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Comparison of the efficacy of thymosin alpha-1 and interferon alpha in the treatment of chronic hepatitis B: A meta-analysis

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Abstract

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. Thymosin alpha-1 (Tα1) is an immune modifier that has been shown to be effective for chronic hepatitis B (CHB) in some trials. But the trials comparing Tα1 vs. interferon alpha (IFNα) treatment in CHB have been small and the results have been inconsistent. So we conducted a meta-analysis to compare the efficacy of Tα1 and IFNα in the treatment of CHB. Generally, four randomized controlled trials including 199 CHB patients who received Tα1 or IFNα treatment were identified through MEDLINE and EMBASE online search. Virological (for hepatitis B e antigen (HBeAg) positive patients, loss of HBV DNA and HBeAg; for HBeAg negative patients, loss of HBV DNA), biochemical (normalization of transaminases) and complete responses (fulfill criteria of biochemical and virological response simultaneously) were analyzed using the intention-to-treat method. The odds ratio (OR) was used to measure the magnitude of the efficacy. The ORs (95% confidence interval) of the virological response, biochemical response and complete response of Tα1 over IFNα at the end of 6 months treatment were 0.62 (0.35, 1.10), 0.60 (0.34, 1.05) and 0.54 (0.30, 0.97), respectively. The ORs (95% confidence interval) of the virological response, biochemical response and complete response of Tα1 over IFNα at the end of follow-up (6 months post-treatment) were 3.71 (2.05, 6.71), 3.12 (1.74, 5.62) and 2.69 (1.47, 4.91), respectively. These data showed that compared with IFNα, the benefit of Tα1 was not immediately significant at the end of therapy, but virological, biochemical and complete response had a tendency to increase or accumulate gradually after the therapy. For three of the four trials that studied HBeAg-negative patients, the results are mostly applicable to HBeAg-negative CHB.

Keywords: Thymosin; Interferon; Hepatitis B; Meta-analysis

1. Introduction

Hepatitis B virus (HBV) infection is a global public health problem as it is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) with up to one million HBV carriers dying of HBV associated liver disease annually (Safioleas et al., 2007). Several major advances in the treatment of chronic hepatitis B have been made over the last several years. Currently, interferon alpha (IFNα) and four nucleoside analogue (NA): lamivudine, adefovir dipivoxil, entecavir, and most recently, telbivudine have been approved for the treatment of chronic hepatitis B (Lok and McMahon, 2007).

IFNα has reasonably good efficacy with initial response rates of 30–40% compared with 10–20% among untreated controls. However, of those who responded to IFNα therapy, 56% relapsed within the first year after discontinuation of therapy (median 3.1 months) (Manesis and Hadziyannis, 2001). In addition, IFNα has a poor side-effect profile, leading to inadequate compliance and frequent need for dose reduction (Manesis and Hadziyannis, 2001; Liaw, 2002). Once-daily nucleoside analogue rapidly produces a suppression of HBV DNA replication. However, most of patients relapse once therapy is stopped (Lok and McMahon, 2007).

Thymosin alpha-1 (Tα1) is an immunemodulating peptide that has been shown to enhance Th1 cytokine production as well as T-cell differentiation and maturation (Rasi et al., 2003). Tα1 therapy is used in many countries worldwide for the treatment of chronic hepatitis B. Several clinical studies have shown

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that treatment with Tα1 monotherapy results in significantly higher sustained response rates when compared with controls and exhibits no significant side effects. Moreover, complete virological response tends to increase or accumulate gradually after the cessation of Tα1 therapy (Zavaglia et al., 2000; Mutchnick et al., 1999, 1991; Chien et al., 1998). A meta-analysis conducted by Chan et al. (2001) has shown that compared with no treatment, thymosin is effective in suppressing viral replication in chronic HBV infection. And recently, some randomized controlled clinical trials compared the efficacy of Tα1 and IFNα in the treatment of chronic hepatitis B. Thus, we conducted this meta-analysis of these trials to assess the evidence obtained on the efficacy of Tα1 treatment in chronic HBV infection.

2. Methods

2.1. Literature search and data extraction

All English articles were retrieved by using searches of MEDLINE and EMBASE. Included terms were *thymosin* and *hepatitis B* or *HBV*. Our search was limited to human studies. All articles were identified by a search from 1966 to August 2007. Additional studies were identified by scrutiny of the reference lists of trial publications and review articles and by writing to principal investigators of identified eligible trials. The selection of papers and data extraction using the same data extraction form was conducted independently by two investigators. Basic information obtained from each eligible trial included the number of patients randomized into each compared group at the outset of the trial, the treatment regime, duration of follow-up and the treatment outcomes at the end of treatment and/or during the post-treatment follow-up period. Articles were examined to eliminate duplicate reports of the same trial, and uncertainties in the data were clarified by contacting the principal investigators through writing when necessary.

2.2. Inclusion and exclusion criteria

Prospective, randomized controlled trials comparing Tα1 vs. IFNα in the treatment of chronic hepatitis B were considered for analysis. Patients were HBV DNA-positive and had elevated alaninetransaminase (ALT) levels. Studies were included in the meta-analysis if they had a minimum treatment duration of 24 weeks and reported end-of-treatment and/or sustained (more than 6 months post-treatment) virological, biochemical and/or complete responses. Trials including patients suffering from other forms of viral hepatitis (hepatitis C or hepatitis D) or receiving antiviral drugs other than Tα1and IFNα were excluded.

2.3. Excluded and included trials

Eleven potentially eligible randomized trials using Tα1 in the treatment of chronic HBV infection were identified (Zavaglia et al., 2000; Mutchnick et al., 1999, 1991; Chien et al., 1998, 2006; Iino et al., 2005; Andreone et al., 1996; Zhuang et al., 2001; You et al., 2001, 2005, 2006). Five comparing Tα1 vs. no treatment or placebo (Zavaglia et al., 2000; Mutchnick et al., 1999, 1991; Chien et al., 1998, 2006) and one comparing different dose of Tα1 (Iino et al., 2005) were excluded. Among other five randomized trials comparing Tα1 vs. IFNα (Andreone et al., 1996; Zhuang et al., 2001; You et al., 2001, 2005, 2006), one duplicate publication was excluded (You et al., 2001).

2.4. Definition of main outcomes

Virological response was defined as the disappearance of HBV DNA in the serum plus the loss of hepatitis B e antigen (HBeAg) if the patients were HBeAg-positive before treatment and the disappearance of HBV DNA if the patients were HBeAg-negative before treatment. Biochemical response was defined as the normalization of the ALT levels. Complete response was defined as fulfilling criteria of biochemical and virological response simultaneously. We analyzed the outcome at the end of treatment and at the end of follow-up (6 months post-treatment).

2.5. Statistical analysis

Virological, biochemical and complete responses were analyzed separately using the intention-to-treat method. We used the ratio of the odds of the main outcomes in the Tα1-treated group over that in the IFNα-treated group as the measure of efficacy. The 95% confidence interval (CI) for the combined odds ratio (OR) is also provided. Meta-analysis was performed using fixed-effect or random-effect methods, depending on absence or presence of significant heterogeneity (DerSimonian and Laird, 1986). Statistical heterogeneity between trials was evaluated by the Cochran χ^2 test and was considered to exist when $P < 0.10$. In the absence of statistically significant heterogeneity, the fixed-effect method was used to combine the results. When the heterogeneity test was statistically significant ($P = 0.10$ or lower), the random-effect method was used. The combined result was an average OR and 95% CI weighted according to the standard error of the OR of the trial, $P < 0.05$ was considered statistically significant. We used funnel plots (i.e. plots of study results against precision) to assess publication bias, and tested the symmetry of the funnel plot using Egger's test (Egger et al., 1997; Sterne and Egger, 2001). Analyses were performed with STATA version 9.0 (Stata Corp, College Station, Tx) and Review Manager version 4.2 (RevMan, The Cochrane Collaboration, Oxford, England).

3. Results

3.1. Description of the included trials

Table 1 shows the characteristics of the four trials included in the meta-analysis, with a total of 199 patients. All patients were anti-HBV treated naïve. At entry, all patients with presence of hepatitis B surface antigen (HBsAg) in serum for at least 12 months, positive serum tests for HBV DNA (one trial by liquid hybridization (Andreone et al., 1996) and the other three trials by polymerase chain reaction (Zhuang et al., 2001; You et al., 2005, 2006)) documented on at least two occasions and at least 3 months apart during the 12 months before entry,

Table 1
Description of included randomized controlled trials

Author	Entry e status	Treatment (months)	Follow-up (months)	Virological end-point	Sample size (*n*)	Age (years)	M/F	ALT (IU/L)
Andreone et al. (1996)	HBeAg(−)	6	6	HBV DNA(−)	T 17	T 40.5 ± 10.4	T 15/2	T 181.5 ± 158.6
	HBeAb(+)				I 16	I 40.2 ± 12.1	I 13/3	I 141.6 ± 76.3
Zhuang et al. (2001)	HBeAg(−)	6	6	HBVDNA(−)	T 18	NS	NS	NS
	HBeAb(+)				I 30			
You et al. (2005)	HBeAg(−)	6	6	HBV DNA(−)	T 26	T 47 ± 12	T 23/3	T 188.7 ± 102.6
	HBeAb(+)				I 30	I 40 ± 11	I 23/7	I 191.5 ± 106.5
You et al. (2006)	HBeAg(+)	6	6	HBV DNA(−)	T 29	T 45 ± 8	T 26/3	T 177.8 ± 58.8
				HBeAg(−)	I 33	I 42 ± 11	I 27/6	I 189.9 ± 63.1

Note: HBeAg, hepatitis B e antigen; HBeAb, hepatitis B e antibody; HBV, hepatitis B virus; T, thymosin alpha-1 group; I, interferon alpha group; M, male; F, female; ALT, alaninetransaminase; NS, no significant difference.

aminotransferase levels higher than 1.5 times the upper normal limit for at least 12 months before entry. Three trials studied HBeAg-negative (and anti-HBe-positive) patients, one trial studied HBeAg-positive patients. None of the trials included patients with decompensated liver disease or complication of portal hypertension. All these four trials used 900 μg/m^2 (or 1.6 mg) Thα1 twice weekly or 500 MU IFNα three times weekly for 6 months, and followed-up for 6 months. All these four trials had clearly stated inclusion and exclusion criteria. In addition, all these four trials had comparable baseline characteristics among the treatment groups, including age, sex, biochemical, histological, serological parameters and number of patients with histological evidence of cirrhosis. However, none of the four trials was conducted in a double-blind manner, and none had clearly mentioned the concealment of allocation in the randomization process.

There was no evidence for publication bias on the funnel plot (data not shown) or by Egger's test ($P > 0.1$).

3.2. Effect of thymosin alpha-1 and interferon alpha on virological response

The virological response at the end of treatment and at the end of 6 months follow-up is shown in Fig. 1. The number of patients included in the analysis was 199 at the two time points. The heterogeneity tests indicated that the variations of trial-specific ORs were not statistically significant at the end of treatment and at the end of follow-up ($P = 1.00$ and 0.91, respectively). The combined OR at the end of treatment was 0.62 (95% CI, 0.35, 1.10), and was not statistically significant ($P = 0.10$). The combined OR at the end of follow-up was 3.71 (95% CI, 2.05, 6.71), and was statistically significant ($P < 0.0001$). In addition, the conclusions were largely unchanged when the random-effect method was used. These results suggest that at the end of 6 months treatment, there was no significant difference between IFNα and Tα1 at the suppression of viral replication. But at the end of 6 months follow-up post-treatment, Tα1 was better than IFNα.

3.3. Effect of thymosin alpha-1 and interferon alpha on biochemical response

The biochemical response at the end of treatment and at the end of 6 months follow-up is shown in Fig. 2. The number of patients included in the analysis was 199 at the two time points. The heterogeneity tests indicated that the variations of trial-specific ORs were not statistically significant at the end of treatment and at the end of follow-up ($P = 0.96$ and 0.75, respec-

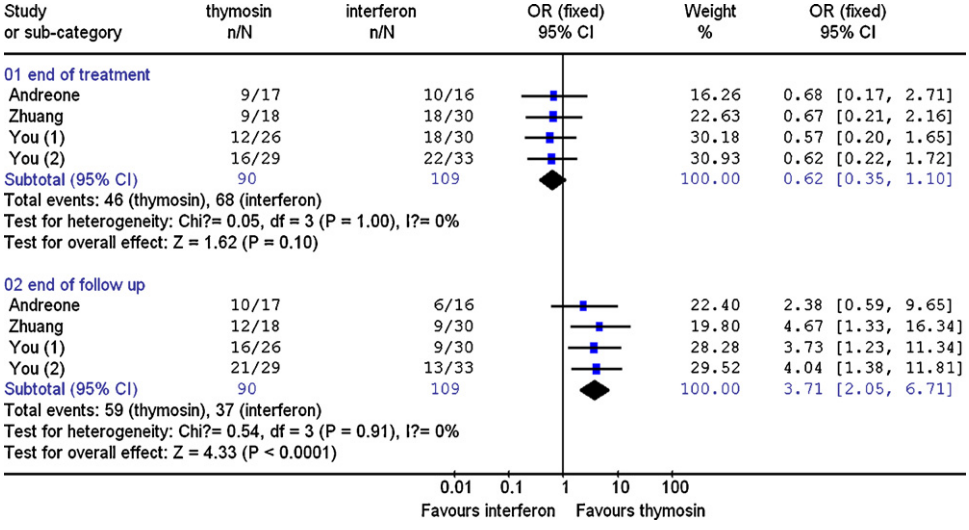

Fig. 1. Effect of thymosin alpha-1 and interferon alpha on virological response of chronic hepatitis B virus infection at the end of treatment and 6 months follow-up.

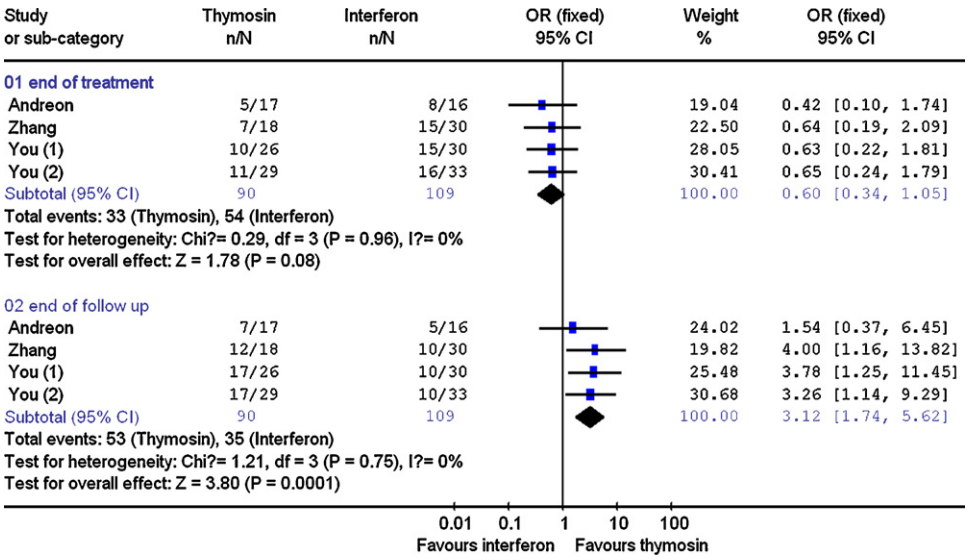

Fig. 2. Effect of thymosin alpha-1 and interferon alpha on biochemical response of chronic hepatitis B virus infection at the end of treatment and 6 months follow-up.

tively). The combined OR at the end of treatment was 0.60 (95% CI, 0.34, 1.05), and was not statistically significant ($P = 0.08$). The combined OR at the end of follow-up was 3.12 (95% CI, 1.74, 5.62), and was statistically significant ($P = 0.0001$). In addition, the conclusions were largely unchanged when the random-effect method was used. These results suggest that at the end of 6 months treatment, there was no significant difference between IFNα and Tα1 on ALT normalization. But at the end of 6 months follow-up post-treatment, Tα1 was better than IFNα at ALT normalization.

3.4. Effect of thymosin alpha-1 and interferon alpha on complete response

The complete response at the end of treatment and at the end of 6 months follow-up is shown in Fig. 3. The number of patients included in the analysis was 199 at the two time points. The heterogeneity tests indicated that the variations of trial-specific ORs were not statistically significant at the end of treatment and at the end of follow-up ($P = 1.00$ and 0.90, respectively). The combined OR at the end of treatment was 0.54 (95% CI, 0.30, 0.97), and was statistically significant ($P = 0.04$). The combined OR at the end of follow-up was 2.69 (95% CI, 1.47, 4.91), and was statistically significant ($P = 0.001$). In addition, the conclusions were largely unchanged when the random-effect method was used. These results suggest that at the end of 6 months treatment, IFNα was better than Tα1 on complete response. But at the end of 6 months follow-up post-treatment, Tα1 was better than IFNα.

3.5. Adverse events

Typical side effects of IFNα treatment, such as flu-like syndrome, fatigue, irritability, headache, and leucocytopenia, were

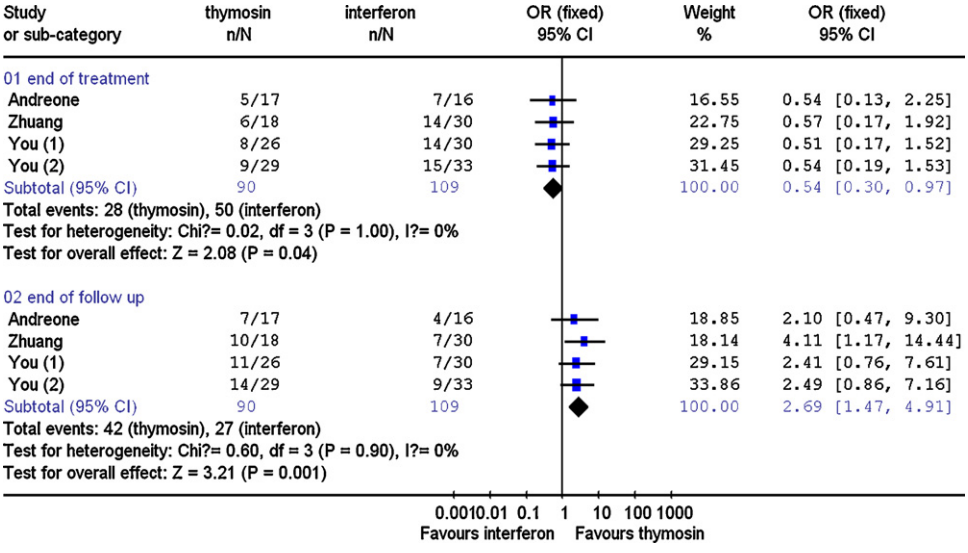

Fig. 3. Effect of thymosin alpha-1 and interferon alpha on complete response of chronic hepatitis B virus infection at the end of treatment and 6 months follow-up.

seen in most of the patients treated with IFNα. However, no serious or long-term side effects were noted and no patients discontinued the treatment in the four trials.

Therapy with Tα1 was well tolerant. Apart from seldom local discomfort at the injection site, no systemic or constitutional symptoms or biochemical abnormalities as a result of Tα1 treatment were reported in these trials.

4. Disscusion

Tα1 belongs to a class of products known as biologic response modifiers (Ancell et al., 2001). *In vitro* studies have shown that Tα1 results in T-cell differentiation and maturation with increases in cluster of differentiation CD4+, CD8+ and CD3+ cells, production of IFNγ, interleukin 2 (IL-2), IL-3 and promotes Th1 type immune response (Sztein et al., 1986). Tα1 also increases natural killer (NK)-cell activity in multiple animal models and normal human subjects (Sztein et al., 1986; Serrate et al., 1987), and enhances expression of class I HLA in culture cells (Giuliani et al., 2000). In addition to its immune-enhancing activities, Tα1 also has antiviral properties. Tα1 treatment leads to the inhibition of chronic viral infection through a mechanism of cellular immune response modulation via an increase in the secretion of IFNα, IFNγ, and cytokines such as IL-2, IL-3, and the differentiation and maturation of T-cells (Rasi et al., 2003; Sugahara et al., 2002). Furthermore, Tα1 has direct antiviral properties as well as it increases the expression of major histocompatibility complex (MHC) class 1 molecules on infected cells (Giuliani et al., 2000).

Tα1 has been clinically used as a 6-month therapy for chronic hepatitis B in many randomized clinical trials (Zavaglia et al., 2000; Mutchnick et al., 1999, 1991; Chien et al., 1998, 2006). In these trials, Mutchnick et al. (1991) found that by the conclusion of the study (1 year), serum aminotransferase levels had improved significantly in Tα1-treated patients, but not in the placebo group, six (86%) of the thymosin treated patients and one (20%) patient given placebo cleared HBV DNA from serum ($P < 0.05$). Chien et al. (1998) found that the complete virological response rate (clearance of serum HBV DNA and HBeAg) was higher in Tα1 group (40.6%) than in blank control group (9.4%) when assessed 18 months after entry. Mutchnick et al. (1999) found that a total of 12 (25%) patients given Tα1 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 ($P < 0.11$). Only one trial conduct by Zavaglia et al. (2000) found that, in anti-HBe, HBV DNA-positive chronic hepatitis B, Tα1 therapy alone did not increases the response rate. In 2001, Chan et al. (2001) conducted a meta-analysis to compare the efficacy of Tα1 vs. placebo or no treatment in chronic hepatitis B, also showed that there was an increasing trend of the virological response with time since the cessation of thymosin treatment ($P < 0.02$), but there was no difference in the biochemical response between the thymosin and placebo groups. What is more, four randomized trials (Andreone et al., 1996; Zhuang et al., 2001; You et al., 2005, 2006) compared efficacy of Tα1 vs. IFNα included in our meta-analysis set a historical control group, in which the patients had never received antiviral therapy and had been followed-up for more than 12 months. All the four trials showed that significantly greater proportion of patients in the Tα1 and IFNα groups than the historical control group had HBV DNA loss and ALT normalization at the end of therapy and follow-up period.

We conducted this meta-analysis to compare antiviral efficacy of Thα1 with IFNα in treatment of chronic hepatitis B; the results showed that at the end of 6 months treatment, IFNα was better than Tα1 at the complete response, but there was no significant difference in the virological and biochemical response. On the other hand, at the end of 6 months follow-up post-treatment, Tα1 was better than IFNα at both suppression of viral replication and ALT normalization. The inter-trial heterogeneity was not statistically significant ($P > 0.1$). The effect remained significant when a random-effects model was used. The benefit of Tα1 was not immediately significant at the end of therapy, but virological, biochemical and complete response had a tendency to increase or accumulate gradually after the therapy. In contrast, the effect of IFNα was relatively more quick but less sustainable. In addition to having better HBV clearance, Tα1 is also more tolerable than IFNα and has fewer side effects.

The methodological limitations of the trials warrant some discussion. First, no study was double-blinded. Although it is true that the tested intervention is difficult to blind, it is unlikely that the lack of blinding could affect the outcomes assessed (i.e. virological response) (Juni et al., 2001). Second, none of the trials described the method used to generate the allocation sequence. Despite these potential sources of bias, randomization was adequate in the four trials as shown by the baseline equivalency of treatment groups. Third, as HBeAg-negative (and anti-HBe-positive) patients were studied in three of the four included trials, it is likely that our results reflect the response of HBeAg-negative patients; whether the efficacy of Tα1 treatment differs from that in HBeAg-positive patients requires further research. In HBeAg-negative patient, the meaning of virological response is different from that in HBeAg-positive patient, as it only requires the loss of HBV DNA at the time of assessment (with no HBeAg seroconversion). Finally, the different HBV DNA assays used in the different trials may also have caused additional variability in the sensitivity of HBV DNA detection and thus in the estimate of efficacy.

Another potentially important limitation of meta-analysis is publication bias, the fact that not all research is published. Compared to positive studies, negative studies may be less likely to be published and more likely to take longer to be published, which can affect the validity of meta-analysis (Thornton and Lee, 2000). One commonly used method to detect publication bias is the 'funnel plot,' which is a scatter plot that displays the relationship between the weight of the study (e.g. study size) and the observed effect. In principle, larger studies should display less variability of the treatment effects. Asymmetric appearance, especially due to the absence of smaller negative studies, can suggest unpublished data. However, neither funnel plots nor Egger's test showed evidence for publication bias.

In conclusion, the results of this meta-analysis indicate that a 6-month Tα1 therapy is safe and effective in inhibiting HBV replication in patients with HBeAg-negative chronic hepatitis

B. Tα1 is better tolerated than IFNα, and may gradually induce more sustained ALT normalization and HBV DNA/HBeAg loss. As Tα1 treatment is relatively free from adverse effects, future research is warranted to study the efficacy of combination therapy of Tα1 with IFNα or antiviral agents in the treatment of chronic HBV infection.

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