

REVIEW ΑΝΑΣΚΟΠΗΣΗ

Treatment optimization of HBeAg-negative chronic viral hepatitis B with thymosin- α 1

Chronic hepatitis B virus (HBV) infection is a widely prevalent and clinically significant condition with profound implications for health care costs worldwide. Interferon- α and direct antiviral nucleos(t)ide analogs such as lamivudine, entecavir, adefovir, telbivudine, tenofovir and clevudine, are effective in the therapy of chronic HBV infection, but efficacy is still found wanting, particularly in HBeAg-negative/anti-HBe-positive cases and in patients with lower levels of alanine aminotransferase. Well documented evidence supports the pivotal role of immunomodulation in the control of hepatitis B virus infection. Thymosin- α 1, a 28-amino acid peptide derived from the amino end of the highly acidic protein prothymosin- α , is an immunomodulatory agent known to increase natural killer (NK), CD4 and CD8 cell counts, shift the immune response towards Th1 cells, increase MHC class I expression on virus infected cells, and inhibit viral replication by direct antiviral action. A differential cellular response has been observed within a range of prothymosin- α concentrations, and optimal concentrations associated with maximal response have been observed. These attributes of the parent molecule prothymosin- α can be assumed to be shared by thymosin- α 1. Treatment of patients with chronic HBV infection with thymosin- α 1, either alone or in combination with interferon- α , has yielded promising results. It is postulated that these results can be significantly improved by optimizing the dose of thymosin- α 1 for individual patients to induce a maximum immunomodulatory response. Drug optimization will be determined by testing the mixed lymphocyte response of each patient to different doses of thymosin- α 1, before the initiation of treatment.

1. INTRODUCTION

Hepatitis B virus (HBV) is a compact DNA virus with an incomplete double helix. Most adult patients with acute hepatitis B infection eliminate the virus through immune mechanisms. By contrast, around 90% of infants infected during the first year of life and 30–50% of children infected between 1 and 4 years of age develop chronic infection. Today, there are over 350 million people with chronic hepatitis B, of whom 20–35% present liver cirrhosis. The group with cirrhosis has an almost 100-fold higher incidence of hepatocellular carcinoma compared with the general population.¹ The evolution of acute to chronic hepatitis B is dependent on a complex interaction between the non-cytopathic HBV and an inadequate immunological response by the patient.

Patients with chronic HBV infection may be antigen e positive (HBeAg-positive) or antigen e negative (HBeAg-

negative), but with positive antibody to the antigen (anti-HBe-positive). The seroconversion of HBeAg to anti-HBe usually indicates a transition to the inactive carrier state, with the viral DNA (HBV DNA) becoming undetectable or barely detectable.

Some patients with chronic HBV infection are anti-HBe-positive but have a fairly high HBV DNA titer in the serum. This is due to the ability of the virus to delete the production of the HBeAg following mutation in the precore region of its genome resulting in continuous or intermittent viral proliferation and development of chronic active hepatitis.²

Currently, HBV infected patients are treated with either interferon, based on its antiviral and immunomodulatory action,³ or nucleos(t)ide analogs, the direct antiviral action of which is mediated by inhibition of the viral DNA polymerase.⁴

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Εξατομικευμένη δοσολογία
θυμοσίνης- α 1, στη θεραπεία
ασθενών με χρόνια ηπατίτιδα Β και
αντίσωμα στο αντιγόνο ΗΒε

Περίληψη στο τέλος του άρθρου

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Interferon therapy is moderately efficacious, with initial response rates of around 30–40%, compared with 10–20% observed among untreated control subjects. However, 56% of the responders relapse within a year after discontinuation of therapy.⁵ Similarly, although nucleos(t)ide analogs rapidly produce a suppression of HBV DNA replication, most patients relapse once treatment is stopped, due to incomplete viral eradication.

Clearly, the currently used drugs do not achieve satisfactory and lasting virological response, as defined by the disappearance of HBV DNA, in anti-HBe-positive patients with chronic active hepatitis B. There is, therefore, a need to develop new drugs or devise new approaches based on existing drugs that would achieve the therapeutic objective, which is eradication of the virus. It is known that patients with HBV infection who become cured clear the virus through an autoimmune mechanism, and that cases with high pre-treatment levels of alanine aminotransferase (ALT) (>five-fold ULN), which are indicative of a stronger immunological reaction, respond better to classical treatment.^{6,7} Based on this knowledge, a case can be made for the use of immunomodulatory compounds in the treatment of chronic HBV infection, either alone as monotherapy or in combination with other therapeutic agents as adjuvant therapy.

2. HYPOTHESIS

Thymosin- α 1 is a 28-amino acid peptide with immunomodulatory effects. It is derived from the amino end of the highly acidic protein prothymosin- α . Originally isolated from calf thymus,⁸ it is now produced by solid phase peptide synthesis. Prothymosin- α is a 109-amino acid polypeptide found in all tissues in man and other mammals, thymus and spleen being the richest sources.^{9–11} Thymosin- α 1 is detected in the serum at concentrations of about 1 ng/mL; its precursor prothymosin- α is found within cells. Both have been shown in *in vitro* and *in vivo* experiments to express immunomodulatory activity.^{8,9,12} The carboxy end (89–109) of prothymosin- α has immunopotentiating properties which have been demonstrated in healthy subjects and in cancer patients.¹³

The immunomodulatory activities of thymosin- α 1 are expressed by a shift of the immune response towards the Th1 cells,¹⁴ enhancement of T cell differentiation and maturation,¹⁵ augmentation of the action of natural killer cells (NK) in autologous and allogeneic mixed lymphocyte reaction,¹⁶ increase in MHC class I expression on virus infected cells,¹⁷ and inhibition of viral replication in the woodchuck model of hepadavirus infection.¹⁸

In vitro studies with mixed lymphocytes from healthy donors have shown a cellular response to prothymosin- α concentrations in the range of 50–750 ng/mL. The response was not continuous, but a bell-shaped response curve was observed, illustrating the immunomodulatory effect of optimum prothymosin- α concentration.¹⁹ These observations raise the question of whether it might be possible to improve the efficacy of treatment by looking for the optimal concentration of prothymosin- α , and by extension of thymosin- α 1, for each patient.

It would be reasonable to postulate that boosting the immune system with thymosin- α 1 could play an important role in the treatment of patients with chronic active HBV infection. More important, this stimulation could be significantly enhanced by optimizing the concentration of the peptide to achieve the maximum immunomodulatory effect. This optimal concentration would be determined by testing the mixed lymphocyte response of each patient to different doses of thymosin- α 1, before the initiation of treatment.

3. DISCUSSION

Recent studies on patients with chronic active HBV infection, in which thymosin- α 1 given as monotherapy was compared with a control group of subjects who received either no treatment or placebo, yielded varying results. Mutchnick et al. reported that 25% of HBeAg-positive patients given thymosin- α 1 and 13% of HBeAg-positive patients given placebo showed a sustained loss of HBV DNA during or following the 12-month study period.²⁰ By contrast, the study of Zavaglia et al. of anti-HBe-positive patients showed that treatment with thymosin- α 1 alone effected no improvement.²¹ Chan et al, in a meta-analysis that compared the efficacy of thymosin- α 1 with that of placebo or no treatment, found that there was an increasing trend of improvement of the virological response with time following the cessation of treatment, but no difference was noted in the biochemical response between the thymosin- α 1 and placebo groups.²²

Studies comparing the efficacy of thymosin- α 1 and interferon- α monotherapy in anti-HBe-positive patients with chronic active HBV infection showed that a significantly higher proportion of patients on either drug had HBV DNA loss and ALT normalization, both at the end of treatment and at follow-up, compared with historical control subjects.^{23,24} A meta-analysis of the antiviral efficacy of thymosin- α 1 and interferon- α in patients with chronic active HBV showed that at the end of a 6-month

course of treatment interferon- α was more effective than thymosin- α 1, according to the complete, that is, both virological and biochemical, response attained. At the end of a 6-month post-treatment follow up period, however, thymosin- α 1 was more effective than interferon- α at both suppression of viral replication and ALT normalization. In addition, thymosin- α 1 was well tolerated and exhibited no significant side effects, and there was no need to reduce the dose during treatment.²⁵

Combination treatment of anti-HBe-positive patients with thymosin- α 1 and interferon- α 2b or thymosin- α 1 and lamivudine has produced significantly higher virological and biochemical response rates than standard therapeutic regimens.^{26,27} This evidence provides good support for the notion that thymosin- α 1 might be an effective therapeutic agent in patients with chronic active HBV infection.²⁸ Based on *in vitro* experiments, it appears that prothymosin- α , the parent molecule, is also a promising candidate drug for these patients.^{19,29}

In all the clinical trials described but one, thymosin- α 1 was administered in a single dose, 1.6 mg subcutaneously, twice weekly, for 24 weeks. In the study of lino et al. two groups of patients received either 0.8 mg or 1.6 mg of thymosin- α 1, six times a week for the first 2 weeks and then twice a week for the subsequent 22 weeks. Both of these dose regimens exhibited long-term efficacy, with a good safety profile.³⁰

The hypothesis bears further consideration. For example, Chien et al reported that HBV genotype, precore mutation and thymosin- α 1 were all independent predictors of a complete response in patients with HBV infection. Specifically, the rates of complete response for patients with genotypes B and C were 64% and 19%, respective-

ly.³¹ Hadziyiannis et al, in their study of anti-HBe-positive patients treated with interferon- α , recorded response rates, in terms of HBsAg seroconversion, of 18%, 2%, 3% and 0% for genotypes A, B, C, and D, respectively.³² The question is whether these results could be improved by thymosin- α 1 titration aimed at inducing the maximum immunomodulatory response.

Although interferon- α is approved for the treatment of HBV infection and is recommended as first-line therapy for certain patients,³³ it is associated with frequent adverse effects that often force a dose reduction.³⁴ In patients with side effects, boosting the immune response with a patient-specific dose of thymosin- α 1 might not only improve the response rates but also permit the use of lower doses of interferon- α without compromising its therapeutic effect.

4. CONCLUSION

In conclusion, the hypothesis regarding effective treatment of patients with chronic HBV infection with thymosin- α 1, as monotherapy or as adjuvant therapy, at optimum serum concentrations for maximum immunomodulatory response, can be supported by research evidence. Augmentation of the immune system by optimum dosage with thymosin- α 1 might be considered for initial treatment, or for cases with a poor response to an approved antiviral agent.

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ΠΕΡΙΛΗΨΗ

Εξατομικευμένη δοσολογία θυμοσίνης- α 1, στη θεραπεία ασθενών με χρόνια ηπατίτιδα Β και αντίσωμα στο αντιγόνο ΗΒε

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Η χρόνια λοίμωξη από τον ιό της ηπατίτιδας Β (ΧΗΒ) αποτελεί σοβαρό παράγοντα νοσηρότητας για τους πάσχοντες, ενώ η προσπάθεια εκρίζωσης του ιού και αντιμετώπισης των επιπλοκών της λοίμωξης συνοδεύεται από υψηλό οικονομικό κόστος για τα ασφαλιστικά ταμεία. Βήματα προόδου ως προς τη θεραπεία των ασθενών με ΧΗΒ έχουν γίνει, με τη χορήγηση ιντερφερόνης- α ή αντι-ιικών φαρμάκων, όπως είναι η λαμβουδίνη, η εντεκαβίρη, η αδεφοβίρη, η τελμπουδίνη, η τενοφοβίρη και η κλεβουδίνη. Όμως, το θεραπευτικό αποτέλεσμα απέχει από το άριστο. Αναζητούνται θεραπευτικές προσεγγίσεις για την επιτυχία ενός άριστου, αλλά και μόνιμου θεραπευτικού αποτελέσματος, ιδιαίτερα

για ασθενείς με ΧΗΒ και ήπια αύξηση των τρανσαμινασών με θετικό anti-HBe. Υπάρχουν ισχυρές ενδείξεις ότι το ανοσοποιητικό σύστημα των ασθενών με ΧΗΒ έχει πρωτεύοντα ρόλο στην αντιμετώπιση της χρόνιας αυτής λοίμωξης. Η θυμοσίνη- α 1 είναι ένα πεπτίδιο αποτελούμενο από 28 αμινοξέα, το οποίο προέρχεται από το αμινοτελικό άκρο της πρωτεΐνης προθυμοσίνης- α . Η θυμοσίνη- α 1 έχει ανοσοτροποποιητική δράση, που εκδηλώνεται με την αύξηση των κυττάρων φυσικών φονέων (NK), με την αύξηση των CD4 και CD8 λεμφοκυττάρων, με την αύξηση της έκφρασης του συμπλέγματος μείζονος ιστοσυμβατότητας (MHC) τάξης I στα κύτταρα που έχουν μολυνθεί από τον ιό. Επίσης, αναστέλλει, με άμεση δράση στους ιούς, τον πολλαπλασιασμό τους και εκτρέπει την ανοσιακή αντίδραση σε Th1-τύπο. Η προθυμοσίνη- α , μητρικό μόριο της θυμοσίνης- α 1, έχει παρατηρηθεί ότι προκαλεί διαφορετική ανταπόκριση των T-λεμφοκυττάρων σε διαφορετικές συγκεντρώσεις της και μάλιστα έχει διαπιστωθεί σε πειράματα η ιδανική συγκέντρωσή της για μέγιστη λεμφοκυτταρική ανταπόκριση. Είναι εύλογο λοιπόν να υποθεθεί ότι η θυμοσίνη- α 1, ως προερχόμενη από την προθυμοσίνη- α , εκφράζει και τις ιδιότητες αυτής. Η χορήγηση της θυμοσίνης- α 1 για τη θεραπεία των ασθενών με ΧΗΒ, ως μονοθεραπεία ή ως θεραπεία συνδυασμού με ιντερφερόνη- α , έχει δώσει ενθαρρυντικά αποτελέσματα, τα οποία ενδεχομένως μπορούν να βελτιωθούν σημαντικά, εάν σε κάθε μεμονωμένο ασθενή χορηγηθεί η ιδανική δοσολογία θυμοσίνης- α 1, ώστε να επιτευχθεί η μέγιστη ανοσοενισχυτική δράση γι' αυτόν. Η ανεύρεση της εξατομικευμένης δόσης της θυμοσίνης- α 1 για κάθε ασθενή πριν από τη θεραπεία, ώστε να επιτευχθεί η ιδανική ανοσοενισχυτική δράση αυτής, μπορεί να γίνει με τον έλεγχο της μεικτής λεμφοκυτταρικής αντίδρασης κάθε ασθενούς σε διαφορετικές δοσολογίες θυμοσίνης- α 1, ώστε να διαπιστωθεί η συγκέντρωση εκείνη που προκαλεί τη μέγιστη λεμφοκυτταρική αντίδραση για τον εν λόγω ασθενή και κατά συνέπεια τη μέγιστη ανοσοτροποποιητική δράση.

Λέξεις ευρητηρίου: Anti-HBe, Θυμοσίνη- α 1, Ιός ηπατίτιδας Β, Χρόνια ηπατίτιδα Β

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