointments.

Liver biopsy, where possible, was repeated within 1 month of completion of the initial study period. The first patients entered the study in January 1990 and the final patient completed the initial study period in April 1994, at which time the treatment code was revealed for all patients.

Biochemical and haematological (complete blood counts with differentials) analyses; evaluation of HBsAg; evaluation of antibodies to HDV, HIV, hepatitis B e antigen (HBeAg) and HBsAg; and detection of antinuclear antibody (ANA) and alphafetoprotein were carried out by a single laboratory (DMC Laboratories, Detroit, MI). Serum HBeAg (IMX; Abbott Laboratories, North Chicago, IL), serum HBV DNA (Abbott Hepatitis B Viral DNA, with a cut-off value of detection at 1.5 pg ml^{-1}; Abbott Laboratories) and anti-HCV (Ortho HCV ELISA Test System verification with Chiron RIBA HCV Test System; Chiron, Emeryville, CA) detection was completed at a single institution (by E. R. Schiff, Center for Liver Diseases, University of Miami, FL).

All liver biopsy specimens were analysed under code by a single pathologist (H.D.A.) for scoring in accordance with both the histological activity index (HAI) [14] and the Scheuer scoring system [15]. The HAI system, as devised by Knodell *et al.* [14] was designed for use in patients with autoimmune chronic hepatitis. In contrast, the Scheuer system was designed for chronic viral hepatitis. The HAI system has four components: periportal/bridging necrosis, lobular necrosis/degeneration, portal inflammation and fibrosis. The Scheuer system has three components. It combines portal inflammation and periportal necrosis into one component and its lobular activity and fibrosis components are similar to the HAI system, although slightly modified.

A complete virological response (CR) to the treatment was defined as non-detectable serum HBV DNA on at least two consecutive occasions, at least 3 months apart, during the initial study period, and non-detectable HBV DNA and negative HBeAg at month 6 post-treatment. A delayed response (DR) was defined as non-detectable HBV DNA on at least two consecutive occasions, first occurring during the long-term follow-up with HBV DNA, and a negative HBeAg value at the last assessment. An incomplete response (IR) was defined as non-detectable HBV DNA on at least two consecutive occasions with continued presence of HBeAg. A biochemical response was defined at month 12 as an ALT value ≤ 1.3 times the upper limit of normal and that was at least 50% lower than that observed at baseline. Increases in ALT ≥ twice the baseline value were defined as 'flares.'

Statistical analysis

The sample size for the number of patients was based on an expected difference in CR rates of at least 30% between patients treated with $T\alpha_1$ and those given placebo. Using $\alpha = 0.05$ and $\beta = 0.20$, 45 patients were required for each randomization arm. The total number of patients of 99 thus allowed for a 10% dropout rate. An intent-to-treat analysis was performed.

The student's two-tailed unpaired *t*-test was used to compare treatment groups. Pre- and post-treatment liver biopsy scores were compared, using both the HAI and Scheuer scoring, by the Student's two-tailed paired *t*-test. Fisher's exact test was used to compare group means.

RESULTS

Data on 97 patients, comprising 49 randomized to $T\alpha_1$ and 48 randomized to placebo, were used in these analyses. At inclusion, the two study groups were similar with respect to biochemical values, serum HBV DNA levels and demographics (Table 1). Three patients in the $T\alpha_1$ group and one patient in the placebo group did not complete the 6-month treatment period. An additional patient treated with $T\alpha_1$ and three patients given placebo did not complete the post-treatment 6-month follow-up period. Therefore, a total of 45 patients treated with $T\alpha_1$ and 44 patients in the placebo group completed the initial 12-month study period.

Initial study period

Seven (14%) patients in the $T\alpha_1$ group and two (4%) patients given placebo demonstrated a complete response to therapy ($P = 0.084$; Table 2). The number

Table 1 Characteristics of study groups at inclusion*

Characteristic	$T\alpha_1$ group ($n = 49$)	Placebo group ($n = 48$)
Age (in years)†	43 ± 14	40 ± 13
Male: female	44: 5	39: 9
Homosexual/bisexual	13 (27%)	13 (27%)
Duration of infection (years)	5.0 ± 4.2	4.2 ± 3.2
Previous IFN therapy	6 (12%)	3 (6%)
Serum ALT (IU l^{-1})‡	147 ± 135	180 ± 117
Serum AST (IU l^{-1})	109 ± 135	123 ± 98
Albumin (g dl^{-1})	4.0 ± 0.4	4.1 ± 0.3
Total bilirubin (mg dl^{-1})	0.8 ± 0.5	0.7 ± 0.3
Serum HBV DNA (pg ml^{-1})	147 ± 190	114 ± 112
No. with baseline HBV DNA		
< 200 pg ml^{-1}	35	36
≥ 200 pg ml^{-1}	14	12
Histology		
Mild chronic hepatitis	13 (27%)	8 (17%)
Moderate/severe chronic hepatitis	28 (57%)	31 (65%)
Active cirrhosis	8 (16%)	9 (19%)

*No significant differences were observed between groups.
†Values represent mean ± SD.
‡Normal values: ALT, < 50 IU l^{-1}; AST, < 50 IU l^{-1}; albumin, 3.5–5.2 g dl^{-1}; total bilirubin, < 1.5 mg dl^{-1}.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; IFN, interferon, $T\alpha_1$, thymosin α_1.

of patients with a delayed or incomplete response are shown in Table 2. The frequency of normalization or near normalization of the ALT level, loss of serum HBV DNA and conversion to an HBeAg-negative status at months 6, 9 and 12 is shown in Table 3. Serum HBV DNA levels in the $T\alpha_1$ group at 6 (115 ± 154 pg ml^{-1}) and 12 months (82 ± 112 pg ml^{-1}) were not significantly different from the corresponding levels in the placebo group (83 ± 122 pg ml^{-1}, 65 ± 98 pg ml^{-1}, respectively).

Table 2 Response to treatment*

Study group (n)	CR†	DR	IR
$T\alpha_1$ (49)	7 (14%)‡	5 (10%)	0
Placebo (48)	2 (4%)	4 (8%)	2 (4%)

*Response was determined based on the results of a 6-month treatment period with a follow-up of at least 6 months.
†For definition of response, see the Subjects and methods.
‡$P = 0.084$, $T\alpha_1$ *vs* placebo.
CR, complete response; DR, delayed response; IR, incomplete response; $T\alpha_1$, thymosin α_1.

Pre- and post-treatment paired liver biopsy specimens were available for 33 patients in the $T\alpha_1$ group and for 32 patients in the placebo group. No significant differences in the Knodell HAI total score or in the total score minus fibrosis were seen between the two groups when pre- and post-treatment biopsy pairs were compared. Using the Scheuer scoring system, significant differences between pre- and post-treatment biopsy pairs were seen in the $T\alpha_1$ responders (Table 4). No significant difference was seen between pre- and post-biopsy pairs in five of the six placebo-treated patients who met the response criteria (Table 4).

ALT flares were seen in 21 of 49 (43%) $T\alpha_1$ recipients and in 18 of 48 (38%) placebo recipients (P =NS). Five of seven $T\alpha_1$ CR and only one of five $T\alpha_1$ DR experienced a flare where the ALT rose to nine times higher than the baseline level before returning to normal. Jaundice did not occur and the patient remained asymptomatic. One of two placebo CR, none of four placebo DR and one of two placebo IR exhibited flares in ALT. Thus, six of the 12 $T\alpha_1$ responders (CR + DR) and only two of the eight placebo responders (CR + DR + IR) experienced ALT flares. Five of the $T\alpha_1$

Table 3 Number of patients (*n*) with an alanine aminotransferase (ALT) response and loss of serum hepatitis B virus (HBV) DNA and hepatitis B e antigen (HBeAg) during the initial study period

	ALT (months)			HBV DNA (months)			HBeAg (months)	
Study group (*n*)	6	9	12	6	9	12	6	12
$T\alpha_1$ (49)	8 (16)*	10 (20)	11 (23)	7 (14)	9 (18)	10 (20)	6 (12)	11 (23)
Placebo (48)	12 (25)	15 (31)	13 (27)	7 (15)	7 (15)	10 (21)	4 (8)	7 (15)

*(), percentage based on intent-to-treat.
$T\alpha_1$, thymosin α_1.

responders experienced flares during the 6-month treatment period as did both placebo patients. Fifteen (41%) of the 37 $T\alpha_1$ NR (non-responders) and 16 (40%) of the 40 placebo NR also experienced ALT flares. Of those individuals who experienced flares, there were no differences between the placebo NR and the $T\alpha_1$ NR in the number of patients experiencing 1, 2, 3 or more ALT flares during the 12-month study (64%, 18% and 18% *vs* 67%, 20% and 13%, respectively).

None of the patients in either treatment group became HBsAg negative during the initial study period.

Of three placebo recipients who had failed previous therapy with IFN-α2b, one was a CR in the present study. Six patients treated with $T\alpha_1$ had previously undergone IFN-α2b treatment without response. One of these six patients developed a CR and the others were $T\alpha_1$ NR.

Long-term follow-up

Of the 89 patients who completed the initial study period (12 months), 14 were unavailable for long-term follow-up. Thirty-eight patients treated with $T\alpha_1$ and 37 patients given placebo entered long-term follow-up (26 ± 8 months, range 14–44 months; and 26 ± 9 months, range 15–44 months; respectively).

All 12 $T\alpha_1$ and six placebo responders (CR + DR) were followed-up for 27 ± 9 months (range 18–44 months) and 26 ± 8 months (range 15–35 months), respectively. Reactivation of disease, as defined by reappearance of serum HBV DNA, occurred in two of the seven $T\alpha_1$ CR and in neither of the two placebo CR. In one $T\alpha_1$ CR with disease reactivation, reappearance of serum HBV DNA at 21 months of follow-up was preceded by detection of HBeAg and ALT elevation at month 18. Recurrent serum HBV DNA in the second $T\alpha_1$ CR at 15 months of follow-up was not accompanied by ALT elevations or detectable HBeAg. One $T\alpha_1$ CR and one placebo DR, neither of whom had previously received IFN-α2b, became HBsAg negative during the long-term follow-up (at months 37 and 30, respectively).

Of the 37 patients who were $T\alpha_1$ NR in the initial study period, three became HBV DNA negative during the long-term follow-up with one patient also

Table 4 Scheuer scoring of paired liver biopsy specimens

Group	*n*	Pretreatment biopsy score	Post-treatment specimen score	*P*-value*
$T\alpha_1$	33	5.7 ± 2.5†	5.8 ± 2.1	NS
Non-responders	23	5.1 ± 2.4	6.0 ± 2.0	NS
CR‡	6	7.0 ± 2.8	5.3 ± 2.7	< 0.02
CR + DR	10	7.0 ± 2.3	5.2 ± 2.2	< 0.01
Placebo	32	5.9 ± 2.2	5.6 ± 2.4	NS
CR + DR	5	6.0 ± 2.4	4.4 ± 3.0	NS

**P*-values were determined using the Student's paired *t*-test.
†Values are expressed as mean ± SD.
‡CR, complete responders; DR, delayed responders; $T\alpha_1$, thymosin α_1.

experiencing loss of HBeAg. Of the 40 placebo NR in the initial study period, three became HBV DNA negative during the long-term follow-up, but all three remained HBeAg positive.

Safety

No serious adverse effects were observed in patients given Tα_1 and no dose adjustment was required. There were two deaths during the initial study period as a result of underlying liver disease. One patient receiving Tα_1 died of complications following a variceal haemorrhage, and a patient given placebo developed spontaneous bacterial peritonitis, became septic, resulting in death.

DISCUSSION

This multicentre study shows that although there was a trend towards efficacy with Tα_1 in the treatment of chronic hepatitis B, statistical significance was not achieved ($P = 0.084$). A sustained and complete virological response (undetectable HBV DNA, negative HBeAg) to Tα_1 was seen in seven (14%) patients at the conclusion of the initial study period in contrast to the spontaneous remission seen in two (4%) patients given placebo. When patients were entered into long-term follow-up, loss of HBeAg and HBV DNA negativity was seen in 12 (25%) Tα_1 recipients and in six (13%) patients given placebo ($P = 0.11$).

The outcome of this phase III study stands in contrast to other clinical trials of chronic hepatitis B where Tα_1 was shown to be both effective and safe when used as single-drug therapy or in combination with IFN [7–10]. In one study, HBeAg-negative and serum HBV DNA-positive chronic hepatitis B patients given Tα_1 had a 29% CR rate at the conclusion of a 6-month treatment period and a 41% CR rate at the end of a 6-month follow-up period [8]. In a recent trial examining the effect of treatment duration on response, clearance of serum HBeAg and HBV DNA was observed in 41% of 32 patients who were given Tα_1 for 6 months and followed-up for an additional 12 months. When patients were treated with Tα_1 for 12 months, a CR was seen in 27% of 34 patients 6 months after stopping treatment [10]. Responders to Tα_1 have been shown to convert to an HBV DNA-negative status in the post-treatment period as well as during treatment [7–10]. The characteristic Tα_1 DR noted in other Tα_1 trials [8,10] was also seen in this study. Seven (58%) of the 12 Tα_1 responders in the current study experienced sustained nondetectable HBV DNA after the 6-month treatment period. Tα_1 dosage, injection schedules and duration of treatment in the present study were similar to those used in other clinical trials [8,10].

Tα_1 is not believed to possess antiviral properties and although it shares some sequence homologies with IFN-α its mechanism of action is unknown [16]. Tα_1 may possess immunoregulatory properties that can promote an endogenous antiviral immune response [8]. Tα_1 has been used to treat patients with cancer or immunodeficiency, resulting in an up-regulation of lymphocyte function to include mitogen responsiveness, T-cell maturation, enhanced T-lymphocyte cytotoxicity and increased B-lymphocyte antibody production [12,17]. Furthermore, Tα_1 is found in and secreted by lymphocytes, justifiably characterizing it as a cytokine [18]. Other thymus-derived compounds have been used in clinical trials. A synthetic octapeptide, THF-γ_2, has been studied in nine patients who had previously failed IFN-α2b treatment [11]. Patients received THF-γ_2 single therapy for 2 months, a combination of THF-γ_2 and IFN-α2b for 2 months, and then IFN-α2b alone for 2 months. Three of the nine patients seroconverted to HBV DNA-negative and HBeAb-positive status. THF-γ_2 appeared to potentiate the suppressive effect of IFN-α2b on HBV replication [11].

Tα_1 has also been used in combination with low-dose lymphoblastoid IFN (3 million units (MU) twice weekly for 6 months) in 15 patients with chronic hepatitis B, including 11 patients who had failed previous IFN-α2b therapy. Nine (60%) of the patients, including six (55%) of the 11 patients previously treated with IFN-α2b, showed a sustained response by losing serum HBV DNA. No reactivation of disease was observed in these patients during a 12-month post-treatment follow-up [9].

The dose, frequency and duration of Tα_1 therapy selected for the present study did not confirm findings of an earlier pilot trial [7]. Clearly, evaluation of dose–response in future trials may demonstrate that Tα_1 retains a potential role as single-drug therapy, particularly given the absence of significant side-effects. It is more likely that the promising results seen in recent trials, where Tα_1 was used in combination with IFN [9], will lead to controlled studies assessing combination Tα_1 therapy with IFN or perhaps with the nucleoside analogue, 3TC. Combination therapy may provide a

more effective approach for the treatment of chronic hepatitis B.

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