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Interferon Therapy of Chronic Hepatitis B

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Key Words

Chronic hepatitis B · Chronic hepatitis B treatment · Interferon · HBsAg quantification · Hepatitis B virus DNA · Immune response

Abstract

Chronic hepatitis B (CHB) results from the inability of the host's immune system to control viral replication. Interferon-α (IFN-α) therapy can convert CHB into inactive hepatitis B virus (HBV) infection in 20–30% of the treated patients. In spite of the low response rate, IFN-α therapy has the advantage of having a limited duration and being effective even after therapy, as demonstrated by a much higher incidence of HBsAg clearance in responders to IFN-α than in naturally occurring inactive HBsAg carriers. IFN-α has multiple antiviral, antiproliferative, and immunomodulatory activities and targets: cellular genes (IFN-stimulated genes) activating different pathways of antiviral defense in infected and noninfected cells, HBV replication blocking the RNA-containing core particle formation and accelerating their decay, degrading pregenomic RNA, and modulating the nuclear viral minichromosome (covalently closed circular DNA) activity by targeting its epigenetic regulation and both innate and adaptive immune response. The interference of viral heterogeneity and genetic polymorphisms of the host on IFN-α susceptibility is under investigation. Only a better understanding of the complex interplay between the different activities of IFN-α would warrant the amelioration of current therapeutic strategies and the design of new therapeutic approaches. The study of on-treatment dynamics of HBV infection by means of combined quantitative monitoring of serum HBV DNA and HBsAg warrant tailoring treatment at the single-patient level and can help to make treatment more cost-effective by using the different combinations of currently available antivirals, including IFN, more appropriately. Integrated molecular and clinical knowledge in a systems medicine fashion is mandatory to further improve antiviral therapy in CHB.

Introduction

Antiviral therapy of chronic hepatitis B (CHB) is aimed to prevent its progression to cirrhosis or, when cirrhosis is already present, to avoid or delay the development of the end-stage complications of liver disease and hepatocellular carcinoma [1]. Disease progression is promoted by persistent liver necroinflammation that results from the

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inability of the host's immune system to control viral replication effectively. Two different therapeutic approaches aim to suppress disease activity: (1) shifting the host-virus equilibrium from the pathogenic active to the nonpathogenic inactive phase with a time-limited course of antiviral treatment capable of inducing sustained off-therapy control of hepatitis B virus (HBV) replication, and (2) suppressing viral replication persistently with continuous antiviral treatment. Interferon (INF) is the major player of the former strategy and its option is preferred in cases of CHB without cirrhosis or early cirrhosis. However, long-term, eventually lifelong treatment using nucleos(t)ide analogues (NUCs), which are direct inhibitors of viral polymerase, are preferred for patients with advanced or decompensated cirrhosis or after failure of IFN-α [2].

The treatment outcomes are much more cost-effective when therapy is started earlier during the precirrhotic phase of CHB and induces an off-therapy control of the infection [3]. This would prevent the development of cirrhosis and warrant a life expectancy comparable with that of noninfected individuals. On the contrary, in the presence of cirrhosis, the cure of CHB reduces the mortality rate significantly, but does not eliminate the risk of hepatocellular carcinoma. Meta-analyses have demonstrated that 4.6% of cirrhotics treated with IFN develop hepatocellular carcinoma versus 9% of controls ($p = 0.006$) with a more significant reduction in patients with earlier cirrhosis [4, 5].

Mechanisms of Action of IFN

IFN-α has multiple mechanisms of action including antiviral, antiproliferative, and immunomodulatory activities; however, the mechanisms of viral antigen decline and off-therapy control of HBV replication are not entirely understood [6]. Antiviral activity is mediated by cellular genes (IFN-stimulated genes) which activate different pathways of antiviral defense in both infected and noninfected cells [7]. IFN-α has been reported to inhibit HBV replication through a variety of mechanisms, including blocking RNA-containing core particle formation, the accelerated decay of replication-competent core particles, and the degradation of pregenomic RNA [8–10]. A recent report proposed an additional direct action on covalently closed circular DNA (cccDNA) degradation mediated by the APOBEC3 family cytidine deaminase A3A [11]. Furthermore, IFN-α appears to inhibit HBV transcription and replication by targeting the epigenetic regulation of cccDNA, the nuclear viral minichromosome [12]. Interestingly, in HBV-transfected HepG2 cells, the pregenomic RNA transcription and cytoplasmic HBV core particle production remained inhibited 48 h after treatment discontinuation, suggesting that IFN-α could induce a persistent 'active epigenetic control' of cccDNA by recruiting corepressors and components of the polycomb repressive complex 2 that targets histone acetylation and methylation [12]. The molecular mechanisms of the inhibition of HBV replication mediated by IFN-α could explain, at least in part, the off-therapy persistent inhibition of viral replication observed in responders to IFN-α. These findings support the view that direct antiviral activity of IFN-α could play a pivotal role in the control of HBV infection; however, the possible links between the overall IFN-α activity and the modulation of innate and adaptive immune-response remain to be clarified. In the natural course of HBV infection, an effective immune response is essential for its control and an immune-modulatory effect of IFN-α had been thought to be crucial for the achievement of a sustained off-therapy response [13, 14]. Nevertheless, the mechanisms underlying the ability of IFN-α to switch an inadequate immune response to sustained off-therapy immune control remain poorly understood, particularly its role in boosting the activity of virus-specific CD8 cells [14]. On the other hand, a vigorous and multispecific CD8 response is detectable in HBsAg carriers who achieve, either spontaneously or after treatment, control of the infection, whereas immune response is weak with HBV-specific T cell hyporesponsiveness in CHB patients [15]. A better understanding of the immunological mechanisms underlying the response to IFN-α is of paramount importance for treatment optimization.

Recent studies on the kinetics of circulating HBV-specific CD8 cell response during IFN-α treatment were unable to show, at least in HBeAg-negative CHB, any restoration of their effector functions during therapy or in the first 6 months after treatment discontinuation [16, 17]. This could be a result of the antiproliferative effect of IFN in a condition of deep functional T cell paralysis that is typical of CHB patients. Nevertheless, the in vitro reactivity of T cells appeared to be restored in HBeAg-negative CHB patients with a long-term response to IFN-α and HBsAg clearance [15]; accordingly, CD8 T cell responses were more frequently detected (after the in vitro use of autologous DCs cells as presenting cells) in responders after therapy withdrawal [18]. Furthermore, patients with higher CD8 function and IFN-α production at baseline were more likely to show complete viral inhibition early during treatment, confirming a major role of adaptive ac-

Table 1. Effectiveness of the multiple synergistic actions of IFN-α in CHB

Activity	Target	Action	Effect	Outcome
Antiviral	Virus	Block of core particle formation Accelerated degradation of core particles and pregenomic RNA Epigenetic regulation of cccDNA ISGs mediated antiviral defense in both infected and noninfected cells	Decline of HBV DNA Decline of HBV antigens expression (HBsAg, HBc/eAg) Reduction of HBV infected cells	Control of HBV infection mediated by an effective adaptive immune response: vigorous multispecific CD8 T response in HBsAg carriers with off-therapy control of HBV infection
Immune modulatory	Host's innate immune response	Increased NK cell TRAIL expression; activation of $CD56^{bright}$ NK cells	Increased production of IFN-γ with direct antiviral effects and promotion of T cell response	
	Host's adaptive immune response	Minimal, if any, increase of CD8 T cell response; modulation of CD4 T cell response		

ISGs = IFN-stimulated genes.

tivity in the overall response to IFN-α treatment [16]. Paradoxically, however, IFN-α treatment could induce an indirect inhibition on T cells by boosting NK activity [17]. This innate immune response boosting, together with the strong antiproliferative effect of IFN-α, could be responsible for the lack of improvement of CD8 cell response during therapy. However, the activation of $CD56^{bright}$ NK cells could activate antiviral mechanisms able to unbalance the virus-host equilibrium as a prerequisite for a later restoration of competent immune response. Accordingly, the stronger the increase of NK cell TRAIL expression during IFN-α treatment, the greater the reduction of viral load and HBsAg serum levels [17].

Overall, these data shed light on some IFN-α activities in the specific setting of CHB (table 1); nevertheless, much clinical evidence remains poorly explained, such as the relatively low proportion of patients who respond persistently to therapy and the strong influence of HBV genotype on the response rate [1]. The possible interference of genetic polymorphisms on IFN-α susceptibility is under investigation. However, preliminary reports on the role of polymorphisms at or near the IL-28B gene, which significantly influence the probability of hepatitis C virus clearance after pegylated IFN (Peg-IFN) and ribavirin in chronic hepatitis C virus patients, are inconclusive, suggesting a minor – if any – role of this SNP in CHB patients [19]. On the contrary, a correlation seems to be present between polymorphisms of the HLA-DP gene and response to therapy; however, the results need further confirmation [20]. Only a better understanding of the complex interactions between the direct antiviral and immunomodulatory activities of IFN-α would warrant the amelioration of current therapeutic strategies with the design of new therapeutic approaches combining IFN-α and NUCs more appropriately.

Efficacy of Treatment

Standard IFN-α was the first treatment option for CHB at the end of the 1980s and was able to induce a progressive decline of serum HBV DNA followed by a slower normalization of serum transaminases (ALT) in both HBeAg-positive and -negative CHB patients [21, 22]. In HBeAg-positive CHB patients, at the end of 4–6 months of therapy 37–56% of patients had undetectable (<1–10 pg/ml) HBV DNA, 33% HBeAg loss, and about 70% ALT normalization. In over 80% of cases, HBeAg to anti-HBe seroconversion was maintained 4–9 years after treatment discontinuation with HBsAg loss in 12–65% of the cases [21]. In anti-HBe-positive CHB, IFN-α was first given with schedules comparable to that of HBeAg-positive patients (5–10 MU every other day for 16–24 weeks) [22]. However, the evidence of extremely high relapse rates (70–90%) in spite of optimal on-treatment responses [up to 70% of the patients with undetectable DNA and normal ALT at the end of therapy (EOT)] suggested longer treatment courses (12–24 months), which were associated with

a higher sustained response rate (22–30% vs. 10–15%) [22]. Among patients with sustained response, the HBsAg clearance rate after 4–7 years posttreatment follow-up ranged from 31.6 to 66.6%, with anti-HBs seroconversion in 50–77% of the cases [22–25]. Interestingly, once highly sensitive assays for HBV DNA detection became available, more than 50% of CHB patients who cleared serum HBsAg showed serum HBV DNA levels <400 cp/ml as compared to 25% of sustained responders without HBsAg loss [23], suggesting a stronger control of HBV infection in patients who cleared HBsAg. Surprisingly, the overall incidence of HBsAg clearance was much higher than in naturally occurring inactive HBsAg carriers [26].

In the last 10 years, standard IFN-α has been substituted by pegylated formulations, where the active drug is conjugated with polyethylene glycol molecules that modify both the pharmacokinetics and pharmacodynamics of IFN, prolonging its half-life and warranting the once weekly administration. Two forms of Peg-IFN are available: α-2b linked to a linear polyethylene glycol molecule of 12 kD, and α-2a linked to a larger branched polyethylene glycol molecule of 40 kD, the only authorized in CHB treatment in Western countries [27].

HBeAg to anti-HBe seroconversion in HBeAg-positive patients treated for 12 months with Peg-IFN-α was achieved in 32% of cases 6 months after treatment discontinuation and in 48% after 1 year (in subgroups of patients with long-term follow-up) [28, 29]. In HBeAg-negative patients, ALT normalization and HBV DNA <2,000 IU/ml was achieved in 38% of the patients 24 weeks after treatment discontinuation, but some of them relapsed and a sustained virological response was maintained after 5 years of posttreatment follow-up in 25% of the patients, with HBsAg clearance in 12% of them [30]. HBsAg loss and anti-HBs seroconversion represent the highest level of immune control of HBV infection and the ultimate goal of antiviral therapy [1]. The evidence that it can be reached progressively during the off-treatment follow-up even years after the end of treatment provides strong evidence of the ability of IFN to induce the curative switch from active CHB to the inactive HBsAg carrier. The rates of HBsAg loss and anti-HBs seroconversion in responders to Peg-IFN-α are significantly higher than in virological responders to NUCs, which pharmacologically induce the inactive HBV carrier status that is rapidly lost when NUCs are withdrawn or once antiviral resistance develops [1].

However, in spite of an ideal clinical outcome, responders to Peg-IFN-α are only about 1/4 to 1/3 of the treated patients. Therefore, the identification of patients with a high probability of response could warrant a more cost-effective approach to IFN-α treatment. Indeed, factors such as viral genotype, sex and age, baseline HBV DNA, and ALT levels correlate with response [1]; however, some of them are invariable or 'constant' during the short treatment timeframe and, in spite of the highly significant statistical association with a sustained virological response, in clinical practice, at the individual patient level, they should guide the treatment decision only if showing very high predictive values. This is not the case with CHB, where such high predictive values are not found for sex, age, or genotype – despite the fact that genotypes A and B are associated with better response rates than genotypes C and D [31, 32]. Nevertheless, these predictors may contribute to treatment tailoring if combined with suitable on-treatment predictors. On the contrary, other factors such as viral load and ALT, resulting from the highly 'dynamic' interplay between the virus and the host's immune system, may vary significantly over time. Therefore, they can be used to select the most appropriate time for initiation of therapy. Accordingly, the available data suggest that low baseline HBV DNA levels ($<10^7$ IU/ml) and high ALT levels (>3 times upper limit of normal) are associated with a higher chance of HBeAg to anti-HBe seroconversion and sustained virological response in HBeAg-positive and -negative CHB patients [1, 31, 32]. In HBeAg-negative CHB, the additional evidence that high baseline IgM hepatitis B core antibody (IgM anti-HBc) levels are associated with higher rates of response [33, 34] suggest that hepatitis exacerbations, which occur in a significant proportion of patients, could identify the right timing for starting treatment.

Overall, there is a compelling need for treatment optimization that can only be pursued by a response-guided treatment tailoring and eventually identifying new treatment options where both Peg-IFN-α and NUCs are used in combination or sequential schedules. The combination of Peg-IFN-α and lamivudine has been attempted in phase III studies; however, the overall rates of sustained response in the posttreatment follow-up were similar in patients treated with IFN-α monotherapy or combination therapy [28, 30]. As a result, combination therapy was no longer recommended for HBeAg-positive or -negative patients [1]. However, a later analysis of the factors influencing the response to Peg-IFN showed that there was a significant interaction between the type of treatment (Peg-IFN-α monotherapy vs. combination) and HBV genotype in HBeAg-negative CHB patients. Interestingly, in genotype D-infected patients, the higher probability of response was observed in the combination

arm, whereas the opposite was true for the genotype B patients [31]. Additionally, in the long-term posttreatment follow-up, HBeAg-positive patients treated with Peg-IFN2b plus LMV achieved higher rates of HBsAg loss and HBV DNA <400 cp/ml as compared to patients treated with Peg-IFN2b monotherapy (15 vs. 8% and 26 vs. 13%, respectively) [32]. In light of these observations, the use of combined or sequential Peg-IFN-α and NUC therapy needs to be revisited to capitalize on the higher antiviral potency of new generation NUCs and to eventually shorten their treatment duration.

Towards Treatment Personalization

For years, viral load has been the only diagnostic tool used for monitoring the efficacy of IFN treatment. Accordingly, current EASL guidelines identify as an on-treatment virological response the drop of viral load below 2,000 IU/ml (the serum HBV DNA cutoff that distinguishes CHB from the inactive carrier) within 24 weeks of treatment and its persistence at 6 and 12 months after stopping therapy as the hallmark of sustained response (in combination with HBeAg to anti-HBe serconversion in HBeAg-positive patients) [1]. However, the evidence that the extent of HBsAg decline from baseline to the end of treatment correlated with HBsAg loss 3 years after treatment, suggested that the on-treatment serum HBsAg kinetics could be an additional viral marker for response-guided IFN treatment [35].

Serum HBsAg circulates in three particulate forms: competent virions (42 nm, Dane particles), 20-nm diameter filaments of variable length, and 20- to 22-nm spherical defective particles, where noninfectious HBsAg particles exceed virions by a factor ranging from 10^2 and 10^5 [36]. Overall, available data suggest that HBsAg serum levels, being the end-product of the transcription of specific subgenomic mRNAs, mirror the complex equilibrium between the virus and immune system rather than viral replication. Even if we cannot exclude a substantial contribution of integrated HBV DNA to the production of HBsAg, its levels appear to be the indirect expression of transcriptionally active cccDNA rather than its total intrahepatic amount [36]. Therefore, the serum HBsAg kinetics can well correlate with direct or immune-mediated antiviral activity of IFN.

Accordingly, in both HBeAg-positive and -negative patients, Peg-IFN caused a significant decline of serum HBsAg levels, strongly associated with off-treatment response [35, 37]. In HBeAg-negative patients, after 1 year the decline in Peg-IFN-α HBsAg serum levels was significantly stronger in patients who achieved a response as compared to nonresponders; end-of-treatment (EOT) HBsAg levels were 10 IU/ml and associated with a 52% probability of HBsAg clearance through 3 years of post-treatment follow-up, compared to only 2% in patients with higher levels [35]. The predictive value of EOT HBsAg levels was higher than that of HBV DNA since HBsAg clearance was achieved in only 15% of the patients with undetectable HBV DNA at week 48. Similarly, HBeAg-positive patients with a virological response to Peg-IFN-α experienced the most pronounced HBsAg declines, whereas nonresponders showed small or absent reductions [37].

Prompted by these promising results on treatment, predictive rules were attempted using HBsAg levels or declines at week 12 or 24; however, none of them showed sufficient power to be successfully applied in clinical practice [38]. The discrepancy and inconsistency of the results between studies depend, at least in part, on the heterogeneity of the patients' cohorts, with the infecting HBV genotype being a major confounding factor. Accordingly, it has recently been shown that HBV genotypes influence HBsAg serum levels both prior to and during Peg-IFN-α, suggesting that the on-treatment timeframe where the HBsAg decline is more likely to be predictive of long-term response differs among viral genotypes [39, 40].

In HBeAg-negative CHB patients, the greatest difference between responders and nonresponders was observed between weeks 12 and 24 in genotype A patients and between baseline and week 12 in genotypes B and D, whereas in genotype C patients it was impossible to differentiate responders from nonresponders because of the minimal HBsAg decline observed in responders. All genotype A and B patients showed a reduction in HBsAg levels during treatment, with a more pronounced decline in responders. However, in genotype D patients the decline occurred only in responders, as HBsAg levels remained steady or increased slightly in nonresponders [39].

At variance with HBeAg-negative CHB, in HBeAg-positive patients HBsAg serum levels declined in all responders, independently of the genotype, but with different kinetics. Accordingly, in genotype A and D the absence of any HBsAg decline from baseline to week 12 was the most accurate predictor of nonresponse (negative predictive value: 97–100%), whereas the persistence of HBsAg levels >20,000 IU/ml was more accurate in genotypes B and C (negative predictive value: 92–98%) [40].

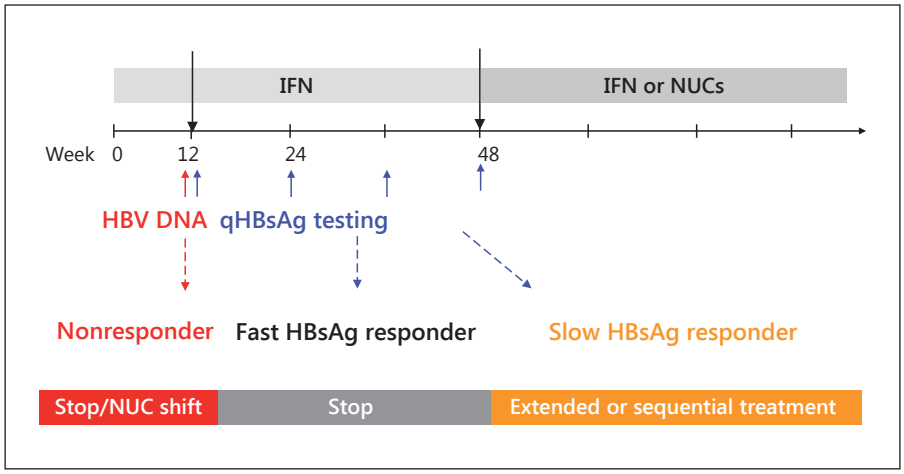

Fig. 1. Combined quantitative monitoring of HBV DNA and HBsAg serum levels from baseline to week 12; identifying nonresponders to IFN warrants an early treatment optimization. HBsAg serum levels at the end of treatment or their decline from baseline to week 48 can help to differentiate patients with a high probability of sustained off-therapy response (who can safely stop therapy) from those at a high risk of relapse (who could benefit from treatment extension or NUC shift) [46, 47].

Consequently, HBsAg genotype-specific algorithms should be designed to warrant response-guided therapy.

A further improvement in the outcome prediction could be obtained by combining the use of both HBV DNA and HBsAg as shown in the critical differential diagnosis between active and inactive HBV infection [41]. In HBeAg-negative CHB patients, the absence of any HBsAg decline together with <2 log IU/ml reduction of HBV DNA after 12 weeks of Peg-IFN-α identifies nonresponders with high accuracy (negative predictive value: 95–100%) [42].

At present, quantitative HBsAg monitoring, eventually combined with that of viral load, proves useful to early identify nonresponders to IFN in both HBeAg-positive and -negative CHB patients [40, 42]. In addition, preliminary reports suggest that EOT HBsAg serum levels could contribute to the identification of patients with a high chance of HBsAg clearance or a sustained virological response [35, 39]. Accordingly, it would be possible to design different treatment strategies with improved cost-efficacy by identifying HBV patients without or with slow versus fast HBsAg decline (fig. 1).

Overall, HBsAg quantification is becoming pivotal for the management of the CHB patient and several assays have been developed. Preliminary data suggest good overall statistical correlations; however, since some assays have shown genotype-dependent differences in analytical sensitivity, clinical decision-making in the single patient should rely only on monitoring performed with the same assay [43].

Finally, recent studies suggest that adding on or shifting to Peg-IFN-α in long-term NUC-treated patients could warrant, at least in a subset of patients, the sustained control of HBV infection as proven by HBeAg to anti-HBe seroconversion or HBsAg loss [44, 45].

In conclusion, new integrated molecular and clinical knowledge in a systems medicine view provides useful means to improve antiviral therapy in CHB. The identification of both viral and host heterogeneity as critical factors of Peg-IFN-α-related outcomes point to a new avenue of personalized medicine. The assessment of on-treatment dynamics of HBV infection by combined quantitative monitoring of HBV DNA and HBsAg and their mathematic modelling will help bring cost-effective actions to the single-patient level by using the different combinations of currently available antivirals, including IFN, more appropriately.

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