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ORIGINAL ARTICLE

Long-Term Effect of Interferon Therapy in Patients with HBeAg Positive Chronic Hepatitis B Infection

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Abstract
Introduction Several studies have reported that interferon therapy increases elimination rate of HBeAg and anti-HBe seroconversion in chronic hepatitis B (CHB) patients. We aimed to evaluate long-term results of interferon-α treatment in HBeAg positive CHB patients in a country with exclusively D genotype.
Methods Seventy-one naive CHB patients (M/F 61/10, mean age 29 ± 12 years, range 16–62) treated with 6 months of interferon-α 2b, 10 MU tiw and had a consequent untreated follow-up period of at least 10 years with positive response were identified and their data were reviewed. The therapy response was defined as HBeAg seroconversion with undetectable HBV-DNA. The responders were followed-up at 3–6-month intervals.
Results Twenty-eight (39%) patients achieved HBeAg seroconversion (25 within the therapy, 3 within the consequent 12 months off-treatment follow-up). The responders were followed-up with a mean period of 152 months (range 123–181). In the follow-up period, 21/25 (84%) initial responders relapsed. On the other hand, 3 patients who did not respond at the end of therapy sustained the response during follow-up. Hence 21/28 total responders relapsed (75%), either with HBeAg reversion (3, 14.3%) or HBV-DNA elevation over 2000 IU/ml (or its equivalent in other types of definitions) and ALT elevation (18, 85.7%). The sustained response was present in 7 patients (9.8%). Serious side effects precluding completion of treatment occurred in three patients (4.2%). In multivariate analysis none of the pre-treatment parameters appeared to be significant in predicting response.
Conclusion Sustained response to interferon treatment is low in HBeAg positive CHB patients with genotype D.

Keywords Chronic hepatitis B · Interferon · HBeAg · Therapy · Genotype · Sustained response

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Introduction

Previous studies from Western European countries [1–4] and the United States [5] reported encouraging results with interferon-α treatment in HBeAg positive chronic hepatitis B (CHB) patients. Nearly 30–40% of patients achieved HBeAg seroconversion with HBsAg loss in up to 70% after 25 years of follow-up [1, 5]. However later studies with pegylated-interferon revealed considerable differences in response rate and durability in reference to the hepatitis B virus (HBV) genotype [6]. While the response rate and durability were best in genotype A, they were worst in genotype D. All the genotypic studies from our country revealed that HBV is exclusively genotype D in Turkey [7].

We aimed to evaluate the long-term results of interferon-α therapy in our HBeAg positive CHB patient group.

Patients and Methods

The data of 71 naive CHB patients treated with 6 months of interferon-α 2b, 10 MU tiw were reviewed. They all had an untreated follow-up of at least 10 years after the therapy.

All patients had CHB diagnosis with elevations of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) for 6 months or more and HBsAg, HBeAg, and HBV-DNA in serum before therapy. Serologic tests for hepatitis markers were performed by ELISA (Abbott Laboratories [EIA], North Chicago, IL, USA). Upon admission, serum HBV-DNA was tested with a molecular hybridization technique without amplification (Digene Hybrid-capture; Murex Diagnostics, Dartford, UK). Pretreatment liver biopsies were also performed. Histological evaluation was done according to Knodell scoring [8] and all patients had histological evidence of CHB (necroinflammatory activity > 3).

The response to treatment was defined as HBeAg seroconversion with undetectable HBV-DNA; the responders were followed-up at 3–6-month intervals with serum ALT testing. An HBV-DNA elevation over the cut-off level of 2000 IU/ml (or its equivalent in other types of definitions) was accepted as relapse, after the therapy response.

Results

A total of 71 CHB patients (61 males, 10 females) were evaluated. The mean age ± standard deviation (SD) at the beginning of treatment was 29 ± 12 years (range 16–62 years). All had elevated ALT of at least two times the upper limit of normal (≥80 U/l) and documented as being HBeAg positive for at least 6 months prior to the initiation of the treatment. Liver biopsies were performed uniformly before treatment. Pre-treatment characteristics of the patients are summarized in Table 1.

Twenty-eight CHB patients (39.4%) achieved HBeAg seroconversion (25 within the therapy period and three within 12 months post treatment). The responders were followed-up for a median period of 152 months (range 123–181) (Fig. 1).

In the follow-up period, 21 of 25 initial responder patients relapsed. None of the three late responder patients relapsed (Table 2). Hence, 21 of 28 total responders relapsed (75%), either with HBeAg reversion (3, 14.3%) or high HBV-DNA and ALT elevation with anti-HBe positivity (18, 85.7%). The mean period ± SD of e-minus relapse was 19 ± 22 months (range 6–92 months). In three of 18 patients HBV-DNA was over 2000 IU/ml, despite ALT levels that were in the reference range at the conclusion of the study. Two HBeAg reversions occurred in the 6 months of post therapy follow-up, and the third relapsed in the ninth month. In one of these patients, the anti-HBe titer was at the borderline level at the end of treatment. The sustained response considering the criteria of inactive carrier state (persistently normal ALT/AST levels and serum HBV DNA $\leq$ 2,000 IU/ml [9]) was, finally, present in seven CHB patients and the rate was 9.8%. While two of seven sustained responders lost HBsAg, one of them developed anti-HBs. Serious side effects precluding completion of treatment occurred in three patients (4.2%). In one, serious nausea and vomiting developed. The side effects were severe pancytopenia in one and serious elevation in ALT (1426 IU/l) and AST (839 IU/l) with hyperbilirubinemia in the other. Flare (intermittent elevations of aminotransferase activity to more than ten times the upper limit of normal and more than twice the baseline value [9]) developed in 17/71 (23.9%) of the patients between 2 and 16 weeks (mean week 8) of treatment. While flare developed in 2/7 (29%) of the sustained responders, it was present in 15/54 (28%) of the others. There were 17 total flare episodes in the 71 patients with seven of the flare episodes occurring within the 28 end-of-treatment responders group. Therefore, treatment-induced flare did

Table 1 Pre-treatment characteristics of all CHB patients

CHB patients treated with interferon	Total CHB patients ($n = 71$)	Nonresponders ($n = 43$)	Initial responders ($n = 28$)	*P*
Mean age ± SD	29 ± 12	30 ± 12	28 ± 11	>0.05
Gender (male/female)	61/10	37/6	24/4	>0.05
Cirrhotic at the beginning of therapy, *n* (%)	5 (7%)	2 (4.7%)	3 (10.7%)	>0.05
ALT (mean ± SD, IU/l)	74 ± 60	68 ± 61	82 ± 58	>0.05
HBV-DNA (mean, IU/ml)	56,102,906	61,183,107	46,619,866	>0.05
Knodell inflammation score (mean ± SD)	7 ± 2.7	6.3 ± 2.8	7.4 ± 2.7	>0.05
Knodell fibrosis score (mean ± SD)	1.25 ± 0.9	1.15 ± 0.9	1.45 ± 0.9	>0.05

CHB chronic hepatitis B, *SD* standard deviation, *ALT* alanine aminotransferase, *HBV* hepatitis B virus

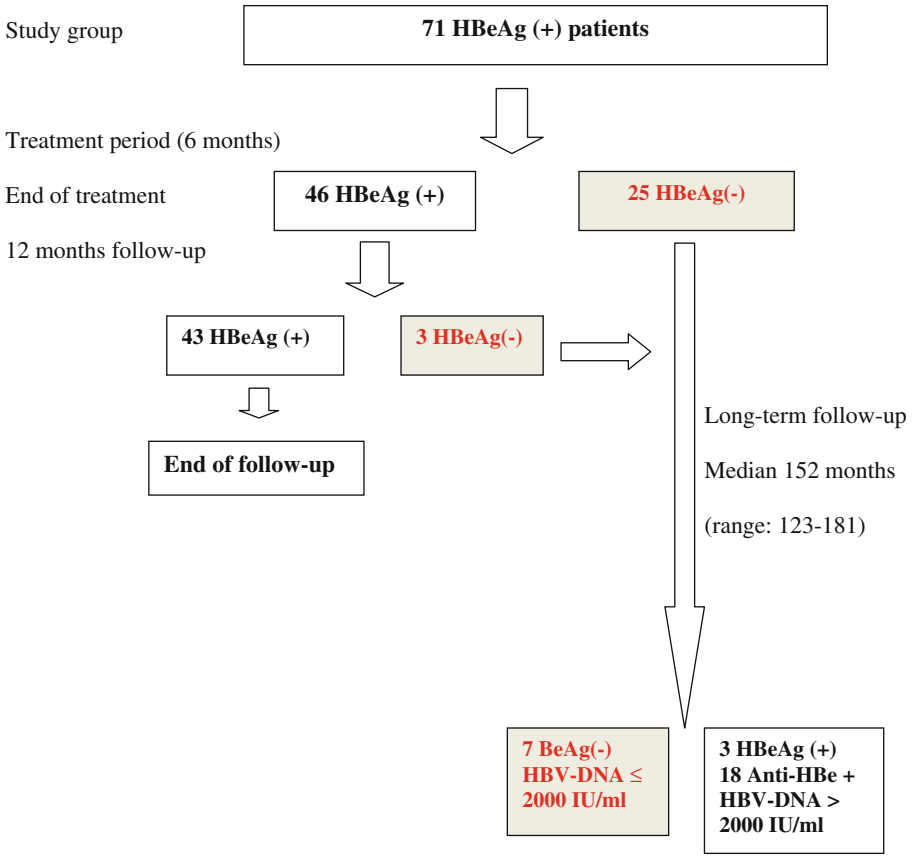

Fig. 1 Summary of results of long-term follow-up

Table 2 Pre-treatment characteristics of the initial responder CHB patients

Initial responder CHB patients	No sustained response ($n = 21$)	Sustained response ($n = 7$)	*P*
Mean age ± SD	27 ± 9	30 ± 14	>0.05
Gender (male/female)	18/3	6/1	>0.05
Cirrhotic at the beginning of therapy, *n* (%)	3 (14%)	0 (0%)	>0.05
ALT (mean ± SD, IU/l)	79 ± 44	86 ± 51	>0.05
HBV-DNA (mean, IU/ml)	52,808,454	39,601,250	>0.05
Knodell inflammation score (mean ± SD)	7.3 ± 2.7	7.7 ± 2.7	>0.05
Knodell fibrosis score (mean ± SD)	1.5 ± 1.1	1.3 ± 0.7	>0.05

CHB chronic hepatitis B, *SD* standard deviation, *ALT* alanine aminotransferase, *HBV* hepatitis B virus

not appear as a significant predictive factor for either end-of treatment or sustained response in this study. Furthermore, two patients who lost HBsAg did not develop flare in the course of treatment. In multivariate analysis none of the studied pre-treatment parameters appeared to be significant in predicting response.

Discussion

Previous studies have suggested that HBeAg seroconversion using interferon-based treatments was a very reliable end-point with long-term durability and eventual HBsAg seroconversion [1, 5, 10–12]. Most of these early studies were from the United States and Western Europe. HBV genotype D is not prevalent in these parts of the world. In most of these studies the follow-up period was too short [10, 12] and HBV-DNA quantitation methods were rather insensitive in comparison to the assays available today. Therefore, some of the patients with a probable recurrence over a longer time may have been missed. Our findings, as well as some of the previous studies, revealed that the occurrence of e-minus (the so-called mutant) infection years after interferon-induced HBeAg seroconversion is not rare [13–15].

Our study questions the significance of interferon-induced HBeAg seroconversion in the management of CHB patients with genotype D. It is generally agreed that HBeAg seroconversion confers long-term benefit in the course of CHB. This is true when it is associated with long-term HBV-DNA below 2000 IU/ml, normal ALT, improved histology, and eventual HBsAg clearance [16]. Taking into account the very low rate of HBsAg seroconversion in our interferon-treated HBeAg positive group in comparison to the studies of western countries, a high rate of recurrence in the form of HBeAg negative CHB is not surprising.

The most recent CHB treatment guideline, EASL 2009, advises consideration of interferon treatment for HBeAg positive CHB cases where serum ALT is over 3 times the upper limit of normal and serum HBV-DNA is higher than 2×10^6 IU/ml [17]. In our study, only one of 71 (1.4%) patients retrospectively met these criteria. This was not unexpected because in most HBeAg positive patients serum HBV-DNA is higher than 2×10^6 IU/ml, or serum ALT is not over three times the upper limit of normal. This particular patient achieved end-of-therapy response, recurrence did not happen and HBsAg was lost, with the emergence of anti-HBs.

In HBeAg positive CHB, the initial end-of-treatment response is usually defined as HBeAg loss, with undetectable HBV-DNA and normal serum ALT. HBeAg seroconversion, reportedly, connotes a more durable response. However, in most of the earlier studies, because of the low sensitivity of HBV-DNA assays, the initial response rate may have been overestimated. The emergence of more sensitive assays was concomitant with the emergence of pegylated-interferon in the treatment of CHB, and examining the HBV-DNA status of pegylated-interferon treated patients showed that in a significant number of end-of-treatment HBeAg seroconverters, serum HBV-DNA was over 10^4 IU/ml and ALT was still elevated, thus they had e-minus chronic hepatitis by definition [18]. Moreover, durability of response was worse in the D genotype, which is exclusive in Turkey [7]. HBsAg seroconversion rate is a controversial issue. In a recent study, 52% of the sustained responders (29% of all-treated) lost HBsAg in 7 years [1]. However, in another study with interferon-α, the rate was poorer [19].

Interestingly, in the initial interferon therapy of CHB trials in Europe, the HBeAg clearance rates were very high. This can be seen in the study by Niederau et al. [2] with 2–5 MU interferon-α 2b for 4–6 months, the sustained HBeAg clearance rate (over 5 years) was 39%, which rose to 56% after a second course with a higher dose of interferon therapy. In this study relapse was non-existent [2]. HBsAg clearance rate in patients with HBsAg seroconversion in 5 years was 19%. The corresponding figures, from different studies of Americans [5] and Chinese [17], were 71 and 17%, respectively.

With pegylated-interferon α-2b, there was a significant difference between genotypes in reference to HBsAg clearance in initial responders in the long-term (around 3 years), e.g. 58% in genotype A, 14% in genotype B, and 6% in genotype D [4]. The same study revealed that in long-term follow-up of initial responders, while HBV-DNA was undetectable in 65% genotype A infected patients, it was 24% in genotype D infected patients. In this study, in 27 of 64 (42%) responders, HBV-DNA was still above 2000 IU/ml at the conclusion of treatment. Thus, in around half of the patients, transition to inactive carrier state did not happen with HBeAg loss. In genotype D, core-promoter mutation was present in 43% and pre-core mutation was present in 41% at the same time point. The corresponding rates were 19 and 22% in genotype A. At the conclusion of the treatment in our cohort, the detectability limit of HBV-DNA was 4 pg/ml, with 1 pg/ml corresponding to 150,000 copies/ml. Therefore, a considerable number of e-responders in our study may have had HBV-DNA levels well over 2000 IU/ml (even over 20,000) and could not have been considered initial responders with current time standards. In light of these findings, we may assume that some of the patients we considered initial responders may have not been so even at the end of therapy response. In a recent study, Buster et al. [6] showed very significant differences between A and D genotypes (30% versus 6%) in reference to short-term sustained response (52 weeks of peginterferon α-2b, 26 weeks untreated follow-up, response defined as HBeAg loss with HBV-DNA $<$ 2000 IU/ml).

In another recent study of Buster et al. [20], the data of the largest randomized trials investigating peginterferon in HBeAg-positive CHB were combined. Sustained response rate was revealed as 37% of patients with genotype A, 25% with genotype B, 20% with genotype C, and only 8% with genotype D. The sustained responder patients in their data were older, more often were female, had lower baseline HBV-DNA and higher ALT levels, and were more likely to have genotype A but less likely to have genotype D infection compared with those patients without sustained response. They stated HBeAg-positive and genotype D CHB patients as poor candidates for peginterferon therapy. The analysis of peginterferon data, at least in reference to genotype D patients, is hardly convincing that peginterferon is more efficacious than interferon-α in the management of chronic hepatitis B infection.

In conclusion, interferon-α treatment is effective in a small minority of HBeAg positive patients with genotype D infection. Continuing or recurring e-minus type infection in untreated follow-up is common in patients after the end-of-treatment HBeAg seroconversion.

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