PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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A randomized controlled clinical trial on the treatment of Thymosin a1 versus interferon-α in patients with hepatitis B

Jing You, Lin Zhuang, Bao Zhang Tang, Wei Bo Yang, Su Ying Ding, Wu Li, Rong Xue Wu, Hong Li Zhang, Yan Mei Zhang, Shao Ming Yan and Lu Zhang

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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 (HCC)[1,2]. The World Health Organization estimates that HBV has infected more than 350 million people worldwide, and up to 20% of those infected with HBV will go on to become chronic carriers and be at significant risk for cirrhosis and HCC. The ultimate goals of therapy for chronic hepatitis B are to prevent progression to cirrhosis and to prevent development of HCC. Over the past 20 years, many antiviral or immunomodulatory agents, or both, have been used in patients with chronic HBV infection[3-9]. Among them, interferon alfa (IFN-α), the standard treatment for chronic HBV infection, has been shown to be effective, which induces an apparent initial response in approximately 40% of treated patients[10,11]. However, the response rate is far from satisfactory, particularly in Asian patients, the relapse rate after treatment withdrawal is high[12].

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[13]. 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[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]. On the basis of these observations, we conducted a randomized, controlled trial to compare the efficacy and the safety of T-a1 versus INF-α therapy in patients with chronic hepatitis B.

MATERIALS AND METHODS

Materials

Fifty-one Chinese patients were enrolled in the study. All patients met the following criteria for entry: age between 18 and 60 years; presence of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) in the serum for at least 12 months; positive serum tests for HBV DNA, documented on at least two occasions, at least 3 months apart, during the 12 months before entry; aminotransferase levels higher than 1.5 times 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 and severe hepatitis B 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, 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, 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

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

[1]Department of Infectious Diseases, The First Affiliated Hospital of Kunming Medical College, Kunming 650032, Yunnan Province, China
[2]Department of Hepatology, Kunming Third Municipal People's Hospital, Kunming 650041, Yunnan Province, China
Jing You, graduated from Kunming Medical College in 1984, now associate professor of internal medicine, specialized in studies on gastroenterology and hepatology, having more than 40 papers published.
Correspondence to: Jing You, Department of Infectious Diseases, The First Affiliated Hospital of Kunming Medical College, 153# Xi Chang Road, Kunming 650032, Yunnan Province, China
Tel. 0086-871-5324888
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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 each day for 15 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, ets.); 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 Igm HBcAb) were detected by enzyme-linked immunosorbent assay (ELISA) method. Serum HBV DNA was detected by polymerase chain reaction (PCR) method.

Responses were evaluated both at the end of therapy and at the end of follow-up. A complete virological response was defined as a sustained loss of serum HBeAg in association with the disappearance of serum HBV DNA during the 12 months study. A biochemical response was defined as sustained normalization of serum ALT. At the end of treatment and follow-up, a complete response was defined as HBV DNA and HBeAg clearance from the serum and normalization of ALT activity. Relapse was assessed on the basis of ALT flare and/or HBV DNA/HBeAg 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 81 patients enrolled in the study, 30 had never been treated for HBV with IFN-α, and 2 were non-responders to earlier IFN-α therapy who received T-a1 in the study. Eighteen patients were randomized to receive T-a1 and 33 to receive IFN-α. All patients completed the 6-month follow-up. 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 Tables 1 and 2. In the group receiving T-a1, serum HBV DNA was negative in 10 of 18 patients at the end of treatment. During the follow-up period, five other patients showed HBV DNA loss at the 2nd, 3rd, 4th (in 2 patients) and 6th month, respectively, whereas HBV DNA reappeared in two (at the 3rd and 5th month, respectively). In the group receiving IFN-α, 22 of 33 patients showed HBV DNA loss at the end of treatment. However, during the 6 months of follow-up, HBV DNA reappeared in 9 patients (in 3 patients at the 1st month, in 4 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 5th, 6th, and 11th month, respectively), whereas HBV DNA reappeared in one (at the 7th month). HBV DNA loss was significantly higher in the T-al and IFN-α groups compared with the HC group both at the end of therapy (χ^2=28.97, P<0.01 and χ^2=23.99, P<0.01, respectively) and follow-up period (χ^2=22.49, P<0.01 and χ^2=9.27, P<0.01, respectively). The rates of seroconvertion of HBVe antigen antibody in the T-al, IFN-α and HC groups at the end of treatment were 33.3% (6/18), 45.5% (15/33) and 3.3% (1/30), respectively; and at the end of follow-up 55.6% (10/18), 27.3% (9/33) and 3.3% (1/30),respectively. Serum ALT levels fell within the normal range in 7 of 18 patients given T-al, in 16 of 33 patients in IFN-α group at the end of treatment and in 5 of 30 of the HC group after 6 months of follow-up. During the follow-up, five patients receiving T-al normalized ALT and one patient showed ALT flare, whereas six patients of the IFN-α group showed ALT flare, and no one normalized ALT. In the HC group, two patients normalized ALT between the 6th and 12th month of follow-up and an ALT flare was seen in the four patients who normalized ALT during the first 6 months of follow-up. At the end of the study period a complete response (ALT normalization and HBV DNA/HBeAg loss) was observed in 10 of 18 (55.6%) patients treated with T-al, in 9 of 33 (27.3%) receiving IFN-α, and in 1 of 30 (3.3%) in HC patients (T-al *vs* IFN-α, HC,P<0.01).

Table 1 Responses to treatment at the end of therapy *n*(%)

	T-a1 (n = 18)	IFN-α (n = 33)	HC (n = 30)
	(after 6 mo of follow-up)		
ALT normalization	7 (38.9)	16 (48.5)	5 (16.7)
HBV DNA-negative	10 (55.6)[b]	22 (66.7)[b]	2 (6.7)
ALT normal / HBV DNA and HBeAg-negative	6 (33.3)	15 (45.5)[b]	1 (3.3)

[b]P<0.01, *vs* HC.

Table 2 Responses to treatment at the end of follow-up *n*(%)

	T-a1 (n = 18)	IFN-α (n = 33)	HC (n = 30)
	(after 12 mo of follow-up)		
ALT normalization	11 (61.1)[a]	10 (30.3)	3 (10)
HBV DNA-negative	13 (72.2)[ab]	13 (39.4)[b]	2 (6.7)
ALT normal / HBV DNA and HBeAg-negative	10 (55.6)[ab]	9 (27.3)	1 (3.3)

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

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-al was not associated with significant side effects. Three patients reported local discomfort at injection sites. No systemic or constitutional symptoms were observed with T-al administrations.

DISCUSSION

The results of the present randomized, controlled trial have shown that T-a1 therapy at a dose of 1.6 mgvia subcutaneous injection twice a week for 6 months is effective and safe in patients with chronic hepatitis B, because nearly 60% of the treated patients became HBeAg- and HBV DNA-seronegative 6 months after the end of therapy. This response rate was 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 (27.3%) 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 rate of response in terms of ALT normalization and/or HBV DNA and/or HBeAg loss was not significantly different in the T-a1 group compared with the IFN-α group at the end of the treatment (P>0.05). But there was significantly difference on the rate of response between the two groups at the end of the follow-up periods (P<0.05). The normalization of serum ALT and loss of HBV DNA and HBeAg were observed more frequently in the IFN-α group at the end of therapy and in the T-a1 group at the end of the follow-up. Furthermore, in the T-a1 group the response to the treatment was observed also 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/HBeAg negativization may spontaneously occur in untreated patients, 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 ± 159 U/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[19]. The reasons for this delayed effect of T-a1 are not clear. The delayed response is not likely a result of direct antiviral effects similar to those of interferons. On speculating, 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-γ production by peripheral blood mononuclear cells during and after the end of T-a1 therapy[14]. 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-ymphocytes through secreting endogenous IFN-α, IFN-γ, interleukin-2, and tumor necrosis factor, and increase lymphocyte interleukin-2 receptor expression[20-29]. Moreover, T-a1 is able to act synergistically with endogenous IFN-α and IFN-β in stimulating natural killer activity[30]. 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[31]. 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[32,33], it is also possible that 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, a 6-month T-a1 therapy is safe and effective in arresting HBV replication and

reducing lobular activity in patients with 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/HBeAg loss, so it might represent an alternative to IFN-α therapy. However, a response rate of 50% is still not sastifactory. 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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