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Preliminary results of Thymosin-a1 versus interferon-α treatment in patients with HBeAg negative and serum HBV DNA positive chronic hepatitis B

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a serious problem because of its worldwide distribution and possible adverse sequelae, such as cirrhosis and hepatocellular carcinoma. The World Health Organization estimates that HBV has infected more than 350 million people worldwide, and up to 20% of them will become chronic carriers and will be at significant risk for cirrhosis and HCC. The ultimate goal of the therapy for chronic hepatitis B is to prevent progression to cirrhosis and to prevent development of HCC. Various subgroups of hepatitis B surface antigen (HBsAg) positive patients with chronic hepatitis have been identified. Typical patients have hepatitis B e antigen (HBeAg) and HBV DNA in serum during the active phase of the disease and usually show remission if they seroconvert to antibody to HBeAg (anti-HBe). However, a subset of patients has been found to be HBeAg negative but instead to have anti-HBe and HBV DNA in serum. This form of hepatitis is characterized by a progressive and relapsing course with fluctuations of viral replication[1,2] and a poor response to interferon α (IFN-α) therapy[3-11].

Thymosin a1 (T-a1) is an immune modifier that has been shown to trigger maturational events in lymphocytes, to augment T-cell function, and to promote reconstitution of immune defects[12]. T-a1 has been shown to promote disease remission and cessation of HBV replication in patients with HBeAg-positive chronic hepatitis B without significant side effects[13,14]. Moreover, clinical trials using T-a1 in the treatment of patients with immunodeficiency or cancer indicate that this agent is nontoxic, enhances immune responsiveness and augments specific lymphocyte functions, including lymphoproliferative responses to mitogens, maturation of T-cells, antibody production, and T-cell mediated cytotoxicity[15,16]. Based on these observations, we conducted a randomized, controlled trial to compare the efficacy and the safety of T-a1 versus IFN-α therapy in anti-HBe and HBV DNA positive chronic hepatitis B.

MATERIALS AND METHODS

Materials

Forty-eight Chinese patients were enrolled in the study. All patients met the following criteria for entry: age between 18 and 60 years; presence of HBsAg in serum for at least 12 months; positive serum tests for anti-HBe and HBV DNA, documented on at least two occasions and at least 3 months apart during the 12 months before entry; aminotransferase levels higher than 1.5 times that the upper normal limit for at least 12 months; and liver biopsy taken within 3 months before enrollment showing chronic hepatitis. Eligible patients with evidence of cirrhosis were also included. Patients treated with immunosuppressive or antiviral therapy within 1 year before entry, and those with concurrent hepatitis C virus, hepatitis delta virus, and human immunodeficiency virus infections, causes of liver disease other than HBV, intravenous drug abuse, pregnancy, malignancy, decompensated liver disease, chronic renal failure, or other serious medical illness that might interfere with this trial were excluded.

Thirty patients with the same virological and clinical characteristics, who were never treated with IFN-α and followed up for at least 12 months, were used as a historical control (HC) group to evaluate the efficacy of the therapies.

Methods

Forty-eight patients were randomly divided into two groups to receive either a 6-month course of T-a1 (Zasaxin, supplied by SciClone Pharmaceuticals Inc., San Mateo, CA) at a dose of 1.6 mg

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subcutaneous injection twice a week or a 6-month course of IFN-α at a dose of 3-5 MU subcutaneous injection daily for fifteen days, then three times weekly. The patients assigned as a historical control group were followed up without specific treatment. All patients were assessed biweekly for the first 2 months of study, and then monthly for a total study duration of 12 months. Clinical and laboratory assessments consisted of a detailed history, including postinjection symptoms and physical examination; routine serum biochemical tests (serum alanine transaminase (ALT), aspartate transaminase (AST), r-glutamine transpeptidase (r-GT), alkaline phosphatase (AKP), albumin, globulin, bilirubin, etc.); complete cell count; markers of HBV replication and urine analysis. All biochemical and hematological tests were performed with routine automated techniques. HBV-markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb and lgm HBcAb) were detected by enzymelinked immunosorbent assay (ELISA) method. Serum HBV DNA was detected by polymerase chain reaction (PCR) method.

Responses were evaluated both at the end of the therapy and at the end of follow-up. A virological response was defined as sustained disappearance of serum HBV DNA, and a biochemical response as sustained normalization of serum ALT. At the end of the treatment and follow-up, a complete response was defined as HBV DNA clearance from the serum and normalization of ALT activity. Relapse was assessed on the basis of ALT flare and/or HBV DNA reappearance during the follow-up period.

Statistical analysis

Analysis of data was accomplished using Chi-square test. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Of the 48 patients enrolled in the study, 18 were randomized to receive T-a1 and 30 to receive IFN-α and all were followed up for 6- months. The three groups were not significantly different in age, sex, biochemical, histological, serological parameters and number of patients with histological evidence of cirrhosis.

The biochemical and virological modifications at the end of treatment and follow-up period in the two treated groups and the biochemical and virological events in the HC group are illustrated in Table 1. In the group receiving T-a1, serum HBV DNA was negative in 9 of 18 patients at the end of treatment. During the follow-up period, five other patients showed HBV DNA loss at the 2nd, 3rd, 5th (in 2 patients) and 6th month, respectively, whereas HBV DNA reappeared in two at the 2nd and 3rd month, respectively. In the group receiving IFN-α, 18 of 30 patients showed HBV DNA loss at the end of treatment. However, during the 6-month follow up, HBV DNA reappeared in 9 patients (in 2 patients at the 1st month, in 5 at the 2nd and in 2 at the 3rd month), while no one lost HBV DNA. In the HC group, HBV DNA became negative in 3 of 30 patients at the 6th (in 2 patients) and 12th month, respectively, whereas HBV DNA reappeared in one at the 8th month. HBV DNA loss was significantly higher in the T-a1 and IFN-α groups compared with the HC group both at the end of therapy (χ^2=11.98, P<0.01 and χ^2=19.21, P<0.01, respectively) and follow-up period (χ^2=19.58, P<0.01 and χ^2=5.46, P<0.05, respectively).

Table 1 Responses to treatment in patients with chronic hepatitis B

	T-a1 (n = 18)	IFN-α (n = 30)	HC (n = 30)
	After 6 mo of treatment		After 6 mo of follow-up
ALT normalization	7 (38.9%)	15 (50%)	3 (10%)
HBV DNA-negative	9 (50%)[b]	18 (60%)[b]	2 (6.7%)
ALT normal / HBV DNA-negative	6 (33.3%)	14 (46.7%)[b]	1 (3.3%)
	After 6 mo of follow-up		After 12 mo of follow-up
ALT normalization	12 (66.7%)[ab]	10 (33.3%)	2 (6.7%)
HBV DNA-negative	12 (66.7%)[ab]	9 (30%)	2 (6.7%)
ALT normal / HBV DNA-negative	10 (55.6%)[ab]	7 (23.3%)	1 (3.3%)

[a]P<0.05, *vs* IFN-α; [b]P<0.01, *vs* HC.

Serum ALT levels fell within the normal range in 7 of 18 patients given T-a1, in 15 of 30 patients in IFN-α group at the end of treatment and in 3 of 30 of the HC group after 6 months of follow-up. During the follow-up, ALT became normal in six patients receiving T-a1 and ALT flare occurred in one patient, whereas five patients of the IFN-α group showed ALT flare, and no one had normal ALT. In the HC group, ALT was normal in two patients between the 6th and 12th month of follow-up and ALT flare was seen in the three patients who had normal ALT during the first 6 months of follow-up. At the end of the study period, a complete response (ALT normalization and HBV DNA loss) was observed in 10 (55.6%) of 18 patients treated with T-a1, in 7 (23.3%) of 30 receiving IFN-α, and in 1 (3.3%) of 30 in HC patients (T-a1 *vs* IFN-α, P<0.05 and T-a1 *vs* HC, P<0.01).

Typical side effects of IFN-α treatment, such as flu-like syndrome, fatigue, irritability, and headache, were seen in most of the patients treated with IFN-α. However, no serious or long-term side effects were noted and no patients discontinued the treatment. Therapy with T-a1 was not associated with significant side effects. Only one patient reported local discomfort at injection sites. No systemic or constitutional symptoms were observed with T-a1 administrations.

DISCUSSION

Anti-HBe and HBV DNA-positive chronic hepatitis B is a clinical entity distinct from classical HbeAg positive chronic hepatitis B. This peculiar form of hepatitis B is usually characterized by a severe progressive outcome often leading to cirrhosis and only occasionally shows spontaneous remission. These patients present fluctuations of viral replication in which relapses of hepatitis and periods of biochemical remission and HBV DNA negativity may occur. IFN-α treatment does not appear to be as successful as in HBeAg positive disease[3-11]. Clinical studies suggested that IFN-α at the dosage ranging from 3MU to 9MU three times a week for 6months is able to suppress HBV replication in more than 50% of treated patients, but the relapse rate after treatment withdrawal is high[3-11]. In our study, a complete response was seen in 46.7% of the patients at the end of treatment and in 23.3% at the end of follow-up. Our results are similar to those previously reported, and the response rate is far from satisfactory.

The results of the present randomized, controlled trial have shown that T-a1 therapy at a dose of 1.6 mg via subcutaneous injection twice a week for 6 months is effective and safe in anti-HBe and HBV DNA-positive chronic hepatitis B, because nearly 60% of the treated patients became HBV DNA-seronegative 6 months after the end of therapy. This response rate is not only significantly higher than that of the spontaneous seroconversion rate (3.3% in this study), but also obviously higher than the response to IFN-α therapy alone (23.3%) assessed 6 months after the end of therapy. The study showed that, at the dose tested, T-a1 has the same efficacy as IFN-α in inducing clinical and virological remission. The response rate in terms of ALT normalization and/or HBV DNA loss was not significantly different in the T-a1 group as compared with the IFN-α group at the end of the treatment ($P>0.05$). But there was significantly difference in the response rate between the two groups at the end of the follow-up ($P<0.05$). The normalization of serum ALT and loss of HBV DNA were observed more frequently in the IFN-α group at the end of therapy and in the T-a1 group at the end of follow-up. Furthermore, in the T-a1 group, the response to the treatment was also observed during the follow-up period, but not in the IFN-α group. On the basis of these results and considering that ALT normalization and HBV DNA negativization may spontaneously occur in the untreated patients infected by the precore mutant virus, we retrospectively compared the two treated groups with a group of untreated patients followed for at least 12 months. The results showed that a significant higher rate of complete response occurred in the IFN-α group at the end of therapy and in the T-a1 group at the end of follow-up compared with the HC group.

It is noted that the benefit of T-a1 was not immediately apparent at the end of therapy. There was a trend for complete virological response to increase or accumulate gradually after the end of thymosin therapy. This trend was also reported in a multicenter American trial in which 5 of the 12 responders to T-a1 therapy showed a delayed response [17]. This is in contrast to therapy with IFN-α, in which responses usually occur during the first 4 months of treatment. These contrasting patterns of response were best demonstrated in a recent Italian study involving HbeAg negative, HBV DNA-positive, interferon naive patients with higher ALT level (181 ± 159U/L), in which the complete response (ALT normalization and HBV DNA loss) rate increased gradually from 29.4% at the end of therapy to 41.2% 6 months after the end of T-a1 therapy. In that study, the response to interferon therapy decreased from 43. 8% at the end of therapy to 25% 6 months after the end of therapy[18]. This trend of delayed effect of T-a1 was also reported by Chien *et al*. in patients with chronic hepatitis B recently[14]. The reasons for this delayed effect of T-a1 are not clear. The delayed response is not likely a result of direct antiviram effects similar to those of interferons. T-a1 may exert an immunoregulatory function that promotes the endogenous antiviral immune response, as previously suggested, improving the effectiveness and coordination of the host cellular immune mechanisms in clearing HBV infected hepatocytes.

It has been shown that patients treated with T-a1 have a higher peripheral blood helper T cell count (CD4) and IFN-α production by peripheral blood mononuclear cells during and after the end of T-a1 therapy[13]. In view of the immune mechanisms involved in the pathogenesis of liver injuries in chronic HBV infection, it is possible that T-a1 may activate viral-specific helper T cells and result in the amplification of the humoral immune response to viral proteins and the induction of viral antigen-specific cytotoxic T lymphocytes through secreting endogenous IFN-α, IFN-γ, interleukin-2, and tumor necrosis factor, and increase lymphocyte interleukin-2 receptor expression[19-28]. Moreover, T-a1 is able to act synergistically with endogenous IFN-α and IFN-β in stimulating natural killer activity[29]. Although T-a1 is not known to possess antiviral properties, a preliminary report showed that this agent is able to inhibit woodchuck hepatitis virus replication[30]. Hence, the delayed effect after the end of T-a1 therapy in the present study was possibly caused by the immunomodulating effect of T-a1 that induced persistently higher helper T-cell function. Because noncytolytic inhibition of HBV RNA, nucleocapsid particles, and replicative DNA intermediates by cytotoxic T lymphocytes has been described in the transgenic mouse model[31,32], it is also possible the viral clearance after T-a1 therapy,

particularly those without preceeding ALT flaring, may be mediated by noncytolytic antiviral effects of cytotoxic T lymphocytes. Clearly, further studies are needed to elucidate the possible mechanisms.

The tolerability of T-a1 was excellent and without side effects. This finding together with a lower number of weekly injections could favor better patient compliance.

In conclusion, the results of this trial indicate that, at the dosage tested, T-a1 is of potential interest in patients with anti-HBe and HBV DNA-positive chronic hepatitis B. Furthermore, compared with IFN-α, T-a1 is better tolerated and seems to induce a gradual and more sustained ALT normalization and HBV DNA negativization, so it might represent an alternative to IFN-α therapy. However, a response rate of 55.6% is still less than ideal. A more effective therapeutic approach, such as combination therapy using the immunomodulating effect of T-a1 and antiviral effect of interferon or nucleoside analogues (such as lamivudine, famciclovir, etc.), warrants further studies.

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