Interferon Alfa for Chronic Hepatitis B Infection: Increased Efficacy of Prolonged Treatment

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Interferon alfa (IFN-α) is the primary treatment for chronic hepatitis B. The standard duration of IFN-α therapy is considered 16 weeks; however, the optimal treatment length is still poorly defined. We evaluated the efficacy and acceptability of prolonged IFN-α treatment in patients with chronic hepatitis B. To investigate whether treatment prolongation could enhance the rate of hepatitis B e antigen (HBeAg) seroconversion, we conducted a prospective, controlled, multicenter trial in which all patients were treated with a standard regimen of 10 million units IFN-α 3 times per week over 16 weeks. Patients who were still HBeAg-positive after 16 weeks of therapy were randomized to prolongation of the identical regimen up to 32 weeks (prolonged therapy) or discontinuation of treatment (standard therapy). Among the 162 patients who entered the study, 27 (17%) were HBeAg-negative after the first 16 weeks of treatment, and 118 were randomized to standard or prolonged therapy. After randomization, a response (HBeAg seroconversion and sustained hepatitis B virus [HBV]-DNA negativity) was observed in 7 of the 57 (12%) patients assigned to standard therapy versus 17 of the 61 (28%) patients assigned to prolonged therapy ($P = .04$). A low level of viral replication after 16 weeks of treatment, as indicated by serum HBV-DNA values under 10 pg/mL, was found to be the only independent predictor of response (52% vs. 0%; $P < .001$) during prolonged therapy. The prolonged IFN-α schedule was well tolerated in the large majority of patients. In chronic hepatitis B, prolongation of IFN-α therapy up to 32 weeks is superior to a standard course of 16 weeks. Those patients who exhibit a low level of viral replication at the end of the standard regimen benefit most from prolonged treatment. (HEPATOLOGY 1999; 30:238-243.)

Worldwide, chronic hepatitis B virus (HBV) infection is a major cause of cirrhosis and hepatocellular carcinoma. Clearance of hepatitis B e antigen (HBeAg) indicates transition to a state of low-level viral replication that is accompanied by biochemical remission of liver disease and prolonged survival.[1-3] Interferon alfa (IFN-α) has emerged as one of the most effective treatments for chronic hepatitis B, inducing HBeAg seroconversion in about one third of the patients.[4,5] Many attempts to enhance this response rate have been unsuccessful. Treatment with nucleoside analogues, such as lamivudine, or combination of these agents with IFN-α, does not yet appear to significantly increase the HBeAg seroconversion rate,[6-8] whereas the use of priming therapy with prednisone has not been generally accepted as a result of a lack of consistent results and the risk of hepatic decompensation.[4,9] How long we should treat chronic HBV patients with IFN-α is still not established. The standard duration of treatment is considered 16 weeks. In several studies, IFN-α was given for a longer period, and some suggest additional benefit of prolonged therapy.[10,11] However, these studies contain a small number of patients, show considerable heterogeneity in patient population and response rates, and the results should therefore be interpreted with caution. In an uncontrolled pilot study, we successfully prolonged IFN-α treatment in those patients who approximated HBeAg seroconversion, as demonstrated by a continuous decrease of quantified serum HBV-DNA and HBeAg values, at the end of standard IFN-α therapy.[12] Therefore, we initiated a large, prospective, randomized, controlled trial investigating the efficacy of treatment prolongation with an additional 16 weeks in those patients who did not respond with HBeAg seroconversion during a standard 16-week IFN-α course.

PATIENTS AND METHODS

Patients. One hundred sixty-two patients from 16 European hepatologic centers (EUROHEP)[13] were enrolled after central evaluation of their eligibility. The inclusion criteria were age between 18

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; IFN-α, interferon alfa; HBsAg, hepatitis B surface antigen; AST, aspartate transaminase; ALT, alanine transaminase.

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and 70 years; hepatitis B surface antigen (HBsAg) positivity in serum for at least 6 months; presence of HBeAg and HBV DNA (by hybridization methodology) in serum, and elevation of either serum aspartate transaminase (AST) or alanine transaminase (ALT) values, as documented on 3 occasions in the 3 months before entry; and histological evidence of chronic hepatitis on a liver biopsy taken in the 6 months preceding enrollment. Patients were excluded for the following reasons: presence of serum antibodies against hepatitis delta, hepatitis C, or human immunodeficiency virus; recent alcohol abuse or drug addiction; previous IFN-α course with a minimum duration of 12 weeks using at least 30 million units (MU) per week; any antiviral or immune modulatory therapy in the preceding 6 months; immunocompromised patients; pregnancy; females of fertile age with inadequate contraception; significant medical illness potentially interfering with completion of the study; inadequate levels of hemoglobin (<6 mmol/L), platelets ($<70 \times 10^9$/L), or leukocytes (3×10^9/L); decompensated liver disease with a bilirubin > 34 μmol/L, albumin < 32 g/L, or history of either ascites, bleeding esophageal varices, and hepatic encephalopathy. During the screening period, 1 patient died in a car accident, and 7 others were excluded from the start of treatment after initial approval of their eligibility: 4 decided not to participate in the study, 2 lost serum HBeAg, and 1 became pregnant. The study was formally approved by the ethical committee of each participating center, and the patients were required to give written informed consent before entering the study.

Design of the Study. Recombinant IFN-α (alfa-2b, Intron-A; Schering-Plough, Kenilworth, NJ) was administered subcutaneously to all patients at a dose of 10 MU three times per week during a 16-week period (phase A). Patients who were HBeAg-seropositive after 16 weeks of treatment were randomly assigned to either prolongation of the same IFN-α regimen up to 32 weeks (prolonged therapy) or discontinuation of IFN-α after 16 weeks (standard therapy) (phase B). Those not randomized were followed without further therapy (Fig. 1). Patients were instructed to self-administer IFN-α to make further treatment on an outpatient basis feasible. All patients were seen at the outpatient clinic for routine examination and laboratory assessment at weeks −8, −4, 0, 2, and 4 of therapy, and monthly in the period thereafter up to 32 weeks; after 32 weeks, patients were seen every 4 to 8 weeks until 52 weeks after the start of therapy. On all occasions, hematological studies (hemoglobin, leukocytes, platelets), and serum markers of viral replication (HBeAg and HBV DNA) and hepatic inflammation (AST) were assessed; every 2 months, additional laboratory investigations including measurements of serum bilirubin, albumin, ALT, prothrombin time, HBsAg, and antibodies against HBeAg and HBsAg were performed. To score acceptability and safety of prolonged IFN-α treatment, a standardized questionnaire, in which patients could notify the intensity of side-effects in 3 scores (0 = none, 1 = mild, 2 = moderate), was completed every 2 months. Predefined clinical and laboratory toxicity criteria were used for dose reduction and discontinuation of IFN-α.

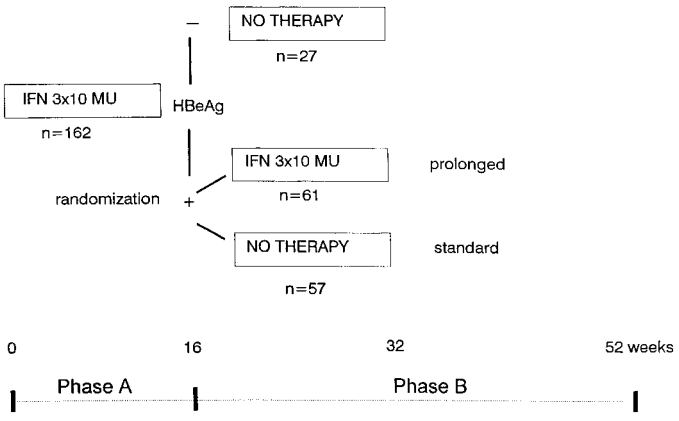

FIG. 1. Design of the study.

Laboratory Evaluation. Routine biochemical and hematologic tests were performed at the participating centers using automated techniques. To correct for heterogeneity of local assays and to ensure standardized results, levels of AST and ALT were expressed as values representing a ratio to the local upper limit of normal. All HBV markers were assessed centrally at the Department of Virology, Erasmus University in Rotterdam. HBeAg, antibodies against HBeAg, HBsAg, and antibodies against HBsAg were measured using routine commercially available enzyme immunoassays (Abbott Laboratories, North Chicago, IL). HBV DNA was determined by a solution hybridization assay that uses a ^{125}I-labeled probe (cut-off: 1.7 pg/mL; Genostics, Abbott Laboratories). The criteria for response to therapy were defined as loss of HBeAg and HBV DNA that sustained until the end of follow-up at week 52. All other patients were considered nonresponders.

All patients underwent a liver biopsy in the 6 months before treatment; a second liver biopsy was proposed to each patient at 1 year after the start of therapy. Biopsy specimens were evaluated by a single experienced pathologist who was blinded with respect to the treatment regimen and chronological order of the biopsies. Histological scoring was performed according to the Desmet modification of the histological activity index as described by Knodell et al.[14,15]

Randomization and Statistical Analysis. To enable optimal intention-to-treat analysis, we chose to randomize as late as possible within the initial 16-week course of treatment (phase A). The randomization was performed centrally after 14 weeks of treatment only for those patients being HBeAg-positive after the first 12 weeks of treatment. Criteria other than HBeAg negativity that precluded randomization were discontinuation of IFN-α during the first 12 weeks, unwillingness of the patient to continue treatment beyond 16 weeks, and incompleteness of collected data. To balance the pretreatment response probability, the randomized groups were stratified according to serum HBV-DNA level (more or less than 150 pg/mL) and AST level (more or less than twice the upper limit of normal) at entry. Within each stratum, patients were randomly assigned to standard or prolonged IFN-α therapy using consecutively numbered, sealed, opaque envelopes. The result of the randomization procedure was sent by fax to the participating centers on the same day. It was pre-established that patients who became HBeAg-negative between weeks 12 and 16, and thus in retrospection, not eligible for phase B, were withdrawn from the randomized study and followed without further therapy until 52 weeks after the start of treatment. To achieve a comparable observation time after cessation of therapy, we separately analyzed the results for the responders of the prolonged treatment group after extension of the follow-up period with 16 weeks.

To obtain a power (1-β) of greater than 80% ($\alpha = .05$), an estimated number of 120 patients should be eligible for randomization after 16 weeks, assuming an HBeAg seroconversion rate of 5% for standard treatment (no further therapy after randomization) and 25% for prolonged treatment (another 16 weeks of IFN-α after randomization), and a 5% drop-out after randomization. Intention-to-treat analysis was performed for randomized patients who were HBeAg-positive at week 16. The Wilcoxon rank sum and signed rank tests were used to compare unpaired and paired observations, respectively. Comparison of discrete variables was accomplished using the χ^2 test or Fisher's exact test. Rate of response to therapy was calculated with life-table analysis. Actuarial curves of response were compared by log rank testing, and independent prediction of response was evaluated by stepwise logistic regression analysis. Before multivariate analysis, logarithmic transformation was applied for HBV DNA and AST because of skewed distribution.

RESULTS

Twenty-seven of the 162 (17%) patients who started IFN-α therapy exhibited clearance of HBeAg and HBV DNA during

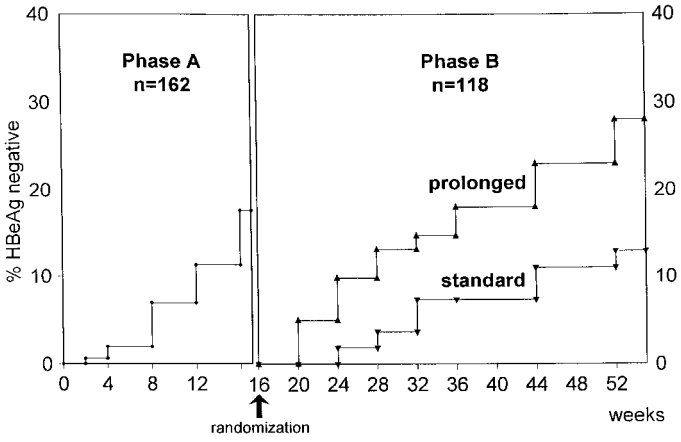

FIG. 2. Kaplan-Meier estimate of HBeAg disappearance during the initial 16 weeks of IFN-α therapy (phase A) among all patients who started treatment (n = 162; *left*), and of sustained HBeAg disappearance (response) for patients being HBeAg-positive at week 16 and randomized to standard (no further therapy after randomization) or prolonged treatment (another 16 weeks of IFN-α after randomization) (phase B; n = 118; *right*).

phase A (Fig. 2). Nine of these 27 patients were randomized (standard therapy n = 7; prolonged therapy n = 2) but appeared to be HBeAg-negative at week 16, and were thus excluded from further analysis of phase B. Seventeen other patients were not eligible for randomization, because they either discontinued treatment in phase A (n = 12), refused to continue therapy in phase B (n = 4), or were in retrospect HBV-DNA–negative at the start of therapy (n = 1). Of the 118 patients analyzed after randomization (phase B), 4 (7%) assigned to standard therapy and 1 (2%) to prolonged therapy did not complete the entire follow-up period of 1 year. These 5 patients were considered nonresponders, because all final samples obtained showed HBeAg positivity.

Patient characteristics of the 162 patients who started treatment (phase A) and the 118 patients who were analyzed after randomization (phase B; standard therapy n = 57, prolonged therapy n = 61) are shown in Table 1. The 2 randomized groups were similar with respect to age, sex, race, sexual preference, history of acute hepatitis, known duration of hepatitis, presence of cirrhosis, and levels of HBV DNA and transaminases, both at entry and at the time of randomization. After randomization, a response (sustained HBeAg and HBV-DNA negativity) was observed in 7 of the 57 (12%) patients of the standard-treatment group and in 17 of the 61 (28%) patients of the prolonged treatment group (P = .04) (Fig. 2). The median time interval between randomization and response was 16 weeks for both the standard and prolonged-treatment group. In the prolonged-treatment group, 56% of the patients who responded lost HBeAg while still receiving IFN-α. Twenty-three of the 24 responders (96%) developed antibodies against HBeAg. Two patients in the standard-treatment group and 1 in the prolonged-treatment group demonstrated clearance of HBeAg with subsequent HBeAg reappearance during posttreatment follow-up. The HBeAg response in the prolonged-treatment group remained unchanged after an extended follow-up of 16 weeks, because HBeAg reactivation did not occur during this period. Of the 27 patients who became HBeAg-negative during phase A, 6 exhibited an HBeAg reactivation, leaving 21 responders at 52 weeks. Combining the response rate of phase A (13%) with the response rate of phase B (12% for standard therapy and 28% for prolonged therapy), the cumulative response probability obtained for standard and prolonged therapy equals 24% and 37%, respectively. Among the patients who were HBV-DNA–positive at randomization (Table 1), sustained negativity of HBV DNA with persistent HBeAg positivity was observed in 4 of the 45 (9%) of the standard-treatment group and in 8 of the 44 (18%) of the prolonged-treatment group

TABLE 1. Characteristics of All Patients and of the Patients Randomized to Standard and Prolonged IFN-α Treatment at Entry and After Randomization at the Start of Phase B

		Randomized Group (n = 118)	
	Total Group (n = 162)	Standard (n = 57)	Prolonged (n = 61)
At entry			
Age (yr)*†	34 (16-70)	31 (17-70)	33 (16-64)
Male (%)	121 (75)	45 (79)	40 (66)
Race			
White (%)	130 (80)	47 (82)	48 (79)
Asian (%)	23 (14)	5 (9)	11 (18)
Other (%)	9 (6)	5 (9)	2 (3)
Duration HBsAg positivity (yr)*	2.1 (0.5-20)	2.0 (0.5-20)	2.0 (0.5-20)
History acute hepatitis (%)	18 (11)	4 (7)	8 (13)
Cirrhosis (%)	27 (17)	6 (11)	9 (15)
HBV DNA in serum (pg/mL)*	115 (2-1065)	144 (2-883)	117 (2-1065)
AST in serum*‡	1.64 (0.61-10.53)	1.40 (0.61-6.13)	1.61 (0.67-10.53)
ALT in serum*‡	3.31 (0.72-20.09)	2.96 (0.72-15.00)	3.06 (0.94-20.09)
After randomization (week 16)			
HBeAg positivity (%)	—	57 (100)	61 (100)
HBV-DNA positivity (%)	—	45 (79)	44 (72)
HBV-DNA in serum (pg/mL)*	—	37 (2-503)	30 (2-826)
AST in serum*‡	—	1.17 (0.40-16.76)	1.18 (0.50-11.11)

*Median (range).

†Five patients (3 in the prolonged-therapy group and 2 in the standard-therapy group) were younger than 18 years old. This protocol violation was considered minor, and these patients were thus retained in the study.

‡Elevation of AST or ALT/upper limit of normal range.

TABLE 2. Mean Histological Scoring ± SEM of Paired Liver Biopsies Obtained From Patients Randomized to Standard and Prolonged IFN-α Treatment

		Grading Score		Fibrosis Score		HAI Score	
	n	Entry	Follow-up	Entry	Follow-up	Entry	Follow-up
Standard therapy							
Nonresponse	15	3.3 ± 0.7	2.9 ± 0.6	1.5 ± 1.0	1.8 ± 0.2	4.8 ± 0.9	4.7 ± 0.6
Response	4	3.3 ± 2.4	1.3 ± 0.5	2.0 ± 0.9	1.8 ± 0.8	5.3 ± 3.2	3.0 ± 2.2
Prolonged therapy							
Nonresponse	18	3.4 ± 0.5	2.6 ± 0.3	1.7 ± 0.9	1.8 ± 0.3	5.0 ± 0.7	4.3 ± 0.5
Response	13	4.5 ± 0.9	1.6 ± 0.3	2.2 ± 0.4	2.2 ± 0.3	6.6 ± 1.0	3.8 ± 0.5

NOTE. Grading score: sum of periportal hepatitis, confluent necrosis, focal necrosis, and portal inflammation.
Abbreviation: HAI, histological activity index; sum of grading and fibrosis score.

($P = .11$). None of the patients allocated to standard treatment lost HBsAg, whereas 3 (5%) patients in the prolonged group did so. At the last observation, 76% of the responders and 33% of the nonresponders had normal AST levels ($P < .001$). Irrespective of response, 10 of the 32 patients (31%) with elevated AST levels at randomization in the standard-therapy group versus 16 of the 39 patients (41%) in the prolonged-therapy group exhibited persistent normalization of AST ($P = .27$).

Histological scoring of pretreatment biopsies was performed for 147 of the 162 (91%) patients who entered the study and for 108 of the 118 (92%) randomized patients. Twelve initial biopsy specimens had not been received by the consulting pathologist, and 3 were considered inadequate for histological grading and staging. No significant difference in histological activity index score was found between the randomized groups in initial biopsy specimens (standard therapy n = 53; mean score: 4.9 ± 0.5 vs. prolonged therapy n = 55; mean score: 5.6 ± 0.4; $P = .13$). Paired liver biopsies were available for 50 (42%) of the 118 randomized patients. A marked histological improvement after IFN-α therapy was observed among responders of both the standard- and prolonged-therapy groups, primarily as a result of regression of portal, periportal, and lobular inflammation (Table 2). There was no change in degree of fibrosis and confluent necrosis. Because there appeared to be an association between the probability of response and the likelihood of undergoing a posttreatment biopsy (79% among responders vs. 37% among nonresponders), correlation of histological changes with scheme of therapy was not considered feasible.

To assess which patients may benefit from prolonged IFN-α treatment, a response prediction analysis was performed for the patients assigned to prolonged therapy (Table 3). In the univariate analysis, patients with higher age, presence of cirrhosis, and high AST values showed a better response rate during prolonged therapy. These differences, however, did not reach statistical significance. Low HBV-DNA level at randomization was strongly associated with response during prolonged therapy ($P < .001$), while low HBV DNA at entry tended toward significance ($P = .07$). By multivariate analysis, only HBV-DNA level at randomization was shown to be predictive of response ($P < .001$). In fact, all patients responding in the prolonged-treatment regimen had HBV-DNA levels below 10 pg/mL at randomization (Fig 3).

A transient flu-like syndrome with fever and myalgia was seen in nearly all of the patients during the initial days of IFN-α treatment. Other frequent side-effects, encountered in at least 20% of the patients, were fatigue, asthenia, anorexia, arthralgia, and various neuropsychiatric symptoms including depression and irritability. During the initial 16 weeks of therapy (phase A), dose modification as a result of adverse effects was required in 16 of the 162 patients (10%). Eleven of these 16 patients stopped treatment before randomization. The reasons for discontinuation were flu-like symptoms in 3 patients, psychosis or depression in 3, dizziness in 2, fatigue in 1, thrombocytopenia in 1, and exacerbation of the HBV

TABLE 3. Characteristics of Patients Assigned to Prolonged IFN-α Treatment (n = 61) in Relation to HBeAg Response

	n	HBeAg Response	*P*
Sex			
Male	40	28% (9%)	.93
Female	21	29% (25%)	
Age			
≤35 years	34	21% (10%)	.14
>35 years	27	37% (15%)	
HBsAg positivity			
≤2 years	33	24% (15%)	.56
>2 years	28	32% (10%)	
History of acute hepatitis			
No	53	30% (11%)	.33
Yes	8	13% (25%)	
Asian origin			
No	50	30% (13%)	.39
Yes	11	18% (0%)	
Cirrhosis			
No	52	25% (12%)	.20
Yes	9	44% (17%)	
AST at entry			
≤1.5 normal	24	20% (13%)	.26
>1.5 normal	37	32% (12%)	
AST at randomization			
≤1.5 normal	35	31% (16%)	.56
>1.5 normal	26	23% (5%)	
HBV DNA at entry			
≤100 pg/mL	29	38% (16%)	.07
>100 pg/mL	32	19% (11%)	
HBV DNA at randomization			
≤10 pg/mL	33	52% (21%)*	<.001
>10 pg/mL	28	0% (6%)	

NOTE. The given *P* values were calculated from differences in HBeAg response within this group of 61 patients. Percentages in parentheses denote the response rate of patients with similar characteristics randomized to standard IFN-α treatment (n = 57).

*Among patients with HBV DNA ≤10 pg/mL at randomization, the HBeAg response was significantly higher in the group of prolonged therapy as compared with standard therapy ($P = .02$). In patients randomized to standard therapy, 5 of the 24 (21%) with HBV DNA ≤10 pg/mL at randomization versus 2 of the 33 (6%) with HBV DNA >10 pg/mL exhibited a response ($P = .002$).

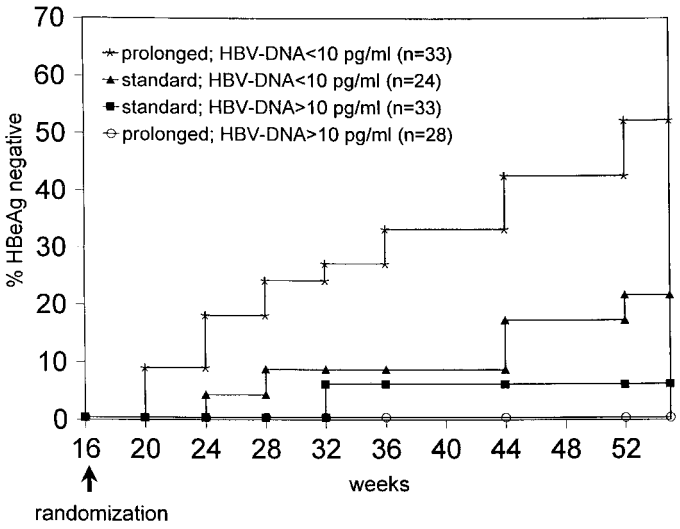

FIG. 3. HBeAg clearance for patients in the standard- and prolonged-therapy groups with HBV-DNA levels above or under 10 pg/mL after 16 weeks of therapy.

infection with hepatic decompensation in 1. In 1 patient, trial medication was interrupted because lung cancer was diagnosed after 12 weeks of therapy. After randomization (phase B), dose reduction was necessary in 7 of the 61 patients (11%) receiving prolonged IFN-α therapy because of depression, fatigue, hair loss, and headache; 3 of the 7 patients discontinued therapy prematurely, all as a result of a combination of these side-effects. One patient fractured a rib after he collapsed as a result of dizziness in week 32 just after the scheduled treatment had been completed. None of the patients died during the study. A standardized scoring system to evaluate the tolerance of prolonged treatment only showed aggravation of hair loss after 32 weeks as compared with 16 weeks of IFN-α therapy (Table 4). There was no significant increase in severity of other side-effects, such as fatigue, depressive feelings, anorexia, and concentration disturbances. Nearly all adverse effects attributed to IFN-α were reversible with dose modification or cessation of therapy.

DISCUSSION

IFN-α is the only agent with an established long-term beneficial effect on the natural history of chronic hepatitis B.[1] The currently recommended IFN-α treatment schedule is either 5 MU daily or 10 MU three times per week for 16 weeks.[16] We demonstrated an increased clearance of both HBeAg and HBV DNA after prolonged therapy as compared with treatment with the standard duration of 16 weeks. The enhanced efficacy of prolonged treatment was so pronounced that in future treatment regimens comprising both IFN-α monotherapy as its combination with other antiviral agents, prolongation of IFN-α therapy up to 32 weeks should be considered.

TABLE 4. Mean Score ± SEM of Side-Effects (0 = none, 1 = mild, 2 = moderate) in Week 16 (randomization), Week 32 (end of therapy), and at the End of the Follow-up Period, as Reported by the Patients Treated With Prolonged IFN-α Treatment (n = 61)

	Randomization	End of Therapy	End of Follow-up
Fatigue	0.98 ± 0.07	0.80 ± 0.08	0.19 ± 0.06
Anorexia	0.46 ± 0.09	0.34 ± 0.08	0.03 ± 0.03
Myalgia	0.48 ± 0.09	0.43 ± 0.08	0.02 ± 0.02
Arthralgia	0.33 ± 0.07	0.30 ± 0.06	0.02 ± 0.02
Depressive feelings	0.29 ± 0.07	0.30 ± 0.07	0.07 ± 0.03
Concentration disturbances	0.25 ± 0.06	0.31 ± 0.07	0.03 ± 0.02
Headache*	0.54 ± 0.08	0.38 ± 0.08	0.02 ± 0.02
Hair loss*	0.43 ± 0.09	0.69 ± 0.10	0.02 ± 0.02

*Significant difference between week 16 and week 32 ($P < .05$).

The beneficial effect of treatment prolongation beyond 4 months has previously been suggested by a time-dependent analysis of individual data of 746 patients from 10 clinical controlled trials.[17] The concept, still propagated by many physicians, that IFN-α treatment for chronic hepatitis B becomes ineffective after a certain total dose has been administered, was not supported by that study. Individual studies comparing the different lengths of IFN-α therapy in chronic hepatitis B are scarce. In a study from the United Kingdom that compared the effectiveness of 12 and 24 weeks of lymphoblastoid interferon, no additional benefit was observed with prolonged treatment.[18] The lack of favorable response in patients scheduled for the 24 weeks of therapy could well have been caused by a poor tolerance of the longer course. Only 8 of the 20 patients in that study were able to complete the full 24 weeks of treatment, and none were able to continue on full dose. In the present study, this problem was, to a large extent, circumvented by randomizing only those patients willing to continue therapy after 16 weeks of treatment.

The pretreatment level of viral replication as indicated by quantitative HBV-DNA values in serum has been implicated as a strong response predictive variable in many studies on IFN-α therapy of chronic hepatitis B.[4,19] In the present study, serum HBV-DNA level after 16 weeks of treatment was the only significant feature to predict beneficial response as a result of prolongation of IFN-α therapy. In fact, only patients with HBV-DNA levels under 10 pg/mL experienced a sustained response after extension of treatment. This HBV-DNA cut-off value of 10 pg/mL after 16 weeks of treatment should be an easy indicator for physicians to select patients eligible for treatment prolongation. We were unable to obtain a significant independent predictive value from the relative decrease of serum HBV DNA during the first 16 weeks of therapy. However, the HBV-DNA assay used in this study, employing hybridization in solution, generates a detection limit of 10^5 to 10^7 HBV copies per milliliter.[20,21] It may well be that recently developed, more sensitive HBV-DNA assays or quantitative HBeAg assays could further optimize response prediction by indicating an approximating response in case of continuously decreasing serum HBV-DNA or HBeAg values.[22] Therefore, close monitoring of the effects of IFN-α on quantified markers of viral replication appears essential for an effective therapeutic strategy.

In spite of the relatively high scheduled dose of IFN-α, the prolonged-treatment course was, in general, well tolerated. Serious adverse events including hepatic decompensation, psychosis, and overt depression were rare, and mainly occurred during the first 16 weeks of therapy. Frequently reported side-effects of IFN-α, such as fever, myalgia, mood changes, and fatigue, are often proclaimed to be minor, but can be invalidating in personal life and lead to temporary discontinuation of work. Most of these side-effects did not become more intense during the period of prolonged treatment. On the contrary, many patients reported to become

somehow accustomed to the toxic effects of IFN-α, and during the phase of prolonged therapy IFN-α had to be stopped early in only 3 of the 61 patients. The absence of profound treatment intolerance during the prolonged IFN-α regimen is probably related to the design of this study in which only those patients without major side-effects during the first 16-week course were randomized to further or no further treatment.

In conclusion, prolongation of IFN-α treatment significantly enhances the response rate in chronic hepatitis B patients with persistent HBeAg, but a low level of viral replication (HBV DNA $<$ 10 pg/mL) at the end of the standard 16-week regimen. We propose, in contrast to the current concept of a fixed treatment course, an IFN-α regimen in which treatment duration is modified by on-line measurement of quantified viral markers. Based on the results of the present study, patients with HBeAg but low HBV-DNA levels after 16 weeks of IFN-α therapy should be offered treatment prolongation.

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APPENDIX

The following centers and investigators participated in this study (values in parenthesis denote the number of patients recruited): the Netherlands: University Hospital Dijkzigt, Rotterdam: H. L. A. Janssen, P. Honkoop, H. Snobl, S. W. Schalm (27); Rijnstate Hospital, Arnhem: R. A. de Vries, C. J. J. Mulder (9); University Hospital Utrecht, Utrecht: J. van Hattum, M.C. Rasch (8). Germany: Johannes Gutenberg Universität, Mainz: G. Gerken, G. Teuber (33); Medizinische Hochschule Hannover, Hannover: M. P. Manns (1). Belgium: University Hospital Antwerp, Antwerp: P. Michielsen (5); Hôpital Erasme, Brussels: M. Adler (5); University Hospital Gasthuisberg, Leuven: F. Nevens, J. Fevery (4); Academic Hospital Free University, Brussels: M. L. Hautekeetet (3); Hôpitaux St. Joseph, Gilly: R. Brenard (2). United Kingdom: King's College, London: N. Naoumov, R. Williams (8). Denmark: Rigshospitalet, Copenhagen: H. Ring-Larsen (8); Marselisborg Hospital, Århus: M. R. Buhl (2). France: Hôpital Beaujon, Clichy: P. Marcellin, N. Boyer (12); Hôtel Dieu, Lyon: C. Trepo (2). Spain: Fundacion Jiminez Diaz, Madrid: V. Carreño, J. A. Quiroga, M. Pardo (12). Italy: Ospedale V Cervello, Palermo: A. Craxi, O. Lo Iacono, P. Almasio (10); Clinica Medica II Universita, Padova: G. Giustina, G. Fattovich (3). Greece: General Regional Hospital, Thessaloniki: G. E. Kitis (8).

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