Antiviral effect of prolonged intermittent lymphoblastoid alpha interferon treatment in chronic hepatitis B

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Abstract

In a European multicentre study 40 patients
with HBeAg positive chronic hepatitis B virus
(HBV) infection were treated with 5 mega units
of lymphoblastoid α-interferon daily according
to the following regimen: a four week primer
course, four weeks of rest and a second course
lasting 16 to 30 weeks. After 52 weeks of follow
up, a response (HBeAg seroconversion and
HBV-DNA negativity) was observed in 22
patients (55%). HBsAg seroconversion occur-
red in five patients (12·5%). One patient
exhibited a relapse for serum HBeAg and
HBV-DNA after cessation of treatment.
According to a response prediction model, the
observed response rate was not related to the
selection of patients likely to respond. The
initial interferon course induced a reduction of
the serum HBV-DNA and HBeAg levels of
87% and 18%, respectively, leading to a
significantly lower level of viral replication
activity at the start of the second longterm
course compared with baseline. After 24 weeks
of follow up (week 16 of the second course),
19 (48%) patients exhibited a response, 13
(32%) a partial response (HBeAg <50% of
initial level or HBV-DNA negative) and 8
(20%) no response. For eight of the 13 partial
responders treatment was stopped at week 24
and viral replication rebounded to pretreat-
ment values. In the last five partial responders
prolongation of therapy up to week 38 led to a
definite response and HBsAg seroconversion
in three of the five patients. The results of this
study suggest that a short primer course and
prolongation of therapy may help to enhance
the response rate of α-interferon therapy for
chronic hepatitis type B.

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In patients with chronic hepatitis B virus (HBV)
infection active viral replication is usually associ-
ated with continuing inflammatory activity and
progression of liver disease. Transition to viral
latency, as indicated by hepatitis B e antigen
(HBeAg) seroconversion and clearance of serum
HBV-DNA, is accompanied by biochemical and
histological regression of liver disease activity.[1-3]
With the availability of α-interferon, termination
of the viral replicating phase can be induced in
about 30 to 40% of the patients.[4-6] Usually
interferon is given in a single continuous course.

From a pilot study comparing the antiviral
effect of interferon, acyclovir and a combination
of interferon and acyclovir, it was concluded that

combination therapy appears to be a highly
promising treatment for chronic hepatitis B.[7] As
more recent controlled studies have shown that
acyclovir does not enhance the therapeutic effect
of interferon alone,[8] the results of the pilot study
could also be interpreted as indicating that a
second course of interferon after short term
primer interferon therapy is more effective than
a single course. Therefore we investigated the
effect of intermittent interferon treatment on
termination of herpatitis B viral replication,
measured by HBeAg seroconversion and HBV-
DNA clearance from serum.

Methods

PATIENTS AND TREATMENT

Forty four patients from six European hospitals
were enrolled in the study after central evalua-
tion of their eligibility. At entry all patients had
been seropositive for hepatitis B surface antigen
(HBsAg) and HBeAg for at least six months.
Histological evidence of chronic hepatitis was
present in all patients who underwent a liver
biopsy (n=43). Patients were excluded on
grounds of the following criteria: age under 18 or
over 70 years; presence of hepatitis delta anti-
bodies in serum; antiviral or immune modula-
tory therapies in the preceding six months;
history or presence of decompensated liver
disease (ascites, encephalopathy, variceal
haemorrhage); pregnancy; impaired immunity,
including seropositivity for human immuno-
deficiency virus with T4 cells <400/mm³; inade-
quate levels of platelets (<70×10⁹/l) or white
blood cells (<3×10⁹/l); recent drug or alcohol
abuse; presence of significant other disease that
might interfere with the study. The study was
approved by the ethical committees of all partici-
pating centres and written informed consent was
obtained from each patient who entered the
study.

Lymphoblastoid α-IFN (Wellferon, Well-
come, Beckenham, UK) was given at a dose of
5 mega units daily according to the following
regimen: a four week primer course, a four week
rest period and a second interferon course lasting
16 weeks; for five patients from Rotterdam the
duration of the second course was prolonged
up to 30 weeks. Patients were taught to self-
administer interferon subcutaneously. During
the first four days of interferon therapy indo-
methacin or paracetamol was given to suppress
early side effects. Interferon treatment was dis-
continued in the event of absence of HBeAg on

two successive occasions or intolerable side effects. Follow up began at the start of therapy and was continued for 52 weeks.

CLINICAL AND LABORATORY EVALUATION

Patients were seen, and if indicated examined, at the outpatient clinic every two to four weeks during treatment and every four to eight weeks during the period thereafter. Laboratory assessment was performed every two weeks during the treatment period and every four to six weeks after discontinuation of therapy. On these occasions routine haematological studies were performed and serum markers of viral replication (HBeAg and HBV-DNA) and aspartate aminotransferase activity were measured. Every three months additional biochemical and virological measurements including the prothrombin time and the levels of alanine aminotransferase, albumin, bilirubin, HBsAg, and antibodies against HBsAg as well as HBeAg were performed. Liver biopsies were taken within six months of entry to the study for histological assessment.

All virological parameters were determined centrally at the Rotterdam hepatitis laboratory. HBsAg was assessed using a commercial radioimmunoassay kit (Abbott, Ill, USA). HBeAg was measured quantitatively using a radioimmunoassay (Abbott, Ill, USA). For quantification a P/N ratio (counts of patient sample/counts of negative control sample) was determined for each patient in a fixed serum dilution. HBeAg seroconversion was defined as a P/N ratio under 2·1 for undiluted serum from two consecutive blood samples. HBV-DNA was measured by a liquid hybridisation assay using an iodine 125 probe (Abbott, Ill, USA). Antibodies to hepatitis C virus were determined, retrospectively, by enzyme immunoassay (Abbott, Ill, USA); positive results were confirmed by a recombinant immunoblot assay

(Ortho Diagnostics Systems, USA). Routine serobiochemical tests were performed using automated techniques (Coulter, Technicon, NY, USA).

The criteria for response to treatment were: HBeAg seroconversion and serum HBV-DNA negativity. The criteria for partial response to treatment were: a decrease in serum HBeAg of 50% or more of the initial level or serum HBV-DNA negativity with sustained HBeAg positivity after 24 weeks of follow up.

To analyse whether the response rate was related to treatment modification or selection of patients, the actual response was compared with a predicted response that was obtained with a response model developed by Brook et al.[9] The prediction of response was based on pretreatment aspartate aminotransferase levels, the presence of a history of acute hepatitis and HIV-antibody status.

STATISTICAL ANALYSIS

Differences in dichotomous and other discrete variables were analysed by the Fisher exact and χ^2 tests, respectively. For continuous variables medians were used because the results lacked normal distribution. The two sample Wilcoxon's rank sum test was used to analyse unpaired observations and the Wilcoxon's signed rank test to analyse paired observations.

Results

Four of the 44 patients were withdrawn from the study. Two patients were found to be HBeAg negative at the start or within one week of treatment while pre-entry assessments were positive. Two patients were lost to follow up: one withdrew from treatment after two weeks for reasons unrelated to the study protocol and one patient failed to comply with the protocol after cessation of therapy.

Patient characteristics at entry are shown in the Table. Of the 40 patients who were analysed, one was serum HBV-DNA negative on entry to the study.

RESPONSE

A response to treatment (HBeAg seroconversion and serum HBV-DNA negativity) was observed in 22 of the 40 patients (55%; 95% confidence interval (CI) 40–70%). Five patients (12·5%; 95% CI 4–27%) became negative for HBsAg. Sustained aspartate aminotransferase normalisation occurred in 16 of 19 responders with raised aspartate aminotransferase levels on entry to the study. Figure 1 shows the timing of elimination of viral parameters and sustained aspartate aminotransferase normalisation in the response group. All but one patient exhibited the response while on interferon therapy. A characteristic sequence in clearance of HBV-DNA and HBeAg and then normalisation of aspartate aminotransferase levels was observed for the majority of the responders. One responder showed a relapse for serum HBeAg, HBV-DNA and aspartate aminotransferase 20 weeks after cessation of therapy. Of the 18 non-responders, five exhibited normal-

Patients characteristics at entry into the study

	Treatment group (n=40)	Response (n=22)	Non-response (n=18)
Age* (yr)	38·5 (18–67)	42 (27–67)	37 (18–56)
Male/female	32/8	17/5	15/3
Homo/heterosexual	9/31	3/19	6/12
HBsAg duration* (mo)	27 (6–132)	14 (6–132)	33 (12–96)
History acute hepatitis	2	1	1
Histology:			
CPH	8	2	6
CAH	20	12	8
Cirrhosis	11	8	3
Anti-HCV positive	1	1	0
HIV-status:			
Negative	38	21	17
Unknown	2	1	1
HBeAg* (P/N ratio)	12·4 (2·2–18·8)	13·4 (2·5–18·8)	11·9 (2·2–16·4)
HBV-DNA* (pg/ml)	102 (1–1001)	79 (1–730)	127·5 (3–1001)
HBV-DNA			
<50 pg/ml	11	8	3
51–100	9	6	3
101–300	13	5	8
>300	7	3	4
AST* (U/l)	57·5 (15–475)	66·5 (15–475)	51·5 (16–113)
AST			
<30 U/l	7	3	4
31–60	14	7	7
61–100	11	5	6
>100	8	7	1

*Median (range).
AST normal <30 U/l; HBV-DNA cut-off: 1·5 pg/ml; HBeAg cut-off: P/N ratio 2·1.
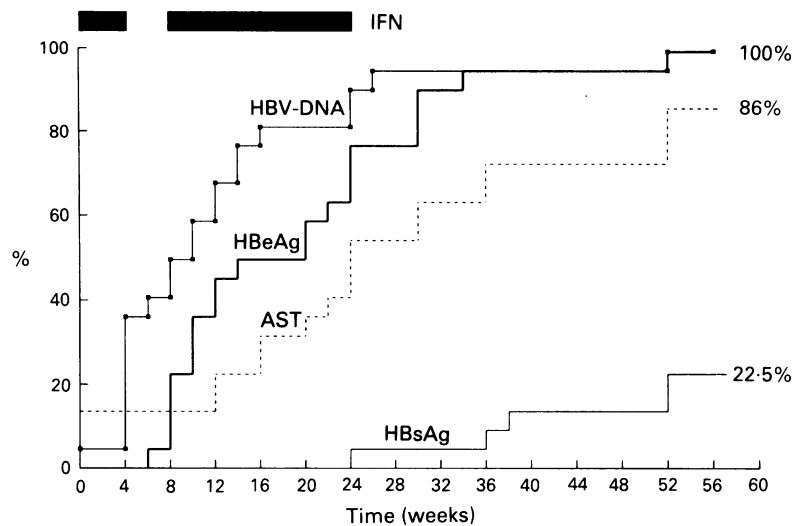AST=aspartate aminotransferase.

Figure 1: Cumulative percentage of clearance of serum HBV-DNA, HBeAg seroconversion, normalisation of serum aspartate aminotransferase and HBsAg seroconversion for patients who responded to therapy (n=22).

isation of aspartate aminotransferase levels and six became, briefly, serum HBV-DNA negative.

Differences in characteristics between responders and non-responders are shown in the Table. The pretreatment serum HBV-DNA level was lower and the aspartate aminotransferase level higher in the response group. Also the duration of HBsAg positivity was shorter and the presence of cirrhosis was more prominent in responders. No statistical difference between responders and non-responders, however, was found for any of these parameters.

According to the Brook model, a response was predicted for 14 of the 40 patients (35%; 95% CI 20–50%), with a positive predictive value of 79% and a negative predictive value of 58%. Compared with this prediction the actual response rate of 55% was significantly higher (p=0·03).

INTERMITTENT TREATMENT

Changes in serum aspartate aminotransferase, HBV-DNA and HBeAg values are shown in Figure 2. The primer interferon course reduced HBV-DNA and HBeAg levels by 87 and 18%, respectively, but the decrease did not continue
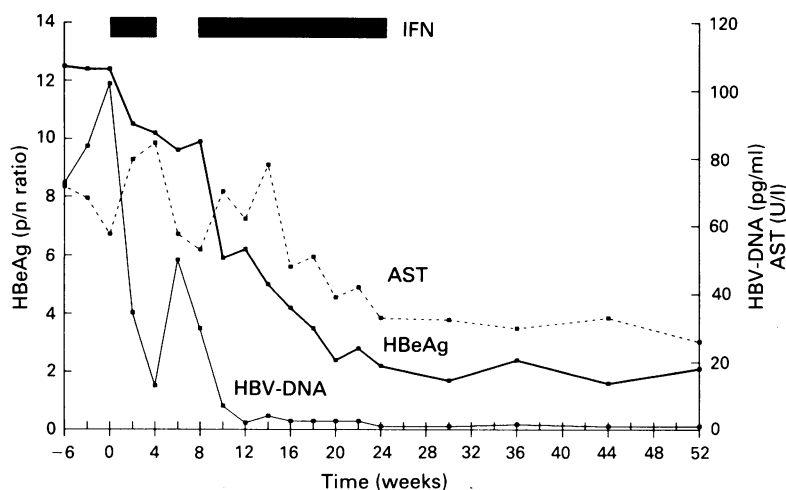

Figure 2: Median levels of HBeAg, HBV-DNA and aspartate aminotransferase in serum (n=40).

during the four weeks without therapy. Nevertheless, at the start of the second interferon course, the serum HBeAg and HBV-DNA levels were significantly lower compared with baseline values. No difference was found in the aspartate aminotransferase levels at the start of the two courses; however, both courses seemed to induce a peak of aspartate aminotransferase values that often preceeds a response to therapy.

PROLONGED TREATMENT

After 24 weeks of follow up (week 16 of the second course), 19 (48%; 95% CI 32–63%) patients exhibited a response, 13 (32%; 95% CI 18–47%) a partial response and eight (20%; 95% CI 8–32%) a definite non-response. For eight of the 13 partial responders, treatment was stopped at 24 weeks. After cessation of therapy the serum HBeAg and HBV-DNA levels in these patients rebounded to baseline levels (Fig 3). For the last five partial responders interferon therapy was prolonged up to week 38. Three of these five exhibited an additional response during prolonged therapy (Fig 4), and all three showed HBsAg seroconversion shortly after the response.

SIDE EFFECTS OF INTERFERON

During the first week of the initial interferon course, a transient flu like syndrome with fever, chills and myalgia was observed in nearly all of the patients. At the beginning of the second course, the majority again reported these symptoms but to a lesser intensity. The predominant adverse effects after the first days of therapy were fatigue (73%), myalgia (60%), anorexia (43%), irritability (30%), and hair loss (15%). Two patients developed an acute psychosis: the first, who had just finished therapy, required intensive psychiatric care for two weeks; the second, who had been treated with interferon for three weeks, fell and fractured the acetabulum. Another patient had a generalised seizure after four and 11 weeks; he was put on anticonvulsive medication and interferon was tapered to 1·25 mega units daily. Side effects led to dose reduction in 11 subjects (28%). The reasons for dose reduction were fatigue in six cases, thrombocytopenia in two, neurotoxicity in two, and leukopenia in one. Four (36%) of these 11 patients exhibited a response.

Discussion

In recent years several strategies have been tested to enhance the efficacy of interferon therapy for chronic hepatitis B. None of these strategies, including additional therapy with agents such as gamma interferon, prednisone, acyclovir or adeninide arabinoside[5][8][10–12]; has proved to be more beneficial than a standard interferon course of 12–16 weeks.

In this uncontrolled pilot study we investigated the effect of prolonged intermittent interferon therapy in 40 patients. Fifty five per cent of the patients responded with HBeAg seroconversion and loss of serum HBV-DNA. HBsAg seroconversion occurred in 12·5% of all treated
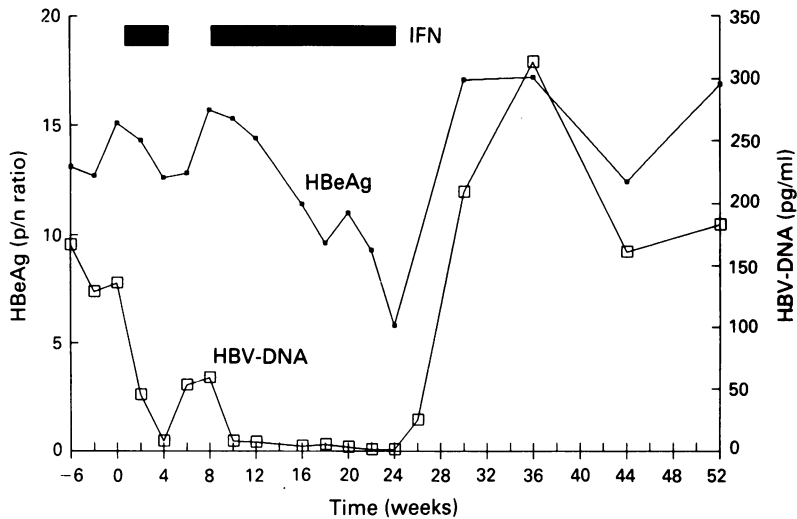
Figure 3: Serum HBeAg and HBV-DNA levels in a partial responder treated until 24 weeks (protocol therapy).

patients and in almost 25% of those who responded. A majority of the responders showed a characteristic sequence in loss of serum HBV-DNA and HBeAg followed by normalisation of the aspartate aminotransferase value. HBeAg seroconversion was often heralded by a rise in transaminase activity and loss of HBV-DNA. In six patients, however, HBV-DNA disappeared from the serum but quickly reappeared after discontinuation of therapy, indicating that persistent absence of HBeAg is the best indicator for termination of the viral replicating phase.

Although several differences in pretreatment characteristics of responders versus non-responders were observed, none of them was found to be significant. The predicted response rate, based on a combination of pretreatment factors,[9] was significantly lower (35%) than the actual response rate (55%). This result suggests that the high response rate was not caused by selection of patients likely to respond but by the treatment modification.

It is difficult to determine the contribution of intermittent therapy to the high response rate obtained in this uncontrolled study. The rationale for the initial four week course was to
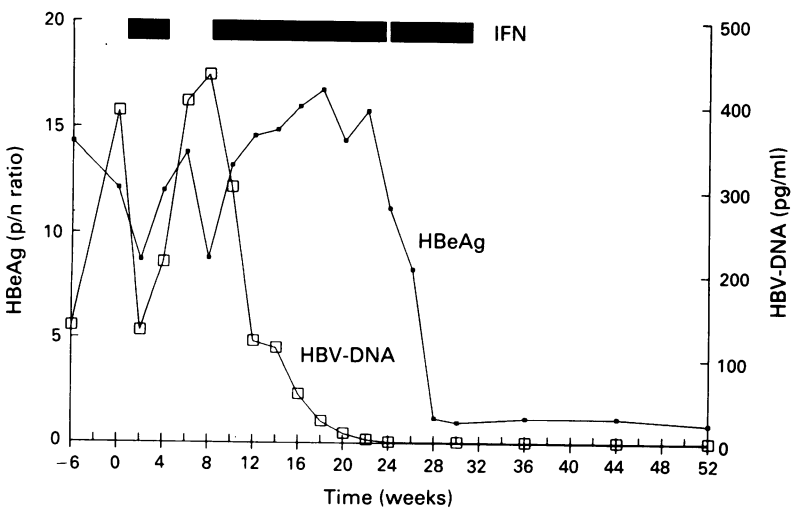

Figure 4: Serum HBeAg and HBV-DNA levels in a partial responder treated until 32 weeks (prolonged therapy) who exhibited an additional response.

decrease viral replication and increase inflammatory activity before the start of the longterm course, thereby enhancing the possibility of response to therapy. At the start of the second interferon course, viral replication, as monitored by serum HBV-DNA and HBeAg levels, was indeed significantly lower compared to baseline values. In addition, both interferon courses induced transient rises in aminotransferase levels. Therefore both courses could have induced a response in some patients and the double transaminase peak could thus be indicative of early and late responders. These interesting observations certainly justify further evaluation of intermittent treatment in a controlled setting.

Close on line monitoring of the effects of interferon on viral replication revealed that in several patients HBeAg and HBV-DNA serum values decreased continuously and were almost negative when treatment was stopped (week 24) at which point a relapse occurred. Therefore, we decided to offer prolonged treatment to the remaining patients who approximated a response at the end of the scheduled therapy (partial response). For three of the five partial responders this approach led to a definite response and subsequently to HBsAg seroconversion. Of the two patients who did not respond to prolonged treatment one had extremely high initial serum HBV-DNA levels (>1000 pg/ml), while both patients had near normal aspartate aminotransferase levels at entry. These features, low serum aminotransferase and high HBV-DNA levels, have been suggested to interfere with the response to interferon treatment.[4 5 9 13] Our findings indicate that prolonging interferon therapy may increase the HBeAg and HBsAg seroconversion rates in the subset of patients that partially respond to standard interferon treatment. To select patients eligible for treatment prolongation, close monitoring of quantified levels of HBeAg and HBV-DNA is essential.

From the literature it is not clear whether prolongation of therapy beyond four months yields a higher response rate. Prolonged therapy can further decrease HBV replication which may trigger the cullular immune response leading to the hepatocytotoxic reaction that eradicates viral replication.[14] In most parts of the world the standard duration of therapy is considered to be 12–16 weeks, usually leading to a response rate of 30–40%,[4–6 15]. Interferon has been given for 24 weeks or longer in a few, mainly Mediterranean, studies (response rates 26% to 70%).[16–18] A controlled trial comparing 12 to 24 weeks of treatment failed to show any beneficial effect of the prolonged therapy.[19] The outcome of that study, however, was markedly influenced by a lack of compliance (eight of the 20 patients withdrew from the longer course). In the present study none of the patients had to withdraw from therapy because of side effects, irrespective of the length of treatment. Both the intermittent treatment schedule and intensive patient monitoring with mental support may have contributed to the good compliance. Nevertheless, three of our patients had major neuropsychiatric side effects (psychosis, seizures). Future studies might elucidate whether these serious side

effects relate to dose and/or duration of the interferon treatment.

In summary, prolonged intermittent interferon therapy resulted in HBeAg seroconversion and serum HBV-DNA negativity in 55% and in HBsAg seroconversion in 12·5% of the patients. The high rate of induced viral latency was probably related first to the short initial interferon course that reduced viral replication significantly before the start of the second long-term course and second to prolongation of therapy in partial responders which induced additional HBeAg seroconversion.

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