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Thymalfasin in the treatment of hepatitis B and C

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Thymalfasin exhibited an immunomodulatory and a direct antiviral mechanism of action. The low rate of sustained response of chronic hepatitis with current therapies, underscores the need for new therapeutic options. It has been suggested that thymalfasin may have efficacy in the treatment of chronic hepatitis B and C. Pilots studies in patients with chronic hepatitis B treated with thymalfasin in combination with interferon or nucleoside analogue, showed a 70% complete sustained response rate. Studies in chronic hepatitis C patients, would indicate that thymalfasin in combination with standard or pegylated interferon with ribavirin may improve response rate in hepatitis C virus (HCV) naïve and nonresponder patients. However, a large phase-III randomized study conducted in Europe in HCV patients nonresponder to Peg-interferon with ribavirin, demonstrated that thymalfasin did not improve the rate of sustained virologic responses, but, in patients who completed therapy, thymalfasin significantly diminished the relapse rate. In conclusion, thymalfasin, in combination with the standard of care, may be helpful as an adjuvant in the treatment of patients with chronic hepatitis B and C.

Keywords: chronic hepatitis; hepatitis B virus; hepatitis C virus; antiviral treatment; thymalfasin

Introduction

Viral hepatitis is an important health issue for the nations worldwide: overall about 200 million subjects are infected with hepatitis C virus (HCV)[1] and more than 350 million with hepatitis B virus (HBV)[2]. Many individuals who become infected with HBV or HCV virus develop liver disease that can lead to serious liver damage. The major goal of antiviral therapy is the suppression of viral replication, to prevent the progression of liver disease, specifically cirrhosis and liver failure, and the development of hepatocellular carcinoma.[3,4] New therapeutic agents, as well as evolving treatment strategies, have emerged over recent years, which have a significant impact on clinical outcomes in both chronic HBV and HCV infection.[5,6] However, despite progress in the management of viral chronic hepatitis, treatment failure still occurs in a distinct percentage of patients who use current therapies. No valid re-treatments are currently available for patients who failed standard treatment, especially for HCV patients.[7]

Thymalfasin ($T\alpha_1$) is a 28 amino acid peptide,[8] characterized by immunomodulatory activities and therapeutic potential in several diseases, including viral hepatitis. Immunomodulating activities are centered primarily on enhancing of T-cell function. Thymosin alpha 1 promotes T-cell differentiation and/or maturation, increases production of nterferons-gamma (IFN-γ), IL-2, IL-3, increases NK-cell activity, and production of migration inhibitory factor (MIF). *In vitro* it was shown to antagonize T-cell apoptosis in thymocytes.

Thymalfasin displays also an important antiviral effect directed to suppress viral replication, through an increase of MHC class I expression and a decrease of viral replication in cultured infected cells.[9,10] In addition to these direct antiviral effects, $T\alpha_1$ leads to increased intracellular glutathione levels, which are necessary to dramatically decrease viral replication.[11]

On this background, thymalfasin has been used in the treatment of hepatitis B and C.

Thymalfasin in hepatitis B

To date, seven agents have been approved for the treatment of subjects infected with HBV. These agents, divided in two main classes as interferons

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(standard interferon α2b and peginterferon α2a) or nucleos(t)ide analogues (lamivudine, adefovir, entecavir, tenofovir, and telbivudine), are currently used in monotherapy or in combination if resistance to monotherapy has emerged. Interferons are administrated for a definite course (48–52 weeks), require subcutaneous injection, and induce systemic side effects (flu-like symptoms, nausea, headache, depression, and blood abnormalities).

Nucleos(t)ide analogues in monotherapy, administered orally, are associated with a high rate of HBV-DNA suppression, but if discontinued are aggravated by a high rate of recurrence of viral replication. They need, therefore, to be given continuously, but their long-term use may lead to the development of resistance. Combination of approved therapies (*de novo* nucleoside + nucleotide or interferon + nuclos(t)ide) should be considered in the future.

Encouraging results have been obtained combining standard of care for chronic hepatitis B with thymalfasin. $T\alpha_1$ was first used in monotherapy, in chronic HBV patients in 1991.[12] The study was a double-blind, placebo-controlled phase-II study, involving 20 HBeAg-positive and -negative patients, that demonstrated a high response rate (75% vs. 25%) with a sustained response maintained for 2 years after the end of therapy in 84% of the patients. In a second study[13] involving 98 HBeAg-positive patients, the same authors demonstrated a gradually increasing response rate in $T\alpha_1$ treated patients (40.6%) 12 months after the end of treatment. A delayed response in patients treated with $T\alpha_1$ monotherapy was also observed in other studies.[14,15] Improved responses with $T\alpha_1$ compared to interferon α were shown in three different randomized studies involving HBeAg -positive and -negative subjects;[14,16–18] the rate of response was 41%, 56%, 48%, and 56%, respectively, compared to 25%, 27%, 27%, and 23% in the interferon monotherapy groups. To enhance the effects of $T\alpha_1$ in the antiviral response, thymosin and interferon were further used.[19–21] A complete response (ALT normalization and HBV-DNA undetectable) was observed in 60% of patients receiving $T\alpha_1$ with lymphoblastoid interferon (3MU b.i.w.)[19] and in 76% of those receiving $T\alpha_1$ with interferon α2b (10 MU t.i.w.)[21]. In a study[22] designed to evaluate the long-term efficacy and safety of combination interferon-α2b and $T\alpha_1$ versus interferon-α2b monotherapy or interferon-α2b plus lamivudine in antiHBe-positive naïve patients with chronic hepatitis B, at the end of follow-up (24 months), a sustained response was observed in 55.5% of patients treated with interferon-α2b plus $T\alpha_1$ group compared to 10% and 13% in the interferon-α2b monotherapy and in the interferon-α2b plus lamivudine group, respectively.

Only a few randomized studies have been conducted to evaluate the efficacy of the combination of nucleoside analogues and $T\alpha_1$ in chronic hepatitis B. An open-label study compared therapy for 26 weeks with $T\alpha_1$ (1.6 mg, sc b.i.w.) plus famciclovir (500 mg daily) versus famciclovir monotherapy in HBeAg-positive patients.[23] At the end of follow-up (52 weeks), 15.6% of patients in the combination group achieved a complete virological response (defined as HBV-DNA undetectable and HBeAg seroconversion to anti-HBe) compared to no patient in monotherapy. Of eight controlled trials designed to study the combination of $T\alpha_1$ (for 24 weeks) and lamivudine (52 weeks) in HBeAg-positive chronic hepatitis B patients, only four reported follow-up results.[24–27] The virological response at the end of 12 months follow-up in the combination therapy group ($T\alpha_1$ 1.6 mg t.i.d., for 24 weeks and lamivudine 100 mg daily for 52 weeks) was 58.3%, 60%, 75.6%, and 89.6%, respectively, while the monotherapy group response rate was 47.2%, 54%, 55%, and 67.7%. The HBeAg to HBeAb seroconversion rate at the end of follow-up was different and statistically significant between the two groups (36.1%, 42.8%, 39%, and 48.2%, respectively, compare to 8.3%, 8.1%, 17.5%, and 6.4% in monotherapy with lamivudine).

In conclusion, the rate of sustained response in patients treated with $T\alpha_1$ monotherapy or in combination with interferon or nucleos(t)ide analogues, and in particular the delay observed in the response rate, demonstrate a participation of $T\alpha_1$, sustaining the viral response, obtained with the standard of care treatment, than its implication in the induction of the primary viral response.

Thymalfasin in hepatitis C

The currently recommended therapy of chronic HCV infection is the combination of a pegylated interferon alfa and ribavirin.

There are two licensed pegylated interferons, peginterferon alfa-2b (Peg-Intron, Schering Plough Corp., Kenilworth, NJ), with a 12-kD linear

polyethylene glycol (PEG) covalently linked to the standard interferon alfa-2b molecule, and peginterferon alfa-2a (Pegasys, Hoffmann-La Roche, Nutley, NJ) with a 40-kD branched PEG covalently linked to the standard interferon alfa-2a molecule.[28,29]

The optimal dose of peginterferon alfa-2b, is 1.5 g/kg/week according to body weight, with ribavirin weight-based (800 mg for patients 65 kg; 1000 mg for patients weighing 65 to 85 kg; 1200 mg for patients weighing 85 to 105 kg; and 1400 mg for patients weighing 105 kg to 125 kg)[30] Alternately Peginterferon alfa-2a is administered subcutaneously at a fixed dose of 180 μg/week in association with ribavirin 1000 (for those who weigh 75 kg) to 1200 mg daily (for those who weigh 75 kg).[31]

The optimal duration of treatment should be based on the viral genotype: patients with genotypes 1 and 4 (and 5 or 6) should be treated for 48 weeks,[32–34] whereas patients with genotypes 2 and 3 could be treated for 24 weeks.[32]

Twenty to fifty percent of patients treated with pegylated interferon and ribavirin will not achieve a sustained viral response (SVR). Failure to achieve an SVR with a course of pegylated interferon and ribavirin can be a consequence of nonresponse, virological breakthrough, or relapse. Poor adherence to the prescribed treatment and inappropriate dose reductions can contribute to poor response rates.

Options for nonresponders to pegylated interferon and ribavirin are limited. Re-treatment with the same regimen leads to an SVR in fewer than 7% of patients and therefore cannot be recommended.[35] There is no convincing evidence that switching to alternative interferons is effective.[36] More prolonged therapy (72 weeks) can achieve response in 16% of treated patients.

New approaches are needed to increase the SVR rates, especially in patients with genotype 1 infection and high viral load or subjects who fail to achieve an SVR using the currently approved treatment regimens.

The development of new hepatitis C antiviral agents is critical. A number of new molecules are under investigation including long-acting interferons, immunomodulators, antifibrotics, specific HCV-derived enzyme inhibitors (antipolymerase and antiprotease), drugs that either block HCV antigen production from RNA or prevent normal processing of HCV proteins, but the majority of case results are discouraging.

While early studies of thymalfasin monotherapy for the treatment of HCV did not show adequate response,[37] an efficacy of thymalfasin in combination with IFN in chronic HCV treatment was demonstrated in different trials.

Three studies of the combination of thymalfasin and standard IFN in chronic hepatitis C demonstrated that this combination was more effective than IFN monotherapy.[38–40] In a study by Rasi *et al.*,[38] 15 patients with chronic hepatitis C (85% genotype 1) were enrolled to be treated with combination $T\alpha_1$ and L-IFN therapy for 1 year. Six months after treatment, six patients (40%), including five with HCV type 1b, have exibited a sustained response; results were similar at 18 months. Good results were obtained also by Sherman *et al.*[40] in a randomized, double-blind, placebo-controlled trial enrolling 109 patients with chronic hepatitis C infection. Patients were randomized to 1.6 mg of $T\alpha_1$ subcutaneously twice weekly and 3 MU of IFNα2b three times weekly, or 3 MU of IFNα2b three times weekly and placebo, or placebo. An end-of-treatment virological response was obtained in about 37% of IFN/$T\alpha_1$-treated patients, in 18.9% of IFN-treated subjects and in 2.7% of untreated controls. The cumulative sustained responses were 14.2% and 8.1% in the IFN/ $T\alpha_1$ and IFN arms, respectively.

These results have prompted a dose-finding study to investigate the effect of three different doses of $T\alpha_1$ (0.8 mg, 1.6 mg, and 3.2 mg) in association with Peg-IFN alfa 2a on 12 week therapy HCV-RNA levels and on peripheral blood T-cell population.[41] The study included 31 genotype 1 nonresponder patients with high viral load. After 12 weeks of therapy, the median reduction in HCV RNA increased with the increased doses of $T\alpha_1$ and at the end of treatment, 36% of patients treated with high dose of $T\alpha_1$ achieved an EVR, compared to 30% and 20% with the other $T\alpha_1$ dosage.

Based on these promising data, three large studies (United States Phase 3 Trial, Mexican pilot study, and European Phase 3 Trial) have been designed to evaluate the efficacy of $T\alpha_1$ given at a dose of 1.6 mg twice weekly to difficult-to-treat patients with chronic hepatitis C that was nonresponding to previous antiviral therapy.

In the U.S. study (unpublished data), HCV-patients who had not responded to IFN standard or Peg-IFN alpha monotherapy or in combination

with ribavirin were allocated to two arms, one re-treated with peg-IFN α2a (180 μg) plus $T\alpha_1$ (1.6 mg), the other re-treated with peg-IFN alpha and placebo; both groups were treated for 48 weeks and followed-up post-therapy till week 72. No ribavirin was given to either arm of the study. The study involved 1000 patients, split into two cohorts: one involved patients who were noncirrhotic to early cirrhosis and the other cohort included patients with bridging fibrosis to cirrhosis.

The primary end-point was the clearance of HCV after 6 months of follow-up and the histological improvement. The results were disappointing. In the noncirrhotic cohort, 5% of the $T\alpha_1$ plus peginterferon-α2a group achieved a SVR *versus* only 1% in the peginterferon-α2a monotherapy group. Presumably the lack of ribavirin influenced the negative results.

The Mexican study by Poo *et al.*[42] has evaluated triple combination therapy with thymalfasin (1.6 mg), peg-IFN-α2a (180 μg), and ribavirin in the treatment of 40 hispanic chronic hepatitis C patients nonresponder to prior IFN/ribavirin combination therapy. Twenty-six patients were infected with the difficult-to-treat genotype 1. An early viral response was observed in 52.5% patients at week 12 and 50% at week 24. Per protocol end-of-treatment response was 52.6% at week 48 and 21.1% treated patients achieved an SVR at week 72. Among genotype 1 patients, 23.5% achieved an SVR at week 72.

The European phase-3 trial (submitted) involved 550 patients who are nonresponders to peg-IFN and ribavirin therapy. These patients were retreated with a triple therapy regimen consisting of 48 weeks of peg-IFN α2a (180 μg) and ribavirin plus $T\alpha_1$ (1.6 mg) or with peg-IFN α2a (180 μg) and ribavirin plus placebo. Patients were randomized according to the viral load (>800,000 or <800,000 IU/mL), previous treatment (peg-IFN -2a/riba or peg-IFN -2b/riba), and the presence or absence of cirrhosis. In the ITT analysis, 86 (31.27%) of the $T\alpha_1$ patients achieved an EOT versus 92 (33.21%) of the placebo patients ($P = 0.6035$ n.s.), and 35 (12.73%) of the $T\alpha_1$ patients achieved a SVR versus 29 (10.47%) in the placebo group [$p = 0.4073$ not significant (n.s.)]. In the analysis of the secondary populations, 34 completer patients (COMP) (subjects who achieved a complete response at week 24 and continued treatment until week 48), achieved an SVR in the $T\alpha_1$ group (40.96%) versus 26 (26.26%) in the placebo group ($P = 0.0478$ significant); in the per-protocol (PP) population 31 (42.47%) of the patients in the $T\alpha_1$ group achieved an SVR versus 24 (28.24%) in the placebo group ($P = 0.079$ n.s.). There was no significant difference in SVR in patients stratified according to the type of previously failed peg-IFN, presence an absence of cirrhosis, low or high viral load. However, at the separate analysis of the stratum of HCV genotype 1 patients with cirrhosis and a high viral load at baseline there was a significant higher rate of SVR among the $T\alpha_1$ group than in the placebo group in all populations this study indicated that adding $T\alpha_1$ to SOC does not improve the induction of a virologic response, but $T\alpha_1$ might improve the ability to sustain a virologic response and play a role as a secondary adjuvant for preventing relapses. As the kinetics of HCV eradication include an early phase of rapid HCV decline from serum (indicated by interferon plus ribavirin), and a second slower phase of progressive elimination of HCV-infected hepatocytes by the immune system;[43] the delayed effect of $T\alpha_1$ would imply a role of the cytokines in completing the eradication of cellular sanctuaries where HCV can hide after seroclearance and from where it can reactivate post-therapy. The role of $T\alpha_1$ in completing the removal of residual infections foci may not be HCV-specific but common to other viral infections, as it was also observed in hepatitis B patients.

Conclusions

Although $T\alpha_1$, alone or in combination with the standard of care, did not increase the primary antiviral effect of therapy against HBV or HCV, it diminished in both infections, the rate of relapser, once a primary virologic response, was obtained. Thymalfasin might therefore be useful as an adjuvant to convalidate response and increase SVR in the treatment of both hepatitis B and C.

Conflicts of interest

The authors declare no conflicts of interest.

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