EFFICACY OF ALPHA-INTERFERON THERAPY OF CHRONIC HEPATITIS PATIENTS INFECTED WITH WILD TYPE HEPATITIS B VIRUS AND HBEAG-MINUS MUTANT

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Summary. – The aim of this study was to evaluate the efficacy of alpha-interferon (alpha-IFN) treatment of 56 chronic hepatitis B (HB) patients positive for HB e antigen (HBeAg), which were previously not treated with alpha-IFN (group A). Seven of them, which did not respond to initial alpha-IFN treatment, were subjected to additional treatment with alpha-IFN (group B). Another 7 patients with chronic HB caused apparently by an HBeAg-minus HB virus (HBV) mutant represented group C. In the alpha-IFN treatment, 5 megaunits (MU) of alpha-IFN were administered subcutaneously three times a week for six months. A trend of improvement of important markers of the disease in the treated patients could be seen with increasing time after completion of the treatment even though it was not statistically significant. In group A, the absence of serum HBV DNA was found in 43% of the patients at the end of the treatment, in 41% 6 months later, and in 46% 12 months later. At the same time intervals group A showed negative HBeAg in 36%, 39% and 46%, positive anti-HBeAg in 36%, 38%, and 46%, negative HBsAg in 9%, 11%, and 14%, and normal level of alanine transaminase (ALT) in 23%, 39%, and 44%, respectively. A trend toward better results of alpha-interferon therapy for the group A patients displaying lower baseline viremia and higher ALT activity could be seen; however, this relationship was not statistically significant. Groups B and C were too small for statistical analysis. Nevertheless, 4 of 7 patients of group B were negative for HBV DNA 12 months after the treatment and HBV DNA was eliminated during the treatment in all patients of group C; however, 3 patients relapsed after the treatment.

Key words: chronic hepatitis B; hepatitis B virus; wild type virus; HBeAg-minus mutant virus; alpha-interferon

Introduction

Viral HB is one of the most serious health problems of recent times, above all in developing countries. It is estimated that two billion people are infected with hepatitis B virus (HBV) in the course of their lives and that 350–400 million people are at present chronically infected. One to two million people die annually as a direct result of HBV infection, suffering from liver cirrhosis, hepatocellular carcinoma or fulminate hepatitis. For these reasons, HB is the ninth leading cause of death on a world scale (Hoofnagle and Di Bisceglie, 1997; Lee, 1997; Rogers *et al.*, 1997; Sherlock and Dooley, 1997; Dove *et al.*, 1998).

Efficient treatment of chronic HB is currently performed with alpha-IFN. It is usually given in doses of 5–10 MU either daily for 4 months or 3 times a week for 6 months. In clinical studies, as primary criteria for successful treatment are generally regarded the elimination of HBV DNA from

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Abbreviations: alpha-IFN = alpha-interferon; ALT = alanine transaminase; anti-HBeAg = antibodies to HBeAg; CAH = chronic active hepatitis; CAH/LC = advanced fibrotic chronic active hepatitis; CPH = chronic persistent hepatitis; ELISA = enzyme-linked immunosorbent assay; HB = hepatitis B; HBeAg = hepatitis B e antigen; HBsAg = hepatitis B s (surface) antigen; HBV = hepatitis B virus; LC = liver cirrhosis; MU = megaunits; ULN = upper limit of normal

serum and the seroconversion of HBeAg to antibodies to HBeAg (anti-HBeAg) (virological remission). This occurs in some patients only in the course of the treatment and may even occur many months later. It is therefore necessary to monitor these parameters at least for 1 year after the treatment (Hoofnagle and Di Bisceglie, 1997; Sherlock and Dooley, 1997; Dove *et al.*, 1998).

Retrospective multivariate analyses have shown that the likelihood of a response to therapy with alpha-IFN correlates with several clinical, biochemical, and serological features that are apparent before the therapy had begun. The features that predict best a beneficial response are high serum transaminase activity, low serum HBV DNA level, high histological activity of liver biopsy, short duration of the disease, and absence of immunosuppression (Hoofnagle and Di Bisceglie, 1997). The literature quotes a reduced effect of the alpha-IFN treatment of patients infected with an HBeAg-minus mutant, necessitating higher dosage or prolongation of the treatment period (Brunetto *et al.*, 1993, 1995; Lai *et al.*, 1994).

Materials and Methods

Patients and alpha-IFN treatment. The effect of antiviral therapy was assessed on 63 patients with chronic HB. Fifty-six of them were infected with HBV and produced HBeAg. All the patients under study underwent an initial treatment with 5 MU of alpha-IFN (a recombinant alpha 2b-IFN, Intron-A™, Schering-Plough Co., Ireland) 3 times a week for 6 months (group A). Seven of them showing persistence of HBV DNA and a seroconversion from HBeAg to anti-HBeAg one year after completion of the treatment were given an additional treatment with the same alpha-IFN for 6 months in the same regimen (group B). Another 7 patients considered infected with an HBeAg-minus HBV mutant (positive for HBsAg, HBV DNA and anti-HBeAg but negative for HBeAg) were also subjected to the treatment (group C).

HBsAg, HBeAg, anti-HBeAg and HBV DNA. Chronic HB infection was identified by enzyme-linked immunosorbent assay (ELISA) of HBsAg (Murex HBsAg Version 3, Murex Biotech Ltd., USA), HBeAg and anti-HBeAg (Monolisa™ HBe, Bio-Rad, France) in serum. Serum HBV DNA, a marker of viremia, was determined by a Digene Hybrid Capture™ System HBV DNA assay (Digene Co., USA). All these markers were determined before and after the treatment and 6 and 12 months later.

ALT in serum was assayed by a standard method (GPT/ALT ISCC System 917, Roche Diagnostic, Germany) before and after the treatment and 6 and 12 months later.

Histological examination of liver biopsy specimens was conducted in standard manner on all patients before the treatment. For the sake of simplicity the histological findings were grouped into four categories. These categories characterized roughly the grade and stage of the liver inflammatory process: chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), advanced fibrotic chronic active hepatitis (CAH/LC) and liver cirrhosis (LC).

Table 1. Histological findings before the initial alpha-IFN treatment

	Group A		Group B		Group C	
	No.	%	No.	%	No.	%
CPH	15	26.8	1	14.3	4	57.1
CAH	30	53.6	5	71 4	3	42.9
CAH/LC	8	14 3	1	14.3	0	0.0
LC	3	5.3	0	0.0	0	0.0
Total	56	100	7	100	7	100

For the abbreviations see their list on the front page.

Statistical analysis. The relative frequencies that occurred in disparate variants nearly covered the whole spectrum of possible values (0–100). The binomial estimates (p) in such extreme variants were then subjected to arcsine and square root transformation prior to any statistical testing (p_{tr} = arcsine $\sqrt{p}$) that brought the underlying distribution to near normal levels. After statistical processing, all the binomial data were transformed back by sin function and expressed in original values (%) with correction for possible bias. Two sample estimates of age were based on independent t-test. Differences with p values under 0.05 were considered significant (Zar, 1984).

Results and Discussion

Baseline characteristics

In terms of age, the groups were comparable (mean age was 44, 42, and 50 years in groups A, B, and C, respectively). Likewise, the gender representation did not differ in a meaningful way in groups A (73% of men) and B (75% of men). The preponderance of men in these groups was, however, statistically significant in comparison to group C (57%). As regards initial histological findings, the active variant of HB (CAH) heavily predominated in groups A and B, while in group C variants CPH and CAH occurred in comparable proportions (Table 1).

Table 2. Baseline viremia and ALT activity

	Group A		Group B		Group C	
	No.	%	No.	%	No.	%
Baseline viremia						
Low (≤200 pg/ml)	9	18.75	0	0.0	7	100
High (>200 pg/ml)	39	81.25	7	100	0	0.0
Total	48	100	7	100	7	100
Baseline ALT						
Low (≤3 × ULN)	33	58.9	1	14.3	1	14.3
High (>3 × ULN)	23	41.1	6	85.7	6	85.7
Total	56	100	7	100	7	100

For the abbreviations see their list on the front page.

Given the expense of this method, viremia could only be established in 48 patients of group A and in all patients of groups B and C. There were 39 patients with serum HBV DNA concentrations >200 pg/ml and only 9 patients with serum HBV DNA levels ≤200 pg/ml in group A. All 7 patients in group B had serum HBV DNA levels above 200 pg/ml. On the contrary all 7 patient in group C had serum HBV DNA concentrations ≤200 pg/ml.

As to the occurrence of high initial ALT values, there were very substantial variances between groups B and C on one hand and group A on the other; between groups B and C there were no differences at all (Table 2).

HBeAg-positive patients – initial alpha-IFN treatment

A summary of results of initial alpha-IFN treatment of previously untreated HBeAg-positive patients infected with HBV (group A) is given in Table 3. A trend of improvement of ALT in the treated patients could be seen with increasing time after completion of the treatment even though it was not statistically significant. It was possible to observe an analogous trend also among other markers, though the differences were not statistically significant as well. Thus the most marked results can be obtained 12 months after completion of the treatment. At that time more than 46% of the patients were free of HBV DNA and HBeAg but became anti-HBeAg-positive. With the exception of one patient, the virological response in all patients was in conjunction with normalization of ALT (25/26, 96%). In addition, HBsAg was eliminated in more than 14% of patients.

According into the literature data alpha-IFN is useful means of treating HBeAg-positive patients with chronic HB. This treatment leads to clinical and virological remission of the illness in 30–40% of patients, while spontaneous remission takes place in 5–15% of cases. Virological remission correlates very well with improvement of biochemical, histological and clinical markers of the patient. About 90% of the patients who become free of serum HBV DNA achieve also normal ALT values. About 10% of patients experience elimination of HBsAg and appearance of anti-HBsAg as a result of the treatment. This situation appears usually several weeks or months after the seroconversion of HBeAg to anti-HBeAg and the elimination of HBV DNA from serum (Hoofnagle and Di Bisceglie, 1997; Lee, 1997; Rogers *et al.*, 1997; Dove *et al.*, 1998).

If we considered our patients in the context of the presence of favorable and unfavorable conditions for successful alpha-IFN treatment, we found that the patients with high baseline viremia, low baseline ALT and high initial inflammatory activity predominated in group A. Baseline viremia influences the probability of successful alpha-IFN treatment; namely, the patients with initial concentration of serum HBV DNA ≤200 pg/ml have a better chance of successful

Table 3. Results of the alpha-IFN treatment of previously untreated HBeAg-positive patients (group A – 56 patients)

	End of treatment		After 6 months		After 12 months	
	No.	%	No.	%	No.	%
HBV DNA-negative	19	33.0	23	41.1	26	46.4
HBeAg-negative	20	35.7	22	39.3	26	46.4
Anti-HBeAg-positive	20	35.7	21	37.5	26	46.4
HBsAg-negative	5	8.9	6	10.7	8	14.3
Normal ALT	13	23.2	22	39.3	25	44.3

For the abbreviations see their list on the front page.

Table 4. Dependence of the response to the alpha-IFN treatment on baseline viremia in previously untreated HBeAg-positive patients (group A – 12 months after the treatment)

	Low viremia		High viremia	
	No.	%	No.	%
HBV DNA-negative	6/9	66.7	15/39	38.5
HBeAg-negative	6/9	66.7	15/39	38.5
Anti-HBeAg-positive	6/9	66.7	15/39	38.5
HBsAg-negative	0/9	0.0	5/39	12.8
Normal ALT	6/9	66.7	14.39	35.9

For the abbreviations see their list on the front page.

treatment than those with the viremia >200 pg/ml (Dove *et al.*, 1998). Table 4 shows the results for patients of group A in relation to high initial viremia. Considering all the markers tested there is an apparent trend toward their improvement with the increasing time after the completion of the treatment. The only statistically significant trend is ALT normalization in patients with low baseline viremia ($p < 0.01$). The statistical significance of the difference between the patients with low and high viremia within the confines of individual time periods was impossible to assess due to the limited number of samples. However, there was a clear overall trend toward better results among patients with low viremia.

Baseline level of serum ALT was determined in all the patients, because the results of clinical studies have shown that high ALT activity (over threefold of the upper limit of normal – in our case, over 108 IU/L for women and over 144 IU/L for men) is often a favorable predictive marker of successful treatment (Dove *et al.*, 1998). Group A consisted of 23 patients with high and 33 patients with low ALT activity. Table 5 shows the difference in the response to the treatment between patients with high and low initial ALT activity. The statistical significance of the difference between both groups within the confines of the individual time periods was impossible to assess due to the limited number of samples. However, there was a clear overall trend toward better results among patients with high ALT activity.

Table 5. Dependence of the response to the alpha-IFN treatment on baseline ALT activity in previously untreated HBeAg-positive patients (group A – 12 months after the treatment)

	Low ALT (≤3 × ULN)		High ALT (>3 × ULN)	
	No.	%	No.	%
HBV DNA-negative	12/33	36.4	14/23	60.9
HBeAg-negative	12/33	36.4	14/23	60.9
Anti-HBeAg-positive	11/33	33.3	15/23	65.2
HBsAg-negative	3/33	9.1	5/23	21.7
Normal ALT	13/33	39.4	12/23	52.2

For the abbreviations see their list on the front page.

Table 6. Dependence of the response to the alpha-IFN treatment on baseline histological activity in previously untreated HBeAg-positive patients (group A – 12 months after therapy)

	CPH		CAH	
	No.	%	No.	%
HBV DNA-negative	6/15	40.0	18/38	47.4
HBeAg-negative	6/15	40.0	18/38	47.4
Anti-HBeAg-positive	6/15	40.0	18/38	47.4
HBsAg-negative	1/15	6.7	7/38	18.4
Normal ALT	5/15	33.3	19/38	50.0

For the abbreviations see their list on the front page.

Table 7. Results of additional alpha-IFN treatment of HBeAg-positive patients (group B – 7 patients)

	End of treatment		After 6 months		After 12 months	
	No.	%	No.	%	No.	%
HBV DNA-negative	4	57.1	5	71.4	4	57.1
HBeAg-negative	2	28.6	4	57.1	3	42.9
Anti-HBeAg-positive	1	14.3	3	42.9	3	42.9
HBsAg-negative	0	0.0	0	0.0	0	0 0
Normal ALT	3	42.9	4	57.1	3	42.9

For the abbreviations see their list on the front page.

Absence of cirrhosis or advanced liver fibrosis is another favorable predictive marker for successful alpha-IFN treatment, as is the heightened activity of inflammatory process before the treatment. Patients with stronger inflammation respond better (CAH and CAH/LC are here considered together under the heading of CAH) than those with lower (CPH). The number of cirrhotic patients was very small. As a result, there was no possibility of comparing treatment results according to the advancement of the liver process. Table 6 shows a comparison of the responses to the treatment in two groups of patients with different baseline histological activity: CPH (15 patients) and CAH (inclusive of CAH/LC, 38 patients). There seemed to be no difference between these groups; also in this case the statistical analysis could not be performed due to an insufficient number of samples.

HBeAg-positive patients – additional alpha-IFN treatment

Table 7 shows the results of an additional treatment of patients who had not responded to the initial treatment (group B). Within twelve months of the treatment, clearance of serum HBV DNA was achieved in 4 patients and a seroconversion from HBeAg to anti-HBeAg and normalization of ALT in 3 of 7 patients treated.

The patients who after the standard IFN treatment do not show a seroconversion from HBeAg to anti-HBeAg and elimination of HBV DNA from serum, present a serious problem in contemporary hepatology. Until the advent of lamivudine, the only efficient drug against HB, an additional IFN treatment in the same or higher doses has been routinely performed (Hoofnagle and Di Bisceglie, 1997; Lee, 1997; Rogers *et al.*, 1997; Dove *et al.*, 1998; Jarvis and Faulds, 1999). We used the same dosage of alpha-IFN for our 7 patients showing high ALT activity and CAH (group B) as that employed in the initial treatment. A low number of patients in groups B and C did not allow statistical analysis; these groups, therefore, had merely an indicative character.

HBeAg-negative patients

Table 8 shows the results of the alpha-IFN treatment of patients infected with an HBeAg-minus mutant of HBV (group C). A definite proof of the presence of a mutant virus could be obtained only by sequencing HBV DNA; however, this was not done. Nevertheless, serological findings (positive for HBsAg, HBV DNA and anti-HBeAg but negative for HBeAg), the sustained elevation of ALT and histological findings make this assumption very probable. Obviously, other possible causes of chronic hepatitis (hepatitis C virus, autoimmune hepatitis, m. Wilson, alpha 1-antitrypsin deficiency) were excluded. Elimination of HBV DNA in all the patients was achieved during the treatment; however, three patients relapsed in the following year.

Table 8. Results of the alpha-IFN treatment of patients infected with an HBeAg-minus mutant HBV (group C – 7 patients)

	End of treatment		After 6 months		After 12 months	
	No.	%	No.	%	No.	%
HBV DNA-negative	7	100	5	71.4	4	57.1
HBsAg-negative	0	0.0	0	0.0	1	14.3
Normal ALT	4	57.1	5	71.4	5	71.4

For the abbreviations see their list on the front page.

Besides, one patient showing a more lasting response also displayed seroconversion from HBsAg to anti-HBsAg.

The patients infected with an HBeAg-minus mutant HBV present a great therapeutical problem. Maximum virological (clearance of serum HBV DNA) as well as biochemical effects (normalization of ALT) take place in the course of administration of IFN (in more than 90% of patients), while most patients (60–100%) relapse after completion of the treatment. The relapse may indeed take place very long after completion of the alpha-IFN treatment; therefore, we must be especially careful in evaluation of lasting effects of the treatment of the patients. Even in our group of patients we recorded reappearance of HBV DNA 18 months after completion of the treatment. Therefore, the real effect of the alpha-IFN therapy is perhaps much smaller than we present here as our data cover a period of one year only. It is possible to lower the probability of relapse by prolonging the administration of alpha-IFN. In the course of a two-year-treatment, a success rate of 28% has been achieved; while that in standard regimen (4–6 months) keeps a position under 10% (Brunetto *et al.*, 1993, 1995; Lai *et al.*, 1994). Therefore, lamivudine (nucleoside analog) becomes more and more the drug of the first choice for these patients (Hoofnagle and Di Bisceglie, 1997; Dienstag *et al.*, 1999; Jarvis and Faulds, 1999).

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