

Original article

## Efficacy of different doses of 12-month Interferon alfa therapy in patients with HBeAg negative chronic hepatitis B: A randomized trial

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### SUMMARY

The best interferon-alfa (IFN $\alpha$ ) regimen for HBeAg negative chronic hepatitis B has not been established yet. We evaluated the efficacy of three regimens of IFN $\alpha$  in 75 patients with histologically documented HBeAg negative chronic hepatitis B, who were randomly allocated to receive IFN $\alpha$ -2b thrice weekly in a dose of: 3MU for 12 months (Group A, n=25); 5 MU for 6 months and 3 MU for another 6 months (Group B, n=25); and 3 MU for 6 months and 1 MU for another 6 months (Group C, n=25). Initial biochemical response was observed in 71% of the 75 patients and initial virological response (undetectable serum HBV DNA by bDNA assay) in 38% of the patients with pre-treatment detectable serum HBV DNA. The decline of HBV DNA levels at the end of therapy was significant in group A (P=0.02) or B (P=0.004), but not in group C. Sustained biochemical and virological response rates (at 6 months after the end of therapy) were 35% and 22% respectively, without any significant difference among the three groups. There was a significant improvement in the grading (P<0.001) and no significant change in the staging

of the post-treatment liver biopsies at 6 months after the end of therapy compared with the pre-treatment histological findings. In conclusion, a 12-month course of IFN $\alpha$  can induce sustained biochemical response in about one third and sustained virological response in approximately one fifth of patients with HBeAg negative chronic hepatitis B. A standard dose of 3 MU IFN $\alpha$  thrice weekly seems to be the most cost effective therapeutic regimen.

**Key words:** Interferon alfa, chronic hepatitis B, sustained response, HBV DNA

HBeAg negative chronic hepatitis B, which is associated with hepatitis B virus (HBV) strains with mutations in the precore or core promoter region,<sup>1</sup> represents a late phase in the course of chronic HBV infection and is associated with frequent progression to cirrhosis and development of hepatocellular carcinoma.<sup>2,3</sup> HBeAg negative chronic hepatitis B is the predominant type of chronic hepatitis B in the Mediterranean and many Far East countries and its frequency is rising worldwide.<sup>4</sup>

Interferon-alfa (IFN $\alpha$ ) has been the treatment of choice for patients with HBeAg positive or negative chronic hepatitis B during the early nineties<sup>5</sup> and remains a therapeutic option for such patients to date, despite the recent introduction of lamivudine, an oral and better tolerated antiviral drug.<sup>6</sup> In particular in HBeAg negative chronic hepatitis B, the long-term efficacy of IFN $\alpha$  therapy was initially not so encouraging. A 4-6 month course with 3-5 MU of IFN $\alpha$  thrice weekly has been found to induce biochemical remission in 50-70% of HBeAg negative chronic hepatitis B patients, but the majority of them relapse after cessation of treatment.<sup>7-9</sup> Thus, a short course of IFN $\alpha$  results in sustained biochemical remission in less than 15% of such patients.<sup>7-9</sup> In contrast, it was recently shown that

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a longer course of IFN $\alpha$  therapy can significantly increase the sustained response rate in this setting.<sup>10</sup> On the other hand, a 12-month course of lamivudine has been shown to be associated with high end of therapy response rates, but with relapses after stopping therapy in the vast majority of HBeAg negative chronic hepatitis B cases.<sup>11-13</sup> In addition, long-term lamivudine monotherapy has been associated with progressively increasing rates of viral resistance and breakthrough phenomena,<sup>14</sup> while there are no data for patients with HBeAg negative chronic hepatitis B discontinuing a long-term course of lamivudine.<sup>1</sup> Thus, IFN $\alpha$  still remains the only antiviral drug that has been associated with induction of sustained remission after stopping of therapy in a proportion of such patients. However, the best IFN $\alpha$  regimen for the treatment of HBeAg negative chronic hepatitis B has not been established yet.

In this randomized trial, we evaluated the efficacy of three different regimens of IFN $\alpha$  given for 12 months in patients with HBeAg negative chronic hepatitis B.

## PATIENTS AND METHODS

### *Patients*

All patients with HBeAg negative chronic hepatitis B who were evaluated at the Western Attica General Hospital and the First Department of Medicine of the Laikon General Hospital of Athens during a two-year period were invited to participate in the study. Inclusion criteria were: HBsAg positive and HBeAg negative for at least 6 months, serum alanine aminotransferase (ALT) higher than 1.5 times the upper limit of normal in at least 3 monthly determinations within the last 6 months, histologically proven chronic hepatitis within the last 6 months, compensated liver disease, age between 18 and 65 years, and patient's willingness to participate in the study. Patients were excluded if they had had treatment with steroids or IFN $\alpha$  or any other antiviral drug within the last 6 months, decompensated liver disease (Child class B or C), anemia (hematocrit < 30%), neutropenia (neutrophils <  $1.5 \times 10^9/L$ ), thrombocytopenia (platelets <  $100 \times 10^9/L$ ), abnormal renal function (creatinine > 1.5 mg/dL), a history of psychiatric disease or severe heart disease, a positive pregnancy test, or positive antibodies against human immunodeficiency virus (HIV) or hepatitis delta virus (HDV) or hepatitis C virus (HCV).

The study was approved by the local Ethics Committees at both hospitals and all patients gave written informed consent.

### *Randomization - Study design*

This was a prospective, open, randomized trial comparing three different regimens of recombinant IFN $\alpha$ -2b (Intron-A, Schering-Plough Co.) always given by thrice weekly subcutaneous injections. Patients were randomly allocated into the following treatment groups: Group A, 3 MU of IFN $\alpha$  for 12 months; Group B, 5 MU of IFN $\alpha$  for the first 6 months and then 3 MU of IFN $\alpha$  for the following 6 months; and Group C, 3 MU of IFN $\alpha$  for the first 6 months and then 1 MU of IFN $\alpha$  for the following 6 months. Randomization was done by a computer generated list, which was kept at the study coordinator center independent of the participating investigators.

All patients were followed by monthly examinations for the first 6 months and by bimonthly examinations for the following 6 months of IFN $\alpha$  treatment. All patients were also followed up by monthly examinations for 6 months after the end of IFN $\alpha$  therapy.

### *Serum assays*

Commercially available enzyme immunoassays were used for the detection of markers of HBV infection (HBsAg, anti-HBs, HBeAg, anti-HBe; Abbott Labs., North Chicago, IL), antibodies to HIV (Abbott Labs., North Chicago, IL), antibodies to HDV (Sorin Biomedica, Saluggia, Italy), and antibodies to HCV (Ortho Diagnostics, Raritan, NJ).

Serum HBV DNA was detected retrospectively in prospectively stored serum samples collected at the start, at the end, and at 6 months after the end of IFN $\alpha$  treatment from 42 patients (14 in each treatment group) of the one participating center (Western Attica General Hospital). A commercially available branched DNA assay was used (bDNA, Chiron) with a sensitivity of  $0.7 \times 10^6$  viral equivalents/mL or 2.5 pg/mL.

### *Liver histology*

All patients had a liver biopsy within the last 6 months before enrollment. At 6 months after the end of treatment, a follow-up liver biopsy was performed in 41 patients of one participating center (Western Attica General Hospital). The histological changes in the pre- and post-treatment liver biopsies were classified into histological grading and staging by a liver pathologist (J.K.D.) according to the classification of chronic hepatitis proposed by Ishak et al (15). The chronic hepatitis grading score (0-18), which represents the necroinflammatory activity, was the sum of piecemeal necrosis score (0-4), confluent necrosis score (0-6), focal lytic necrosis, apoptosis and focal inflammation score (0-6) and portal inflammation score

(0-4). Chronic hepatitis necroinflammatory activity was subdivided into the following categories according to the grading score: a) minimal, grading score=1-3; b) mild, grading score=4-8; c) moderate, grading score=9-12; d) severe, grading score=13-18. The chronic hepatitis staging score (0-6), which is referred to as the fibrosis score, was based on the degree and extent of fibrosis and development of cirrhosis.

### Definitions

An initial biochemical response was defined as normal ALT levels at least on the last two determinations before the end of IFN $\alpha$  therapy. A sustained biochemical response was defined as the maintenance of normal ALT levels throughout the follow-up period after the end of IFN $\alpha$  in patients with an initial response. The virological response was evaluated only in patients with detectable serum HBV DNA by the bDNA assay before IFN $\alpha$ . An initial virological response was defined as undetectable serum HBV DNA by bDNA at the end of IFN $\alpha$  treatment. A sustained virological response was defined as still undetectable serum HBV DNA by bDNA at 6 months after the end of IFN $\alpha$  in patients with initial virological response.

### Statistical analysis

Data were analyzed on an intention to treat basis. Corrected  $\chi^2$  or two-tailed Fisher's exact test and t-test or Mann-Whitney test were used for qualitative or quantitative data, when appropriate. Wilcoxon matched-pairs signed-ranks test was used for evaluation of changes of quantitative variables within the same group and Spear-

man correlation for evaluation of relations between two quantitative variables. A P value of less than 0.05 was considered to be statistically significant.

## RESULTS

Seventy-five patients were randomly allocated into the three treatment groups, with 25 patients in each group. The clinical, virological, and histological baseline characteristics were similar among the patients in the three groups (Table 1). In particular, serum HBV DNA was detectable by bDNA in 37 (88%) of the 42 patients tested.

Side effects, mostly flue-like symptoms were observed in 40%, 48% and 40% of patients in Group A, B and C, respectively, but they were usually mild without requiring dosage modifications. The dose of IFN $\alpha$  had to be decreased in only 2 (2.7%) patients (one in group A and one in group B) and IFN $\alpha$  had to be discontinued in 5 (6.7%) additional patients (one in group A, one in group B and 3 in group C) within the first 6 months of treatment because of side effects. Another patient in group C discontinued IFN $\alpha$  for personal reasons.

### Initial response

Initial biochemical response was observed in 53 (71%) of the 75 patients without significant differences among the three treatment groups (Table 2). Initial virological response was observed in 14 (38%) of the 37 evaluated patients with pre-treatment detectable HBV DNA; it was observed in 50% (6/12) of group A, 55% (6/11) of group B and 36% (5/14) of group C patients (P=0.61) (Table 2). All patients with undetectable HBV DNA at the end of therapy

**Table 1.** Pre-treatment characteristics of 75 patients with HBcAg negative chronic hepatitis B treated with interferon-alfa (IFN $\alpha$ ) given thrice weekly in a dose of 3 MU for 12 months (group A), 5 MU for 6 months and 3 MU for another 6 months (group B), or 3 MU for 6 months and 1 MU for another 6 months (group C)

Pre-treatment patient characteristic	Group A (n=25)	Group B (n=25)	Group C (n=25)	Total (N=75)
Sex, males/females	20/5	23/2	17/8	60/15
Age (years), mean $\pm$ SD	43 $\pm$ 13	45 $\pm$ 10	47 $\pm$ 11	45 $\pm$ 11
ALT (IU/L), median (range)	118 (59-697)	101 (43-800)	115 (42-344)	115 (42-800)
Serum HBV DNA* (x10 <sup>3</sup> pg/mL), median (range)	29.5 (neg.-13000)	440 (neg.-6600)	58 (2.2-15000)	58 (neg.-15000)
Serum HBV DNA* by bDNA, positive/negative	12/2	11/3	14/0	37/5
Histological grading, n (%)				
Mild chronic hepatitis	8 (32)	9 (36)	7 (28)	24 (32)
Moderate/Severe chronic hepatitis	17 (68)	16 (64)	18 (72)	51 (68)
Histological staging, n (%)				
No cirrhosis (fibrosis score =0-4)	20 (80)	19 (76)	17 (68)	56 (75)
Cirrhosis (fibrosis score =5-6)	5 (20)	6 (24)	8 (32)	19 (25)

\*Only in 42 patients, neg. denotes negative by bDNA.

had initial biochemical response. Median serum HBV DNA levels at the end of treatment were significantly lower than the pre-treatment levels in these 37 patients [4.3 (<0.0025-910) vs 150 (2.2-15000)  $\times 10^3$  pg/mL respectively,  $P < 0.001$ ]; the decline of HBV DNA levels was significant in group A [1.3 (<0.0025-93.0) vs 92.5 (4.1-13000)  $\times 10^3$  pg/mL,  $P = 0.02$ ] and in group B [ $< 0.0025$  (<0.0025-440) vs 900 (3.8-6600)  $\times 10^3$  pg/mL,  $P = 0.004$ ], but not in group C [9.3 (<0.0025-910) vs 58.0 (2.2-15000)  $\times 10^3$  pg/mL,  $P = 0.10$ ].

### Sustained response

Sustained biochemical response was observed in 26 (35%) of the 75 patients, without significant differences among the three treatment groups (Table 2). Sustained virological response was observed in 8 (22%) of the 37 evaluated patients; it was observed in 25% (3/12) of group A, 18% (2/11) of group B and 21% (3/14) of group C patients ( $P = 0.92$ ) (Table 2). Seven of the 8 patients with sustained virological response had sustained biochemical response as well. Serum HBV DNA levels increased from the end of treatment to 6 months later; the levels at 6 months after the end of treatment were not significantly different from the pre-treatment values [20.1 (<0.0025-4000) vs 150 (2.2-15000)  $\times 10^3$  pg/mL respectively,  $P = 0.16$ ].

None of the baseline characteristics was associated with sustained biochemical response. Patients with, compared to those without, sustained biochemical response had significantly lower HBV DNA levels at the end of treatment [median (range): <0.0025 (<0.0025-

210) vs 6500 (<0.0025-910)  $\times 10^3$  pg/mL,  $P = 0.04$ ] and at 6 months after the end of treatment [median (range): 1.55 (<0.0025-770) vs 190 (<0.0025-5400)  $\times 10^3$  pg/mL,  $P < 0.001$ ]. Sustained biochemical response was observed in 20% (4/20) of the patients with detectable and in 59% (10/17) of those with undetectable HBV DNA at the end of therapy ( $P = 0.02$ ) as well as in 24% (7/29) of the patients with detectable and in 88% (7/8) of those with undetectable HBV DNA at 6 months after the end of therapy ( $P = 0.002$ ).

### Histological response

A follow-up liver biopsy at 6 months after the end of treatment was available in 41 patients. There was a significant improvement in the total histological grading score as well as in all of its components in the post-treatment liver biopsies compared with the baseline histological findings (Table 3). In contrast, there was no significant change in the histological staging (extent of fibrosis). There was no significant difference in the histological changes after IFN $\alpha$  therapy among the three different treatment groups (data not shown).

Histological grading significantly improved in the post-treatment compared with the pre-treatment biopsies in the 18 patients with sustained biochemical response [from a median of 7 (4-16) to 4.5 (2-7);  $P = 0.003$ ] and in the 15 with initial biochemical response and relapse after the end of therapy [from a median of 8 (4-12) to 5 (2-9);  $P = 0.005$ ], but not in the 8 patients with no response to IFN $\alpha$ . Fibro-

**Table 2.** Initial and sustained biochemical and virological response rates in 75 patients with HBeAg negative chronic hepatitis B treated with interferon-alfa (IFN $\alpha$ ) given thrice weekly in a dose of 3 MU for 12 months (group A), 5 MU for 6 months and 3 MU for another 6 months (group B), or 3 MU for 6 months and 1 MU for another 6 months (group C).

	Group A	Group B	Group C	Total
Initial biochemical response	18/25 (72%)	16/25 (63%)	19/25 (76%)	53/75 (71%)
Initial virological response*	6/12 (50%)	6/11 (55%)	5/14 (36%)	17/37 (46%)
Sustained biochemical response	10/25 (40%)	7/25 (28%)	9/25 (36%)	26/75 (35%)
Sustained virological response*	3/12 (25%)	2/11 (18%)	3/14 (21%)	8/37 (22%)

\*Virological responses were estimated only in the 37 patients with pre-treatment detectable serum HBV DNA (only 42 of the 75 patients were tested for serum HBV DNA).

**Table 3.** Histological changes 6 months after the end of interferon-alfa (IFN $\alpha$ ) therapy in 41 patients with HBeAg negative chronic hepatitis B treated with IFN $\alpha$  for 12 months.

Histological characteristic*	Pre-IFN $\alpha$	Post-IFN $\alpha$	P
Piecemeal necrosis	1 (0-4)	0 (0-3)	0.001
Confluent necrosis	0 (0-5)	0 (0-0)	0.048
Focal lytic necrosis, apoptosis and focal inflammation	2 (0-4)	1 (0-4)	<0.001
Portal inflammation	3 (1-4)	3 (2-4)	0.025
Histological grading	7 (4-16)	5 (2-9)	<0.001
Histological staging (fibrosis)	2 (0-6)	1 (0-6)	0.29

\*All histological characteristics are expressed by medians (ranges).

sis showed a trend for improvement in the patients with sustained biochemical response [from a median of 2 (0-6) to 1 (0-6);  $P=0.13$ ], but not in the patients who relapsed ( $P=0.74$ ) or did not respond at all ( $P=0.56$ ).

## DISCUSSION

Despite previous reports for poor efficacy of IFN $\alpha$  in HBeAg negative chronic hepatitis B,<sup>7-9</sup> our data indicate that a 12-month course of IFN $\alpha$  therapy can induce sustained biochemical remission in about one third of such patients. The better response rate observed in our study may be associated with the duration of IFN $\alpha$  therapy, since IFN $\alpha$  was given for 12 months in ours and for 4-6 months in the previous studies.<sup>7-9</sup> It was recently reported that even high doses of IFN $\alpha$  given for short periods do not seem to achieve satisfactory sustained response rates.<sup>16</sup> A high biochemical sustained response rate similar to ours was observed in an Italian study, in which a long course of IFN $\alpha$  was also used.<sup>17</sup> In the latter study, a 24 month course of 6 MU IFN $\alpha$  resulted in sustained biochemical response in at least 28% of patients with HBeAg negative chronic hepatitis B.<sup>17</sup> Moreover, it was very recently shown that longer compared to shorter courses of IFN $\alpha$  therapy induce significantly more frequently sustained response achieving biochemical remission for a median of 7 years in approximately 20% of such patients.<sup>10</sup> It should be noted that our sustained response rate was evaluated at 6 months after the end of IFN $\alpha$  therapy, which is the post-treatment follow-up period used in most therapeutic trials in chronic viral hepatitis. However, it was recently suggested that, in HBeAg negative chronic hepatitis B, relapses may often occur in up to two years after the end of IFN $\alpha$  therapy.<sup>18</sup> Thus, the sustained response rate observed in our study may decrease with longer post-treatment follow-up.

Serum HBV DNA levels significantly declined during IFN $\alpha$  therapy, but subsequently increased reaching the pre-treatment levels at 6 months after the end of therapy. The decline of viremia levels during therapy was significant in the patients treated with 3 MU IFN $\alpha$  for 12 months or 5 MU for 6 months and 3 MU for another 6 months, but not in the patients who were treated with 3 MU for the first 6 months and then with the low dose of 1 MU for another 6 months. This finding could indicate that the regimen with the low IFN $\alpha$  dose is less efficacious in the treatment of HBeAg negative chronic hepatitis B. Doses of IFN $\alpha$  less than 3 MU thrice weekly have been found ineffective and are not recommended in HBeAg positive chronic hepatitis B or in chronic hepatitis C.<sup>5</sup>

Virological response as defined by undetectable HBV DNA by bDNA was observed in about 50% of patients at the end of IFN $\alpha$  therapy and maintained in 21% of them at 6 months after the end of therapy. In the study by Lampertico et al (17), sustained virological response was observed in 33% of patients, but a smaller number of patients was studied and a less sensitive hybridization assay was used for serum HBV DNA detection.<sup>19</sup> Virological response estimated also by the bDNA assay has been observed in 63% of patients with HBeAg negative chronic hepatitis B at the end of 12 months treatment with lamivudine (11), but it was maintained in only 10% of patients at 6 months after discontinuation of lamivudine.<sup>12</sup>

The clinical significance of virological response, however, and in particular of HBV viremia in the absence of biochemical evidence of necroinflammatory liver activity (elevated ALT), is unclear in the HBeAg negative chronic hepatitis B. The detection of HBV viremia depends on the sensitivity of the method used.<sup>20</sup> Clearance of HBV viremia is almost impossible in patients with HBeAg negative chronic hepatitis B, if very sensitive polymerase chain reaction assays are used.<sup>21,22</sup> On the other hand, a favorable outcome has been observed in patients with chronic hepatitis B who enter and remain in biochemical remission.<sup>23,24</sup> HBsAg carriers with persistently normal liver function tests have an excellent long-term prognosis,<sup>25</sup> although HBV viremia can be detected by sensitive assays.<sup>26</sup> Moreover, it was recently shown that sustained biochemical remission after IFN $\alpha$  therapy can prevent from development of liver decompensation and/or hepatocellular carcinoma and improve survival in such patients.<sup>27</sup> Therefore, a main therapeutic target in patients with HBeAg negative chronic hepatitis B appears to be the sustained normalization of liver enzymes irrespective of viremia.

A promising finding in our study was the significant improvement of the necroinflammatory histological activity in the post-treatment liver biopsies. It should be noted that the follow-up biopsies were performed at 6 months after the end of IFN $\alpha$  and not at the end of therapy, when the histological improvement may have been more marked. Improvement in liver histology was observed only in the patients with sustained biochemical response and in those with initial response and relapse after the end of IFN $\alpha$ , but not in patients without any response to IFN $\alpha$ . Moreover, there was a trend for reduction in the extent of fibrosis in the sustained responders. Since changes in fibrosis may lag behind the changes in necroinflammatory activity, a further improvement in fibrosis later on cannot be excluded, particularly in patients who remain in biochemical and histological remission.

In conclusion, a 12-month course of IFN $\alpha$  can induce sustained biochemical response at 6 months after the end of therapy in about one third and sustained virological response in about one fifth of patients with HBeAg negative chronic hepatitis B. A standard dose of 3 MU IFN $\alpha$  thrice weekly seems to be the best therapeutic regimen, since the regimen with 1 MU IFN $\alpha$  during the second half of treatment may be less efficacious and the regimen with 5 MU IFN $\alpha$  during the first half of treatment is associated with higher cost and potentially more side effects without any evidence for better efficacy. However, the majority of patients with HBeAg negative chronic hepatitis B still does not respond to IFN $\alpha$  monotherapy. Whether other IFN $\alpha$  regimens or nucleoside analogues, alone or in combination with IFN $\alpha$ , can improve the sustained response rate in this atypical form of chronic hepatitis B remains to be evaluated in future studies.

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