High-dose interferon results in high HBsAg seroclearance in children with chronic hepatitis B infection

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Clinical trials for chronic hepatitis B (HBV) infection in children have shown usefulness of interferon alpha 2b (IFN-α) in eliminating HBV replication and in improving liver histology. Although it is not the ultimate goal of the interferon treatment for chronic HBV infection, it has been suggested in adults that HBsAg clearance decreases the likelihood of development of hepatocellular carcinoma, and prolongs the survival. HBV DNA clearance has been shown to be higher with higher doses of interferon in children, but it was rarely associated with HbsAg clearance.

Ten MU/m^2 was tried in 46 children who had biopsy-proven chronic HBV infection. They received IFN-α subcutaneously three times/week for six months. The treatment regimen was completed in 41 children and the second liver hiopsy was carried out one year after the end of the treatment in 30 of 41 patients. With this schedule, 15 (36.6%) children showed persistent loss of HBV DNA 12 months after the cessation of the treatment, 20 (48.7%) lost HBeAg, and eight (19.5%) developed anti-HBs antibody with loss of HBsAg. A significant improvement in liver histology was obtained in children with HBV DNA clearance. Serum ALT levels normalized in all HBeAg seroconverters.

These findings suggested that the 10 MU/m^2 IFN-α treatment was well tolerated and resulted in a high rate of HbsAg clearance in addition to HBV DNA clearance in a group of chidren with chronic HBV infection.

Key words: hepatitis B virus, high-dose interferon, children.

Chronic hepatitis B infection is one of the major causes of cirrhosis in children, and may lead to hepatocellular carcinoma later in life[1,2]. Infection with hepatitis B virus (HBV) more frequently results in chronicity in children than in adults[3]. The aim of treatment of chronic HBV infection is to suppress HBV replication before it leads to irreversible liver damage. Interferon alpha (IFN-α) has been approved for treatment of chronic HBV infection since the 1980s[4]. The effectiveness of IFN-α treatment in chronic HBV has been well established in adults[5-8]. Although HBV infection is less common in children than in adults, the possibility of chronic infection is inversely proportional to age at acquisition of infection[3,9-11]. The purpose of IFN treatment is to eradicate HBV and to prevent or reduce the risk of cirrhosis and hepatocelular carcinoma. Interferon seems to be an effective treatment for children with chronic hepatitis B[11-16]. The response to the interferon treatment was assessed by clearance of HBV DNA, hepatitis B e antigen (HBeAg), and improvement in ALT levels. Studies from European countries have demonstrated that loss of HBV DNA and HBeAg varied between 20 to 50% in treated patients, and between 8-17% among control subjects[11-16]. The percentage of hepatitis B surface antigen (HBsAg) loss has ranged from 0 to 10% with different IFN-α regimens in several studies[4,8,9]. As the dosages of IFN-α used in pediatric patients have been variable, there is not a well defined IFN-α dosage in treatment of chronic HBV infection in children[11-13,16]. In a rather recent consensus report, based on experiences

in European children, it has been stated that the standard schedule is 5 MU/m^2 IFN-α administered thrice weekly for 24 weeks[16]. But there is still controversy on the issue. It has been suggested that the dose of IFN-α should be adjusted according to level of HBV DNA at the third month of treatment. The studies done in Europe have clearly demonstrated that high-dose IFN-α trials have revealed higher percentages of HBV DNA and HBeAg clearance during follow-up when compared with low-dose IFN-α trials[12,14,17]. HBV DNA clearance is not always associated with HBsAg clearance. HBV clearance has been shown to be higher in children who were treated with higher doses of interferon, but HBsAg clearance was not more than 5% with the existing interferon regimens[12,18-20]. Although HBsAg clearance is not one of the major goals of the treatment, it has been clearly demonstrated in a large cohort that the probability of development of hepatocellular carcinoma was lower and survival was longer in adult patients who cleared HBsAg when compared with those in whom HBsAg persisted[21].

The purpose of this study was to evaluate the role of high-dose IFN-α (10 MU/m^2) treatment in HBsAg clearance in addition to HBeAg and HBV DNA clearance in a group of children with chronic HBV infection, and to determine the long-term outcome of children who had HBsAg clearance.

Material and Methods

Forty-six children who had chronic HBV infection, determined by a liver biopsy, were included in the study. All patients had active viral replication (HBV DNA and HBeAg+), and elevated serum ALT levels for at least six months. Children who had previous treatment with antiviral or immune suppressive drugs, decompensated liver disease, antibody to HIV, hepatitis C virus, hepatitis D virus, or other causes of liver disease were excluded from the study. Any with associated viral, toxic, metabolic or autoimmune causes of chronic HBV infection were also excluded. The disease had been discovered by routine screening in all patients.

Liver biopsies were performed percutaneously according to Menghini method using 1.4-1.6 French liver biopsy needle (Hepafix, Liver Biopsy Set according to Menghini, B. Braun Melsungen AG, Germany). The same pathologist who was blind to the chronological order of the biopsies reviewed the biopsy specimens. Histological activity was assessed in each biopsy specimen according to Knodell grading system[22]. HBV DNA was measured quantitatively by molecular hybridisation through hybrid capture (Digene Hybrid Capture System, Murex Diagnostics S.A. France) which detects viral genome levels at or above 1 pg/ml.

Patients received 10 MU IFN-α 2b/m^2 body surface (Intron A, Schering Plough, Kenilworth, NJ) three times a week for 24 weeks by subcutaneous injection. The dosage was maintained for 24 weeks unless side effects occurred. Safety was monitored using clinical and laboratory assessments, and adverse events were graded as mild, moderate, or severe[9]. The dose of IFN was adjusted or discontinued on the basis of the assessment of adverse events, using predetermined clinical and laboratory criteria for drug toxicity. Patients were seen three times during the first week of IFN-α treatment, weekly during the first month and monthly until the end of the treatment. During each visit, patients were asked about symptoms and possible side effects. Blood chemistry and differential cell counts were performed three times in the first week, once weekly for one month, twice in the second month, and once monthly thereafter. After the end of the treatment, biochemical analyses were repeated every three months up to one year. Serological markers were determined at the 3rd and 6th months of the treatment, and every three months thereafter. A control liver biopsy was carried out one year after the completion of IFN-α treatment.

Results

Forty-six children with chronic HBV infection who met the inclusion criteria were included in the study. A total of 30 boys (65.3%) and 16 girls (34.7%) were enrolled; the mean age of patients was 6.9±3.8 years (range 3-14 years). The source of infection was not apparent in 28 children, and the known sources of infection were vertical in 11, intra-familial in five, and after blood transfusion in two patients.

ALT levels were at least 1.6 times to 15.3 times the upper limit of normal. The mean ALT level

was 120.6±80.2 IU/ml (range: 48-363 IU/ml) before the treatment. Only two patients had HBV DNA level at 50 pg/ml; the rest of the patients had HBV DNA greater than 100 pg/ml. The mean HBV DNA level was 1575±1355 pg/ml (range: 50-3681 pg/ml) in the study group. Histopathological evaluation of liver biopsy specimens revealed chronic hepatitis in all patients. The mean hepatitis activity index (HAI) was 6.8±2.4 in the study group (Table I). All patients received IFN for 24 weeks. Treatment regimen and 5.3±1.8 year (2-8 years) follow-up were completed in 41 children, and the second liver biopsy was carried out in 30 of the 41 one year after the end of treatment. Nineteen of 41 IFN-α treated children (46.3%) showed persistent loss of HBV DNA at the end of the treatment. Twenty-one of 41 children (51.2%) lost HBeAg and developed anti-HBe antibodies. All 19 HBV DNA cleared patients were still HBV DNA and HBeAg negative one year after the end of IFN-α treatment. Persistent loss of HBsAg and development of anti-HBs antibodies occurred in eight children (19.5%). All children who developed HBsAg clearance were HBeAg positive at admission. Two patients showed HBsAg clearance six months after the end of treatment, four children cleared HBsAg one year after the end of IFN treatment, and the remaining two developed HBsAg clearance 18 and 24 months after the treatment, respectively. Only two patients in our study group had a low level of HBV DNA (lower than 100 pg/ml), and all of the patients who cleared HBsAg had higher loads of HBV DNA at admission. The basal liver function tests, viral load and histopathological activity of hepatitis were compared in patients with an without HBsAg clearance, and no difference was found between the two groups. Serum aminotransferase levels returned to normal in 68% of patients and in all responders. There was a statistically significant difference in pre-post treatment and pre-1st year ALT levels in responders (151.5±108.2 vs. 96.8±73.7 and 54.7±49.1, respectively) ($p<0.05$, $p<0.05$ ANOVA). Post-treatment liver biopsy was carried out in 30 children one year after the cessation of IFN treatment. When pre- and post-treatment liver biopsy specimens were compared. HAI improved in all children who had a virological and biochemical response, and did not change or became worse in non-responders. The mean HAI was 6.8±2.4 before the treatment and regressed to 3.5±2.2 after the treatment in responders ($p<0.05$), paired t test). It was also demonstrated that HAI regressed more in patients with HBsAg clearance when compared to patients with HBsAg persistence, but it did not reach a statistical significance (2.1±1.0 versus 3.5±2.2, respectively). Serological and virological response was associated with histopathological imp-rovement in portal inflammation and hepatic fibrosis ($p<0.03$, $p<0.01$ respectively, paired Wilcoxon). Piecemeal necrosis and intralobular degeneration were also decreased, but the difference between pre-and post-treatment HAI was not statistically significant. The bicochemical, serological and histopathological responses to the IFN treatment are summarized in Table II.

All of the HBeAg cleared children remained asymptomatic carriers of HBV infection during long-term follow-up. There was no reactivation of the liver disease in responders. Six patients cleared HBsAg and developed anti-HBs antibody one year after the IFN treatment, and two more responders cleared HBsAg within the second

Table I. Characteristics of the Study Group

Age (years)	6.9±3.8 (3-14 years)
Sex (F/M)	16/30
Transmission	
vertical	11 (23.9%)
intrafamilial	5 (10.9%)
transfusion	2 (4.3%)
unknown	28 (60.9%)
ALT (IU/L)	120.6±80.2 IU/L (48-363 IU/ml)
HAI (Knodell)	6.8±2.4
HBV DNA (pg/ml)	1575±1355 pg/ml (50-3681 pg/ml)

ALT: alanine aminotransferase.
HAI: hepatitis activity index.

Table II. Biochemical, Serological and Histological Responses of the Study Group to IFN Treatment

n=41	At the end of IFN treatment	One year after the treatment	Follow-up
ALT and AST normalization	21/41	28/41	28/41
HBeAg clearance	19/41	21/41	21/41
HBsAg clearance	0	6/41	8/41
Anti HBsAg development	0	6/41	8/41
HBV DNA			
Negative	12/41	19/41	19/41
>100 pg/ml	17/41	17/41	20/41
<100 pg/ml	5/41	5/41	2/41
HAI		3.5±2.2	
(6.8±2.4 before treatment)		3.5±2.2	

IFN: interferon alpha ab; HAI: hepatitis activity index; ALT: alanine aminotransferase; AST: aspartate

year of follow-up. No one in the study group developed hepatocellular carcinoma during the eight-year follow-up.

None of the children in the study group experienced a severe degree of adverse events resulting in termination of the treatment (Table III). All of the observed side effects were either mild or moderate. Fever was a common complaint after IFN injections, but it was well tolerated and easily controlled with ibuprofen in all but one patient who developed a moderate degree of side effect. He experienced febrile seizure. The cranial magnetic resonance imaging (MRI) and EEG of this patient were normal. He was given Phenobarbital perorally. IFN-α treatment was discontinued for one month and then restarted by decreasing the dose by 25%. He tolerated this dose of IFN-α very well and completed 24 weeks of IFN-α treatment. The other mild side effects were influenza-like symptoms (95%), musculoskeletal pain (56%), anorexia (38%), and hair loss (10%). White blood cell and platelet counts were decreased during treatment, but none of the patients developed severe leukopenia or thrombocytopenia. No child in the study group developed thyroid disease, autoimmune disease or serious cardiac, pulmonary or renal complications.

Table III. Side Effects Secondary to Interferon Treatment

Flu-like symptoms	44 (95%)
Fever	46 (100%)
Myalgia	26 (56%)
Anorexia	22 (48%)
Weight loss	16 (35%)
Neutropenia	2 (4.3%)
Febrile seizure	1 (2.1%)

Discussion

This prospective study demonstrated that a 24-week course of IFN-α at a dose of 10 MU/m^2 body surface area was effective as treatment in children with chronic HBV infection. The persistent loss of HBV DNA and HBeAg were 46.3% and 51.2%, respectively, and the long-term follow-up of these patients revealed 19.5% of HBsAg clearance, which was higher than the result reported in previous studies[9,12,20]. It has been found that all children who became HBeAg negative with treatment also showed a marked improvement in liver histology.

Interferon has been demonstrated to accelerate the rate of cessation of HBV replication, therefore decreasing the amount of HBV DNA integrated into the host genome, and reducing but not excluding the probability of development of hepatocellular carcinoma (HCC)[16,23]. Da Silva et al.[27] reported that HCC developed in 7.9% of patients in whom HBsAg persisted compared with no patients in whom HBsAg cleared, suggesting that clearance of HBsAg arrested the progression of liver disease. Although HBsAg clearance is not one of the major goals of interferon treatment, it has been stated that the probability of development of hepatocellular carcinoma was lower and survival was longer in adult patients who cleared HBsAg when compared with patients in whom HBsAg persisted[21,24,26]. There are several randomized clinical trials in children with chronic HBV infection, but there are limited data regarding survival, and development of cirrhosis and HCC in the long term[25,27].

Consequently, it can be suggested that HBsAg clearance is not the ultimate goal but may be an advantageous aim of interferon treatment in children. We observed a rather high percentage of HBsAg clearance in our study group, and the histopathological improvement was better in HBsAg cleared patients when compared to ones in whom HBsAg persisted.

Trials with IFN-α performed in children with chronic HBV infection have demonstrated a beneficial effect with loss of HBV DNA and HBeAg in 20-58% of treated patients[10,11,14,17]. IFN-α dosage used in these studies varied greatly, ranging from 3-10 MU/m^2 body surface area[14]. It is still not clear whether prolonged therapy or high doses of IFN-α are better than the standard dose schedules for sustained response. Ruiz-Moreno et al.[12] compared 5 MU/m^2 and 10 MU/m^2 doses of IFN-α in a group of children with chronic HBV hepatitis, and found a slightly higher response rate with the dose of 10 MU/m^2 but the difference was not statistically significant. There was no patient with HBsAg to antiHBs seroconversion in their group. In another study done by Sokal et al.[18] 9 MU/m^2 IFN-α was administered for 16 weeks thrice weekly, and they found persistent loss of HBV DNA in 48%, of HBeAg in 38%, and of HBsAg in 7% in the study group. In a meta-analysis published by Vajro et al.[20] treatment protocols were divided into two categories in which high and low doses of IFN-α had been used for short or long-term treatment. It was found that overall loss of HBV DNA was between 20 and 58% (mean 35.5%) regardless of the dose of IFN-α. This finding was much higher than that observed in controls (mean 11.4%). At the end of treatment, only 0-4% (mean 1.1%) of patients had lost HBsAg, and HBsAg clearance was more pronounced in patients who received higher doses of IFN-α[20]. The benefit of higher doses of interferon was also confirmed by re-treatment studies[28]. Ozen et al.[28] retreated 24 non-responder patients who were previously treated with a standard dose of interferon therapy. They found that 33.3% of patients responded to high-dose interferon treatment, but they could not find any HBsAg clearance in their study group. In our study group, we tried 10 MU/m^2 body surface area IFN-α as the first-line treatment. Persistent loss of HBV DNA and HBeAg was observed in 36.6% and 48.7% of patients, respectively, which was comparable with previous studies done in children[12,17,18,28]. The most striking finding in our study was a high percentage of HBsAg loss. Almost one-fifth of patients had lost HBsAg one year after the cessation of IFN treatment, and all developed HBsAb during the 2-8 year follow-up. This HBsAg clearance was higher than the results reported in children and adults previously[15,16,19,29,30].

A statistically significant decrease in ALT levels and significant improvement in the liver histology were observed in patients who cleared serum HBV DNA or who developed HBeAg seroconversion. After the treatment, a significant decrease in ALT levels was found in responders when compared with baseline values at the end of treatment and one year thereafter. In the literature, histological improvement with a decrease in necro-inflammatory activity and little effect on fibrosis has been documented in individuals with chronic HBV infection who responded to IFN-α treatment[31]. In our study, the histologic imp-rovement was most prominent in regression of portal inflammation and hepatic fibrosis. In contrast to literature, we found a statistically significant improvement in fibrosis when the first and control biopsies were compared.

The benefits of IFN-α in children should be weighed against the side effects of treatment. Serious adverse effects attributable to IFN-α seem to be rare in children[15,29]. Although the dose of IFN-α used in this study was high, the children tolerated IFN-α relatively well. The most important side effect was an episode of febrile seizure observed in one of the children. The other common side effects were fever, fatigue, loss of appetite and hair loss, all of which resolved after the cessation of treatment.

High dose IFN-α is well tolerated in children with chronic HBV infection. A sustained loss of HBV DNA and HBeAg was observed in more than one-third of patients. About one-fifth of children became HBsAg negative and anti-HBs antibody positive within one year of treatment, which is the highest percentage of HBsAg clearance reported to date. Since the long-term consequences of chronic HBV infection are significant and may be life-threatening, a treatment schedule, which achieves a higher rate of HBsAg clearance in addition to HBV clearance, should be considered among

treatment choices. Long-term effects of HBsAg clearance on outcomes of chronic HBV infection need further investigation, in children.

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