Prospective, Randomized Controlled Trial of Interferon-α in Children with Chronic Hepatitis B

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Thirty-six children with chronic hepatitis B were entered into a randomized controlled trial of recombinant human interferon-α. All patients had hepatitis B virus DNA and increased levels of aminotransferases in serum for at least 1 yr. Twelve children received 10 MU of interferon-α 2b/m² body surface area three times a week (group I); 12 children received 5 MU/m² under the same conditions (group II); and 12 children served as controls (group III). During 6 mo of therapy, 12 of 24 (50%) treated patients (7 from group I, 58%, and 5 from group II, 42%) and 2 of 12 (17%) controls lost hepatitis B virus DNA from serum and subsequently remained negative. Comparison of the rate of response in group I vs. controls showed a statistically significant difference (p < 0.05). Eleven of 12 (92%) treated patients who cleared hepatitis B virus DNA from serum lost HBeAg, seroconverted to anti-HBe and had improvement in liver histological findings with loss of hepatitis B virus DNA from liver. In 10, serum ALT levels became normal. Interferon-α was well tolerated and all children finished therapy. These findings indicate that a 6-mo course of interferon-α is effective in inducing a serological, biochemical and histological remission of disease in approximately 50% of children with chronic hepatitis B. (HEPATOLOGY 1991;13:1035-1039.)

Chronic HBV infection can be divided into two clinical and serological phases: (a) an initial phase during which HBV replication is active, HBV DNA is readily detected in serum and liver damage progresses and (b) a second, later phase during which HBV replication is low or absent, HBV DNA is not detected in serum by conventional techniques and liver injury ceases (1, 2).

Children with chronic HBV infection often have high levels of circulating virus, generally have chronic hepatitis and may have cirrhosis (3, 4) or hepatoma (5). An efficient treatment for children with chronic hepatitis B is needed.

The effectiveness of interferon-α as treatment of chronic hepatitis B in adults has been well documented in several studies (6, 7). The role of interferon in treating children is less clear. A controlled trial of recombinant interferon-α 2a in Chinese children with chronic hepatitis B yielded disappointing results because no differences were found in outcome between controls and treated patients (8). In a second controlled trial carried out in white children with the same schedule and duration of therapy (10 MU for 3 mo), initial results were promising, but at the end of the trial the response rates of treated patients and controls were the same (9).

To further assess interferon treatment in children with chronic hepatitis B, we carried out a randomized controlled trial of a 6-mo course of interferon-α 2b in 36 children with this disease.

PATIENTS AND METHODS

Between April 1988 and June 1988, 36 children were entered into the trial. Criteria for entry included a liver biopsy specimen showing chronic hepatitis (33 had CAH and 3 had chronic persistent hepatitis), persistent elevations of serum aminotransferase levels and presence of markers of HBV replication in serum (HBeAg and HBV DNA) for at least 1 yr.

Exclusion criteria included previous antiviral or immunosuppressive therapy, antibody to human immunodeficiency virus (anti-HIV) or hepatitis delta virus (anti-HDV), decompensated liver disease, other serious medical illnesses or evidence of other forms of liver disease.

The source of HBV infection appeared to be vertical transmission in two children. In the remaining 34 patients, the source was unclear but was probably intrafamilially spread in 30 children. In 13 children, vertical transmission was not documented, but their mothers had HBsAg. In 17 other children, other members of the family had HBsAg. The route of infection was not known in the remaining four children (11%) because all members of the family were HBsAg − . Only two children had a clinical history of acute hepatitis; in the rest the disease was discovered incidentally on routine screening.

The study was approved by the hospital Ethics Committee and written consent was obtained from the children's parents.

Children were randomly assigned to three groups. Patients in group I (n = 12) received 10 MU recombinant human interferon-α 2b/m² body surface (Intron A, Schering Plough, Kenilworth, NJ) three times a week for 24 wk by subcutaneous injection. Patients in group II (n = 12) received recombinant human interferon-α 2b at a dose of 5 MU/m² in the same regimen as in group I. Patients in group III (n = 12) were observed without treatment or placebo. Acetaminophen (5 to 10 mg/kg) was given to alleviate side effects of therapy.

HBsAg, HBeAg, anti-HBe, anti-HDV and anti-HIV were

TABLE 1. Initial features in three groups of patients

Analyzed parameters	I (10 MU/m²)	II (5 MU/m²)	III Control	Total	(Range)
Sex (M/F)	7/5	8/4	10/2	25/11	
Age (yr)[a]	8.7 ± 4.2	7.3 ± 2.5	8.5 ± 3.8	8.2 ± 3.7	(1.5-15.5)
Duration of HBsAg (mo)[a]	36 ± 28	27 ± 26	37 ± 30	33 ± 27	(6-126)
Mothers HBsAg+	4/12	7/12	4/12	15/36	
ALT level (IU/l)[a]	147 ± 72	138 ± 90	115 ± 68	133 ± 76	(41-320)
Albumin level (gm/L)[a]	3.9 ± 3	3.7 ± 0.6	3.3 ± 0.3	3.8 ± 0.4	(3.1-4)
γ-Globulin level (gm/L)[a]	1.3 ± 2	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.3	(0.9-1.7)
AST (IU/L)	101.4 ± 56.4	80.8 ± 45.6	82.1 ± 43.6	88.5 ± 48	(35-230)
Bilirubin (mg/dl)	0.6 ± 0.3	0.5 ± 0.2	0.5 ± 0.1	0.55 ± 0.2	(0.2-1.3)
Alkaline phosphatase (IU/L)	276.8 ± 71.8	222.6 ± 43.3	251 ± 64.5	252 ± 63	(124-403)
Prothrombin time	99 ± 3	97 ± 8	97 ± 7	98 ± 6	(75-100)
HBV DNA serum (ng/ml)[a]	0.7 ± 1.0	0.8 ± 0.8	0.6 ± 0.5	0.7 ± 0.8	(0.02-3.3)
Histological activity index	7.7 ± 2.0	8.0 ± 2.0	7.1 ± 3.0	7.5 ± 2.0	(2-12)

[a]Values are given as mean ± S.E.M.

determined by commercial immunoassays (Abbott Laboratories, North Chicago, IL). HBV DNA in serum was tested by dot-blot hybridization according to the method of Berninger et al. (10). For quantitative scoring of the HBV DNA concentration in each serum, the nitrocellulose filter corresponding to the radioactive spots was cut out and counted by liquid scintillation. The values obtained were compared with those of serial dilutions of HBV DNA of known concentration. To avoid interassay variations, all samples from the same patient were quantified in the same run. The sensitivity of this technique is less than 0.1 pg HBV DNA/10 μl of sample.

Liver biopsies were performed before randomization (within 6 mo) and 15 mo from the beginning of the study. The biopsy specimens were reviewed by a single pathologist who was blinded with respect to treatment groups and the chronological order of the biopsies. The histological activity was assessed in each biopsy specimen according to the method of Knodell et al. (11). A fragment of each biopsy was stored in liquid nitrogen for assessment of HBV DNA.

HBV DNA in Liver Biopsy Specimens. Frozen biopsy specimens were thawed and digested three times (10 min each) at 37° C with collagenase (1 mg/ml in PBS plus 200 mmol/L EDTA). The hepatocytes were collected by centrifugation, and the cells were resuspended in 600 μl of TSE buffer (10 mmol/L Tris-HCl, pH 7.5, 10 mmol/L NaCl, 2 mmol/L EDTA), 25 μl of 25% SDS and 70 μl of proteinase K (10 mg/ml in TSE). After 2 hr at 37° C, two phenol/chloroform (24:1) and two ether extractions were carried out, and the aqueous phase was precipitated overnight at -20° C. All the nucleic acids were resuspended in 100 μl of 1× TE buffer (10 mmol/L Tris, pH 7.5, 10 mmol/L EDTA) and were digested with RNase A at a final concentration of 0.1 μg/μl for 1 hr at 37° C. After two phenol/chloroform and two ether extractions, the total DNA was precipitated overnight at -20° C. Finally, the DNA was resuspended in water, and its concentration was determined measuring its absorbance at 260 nm. Between 30 and 40 μg of total DNA was obtained from each biopsy, and 15 to 20 μg (undigested and EcoRI or HindIII digested) was submitted to electrophoresis in a 1% agarose gel and transferred to a nitrocellulose filter. The filters were hybridized with ³²P HBV DNA and autoradiographed at -70° C. The sensitivity of the technique was less than 1 pg of cloned DNA.

Statistical Tests. The results are expressed as means ± SD. The differences between dichotomous variables were analyzed by Fisher's exact test. Continuous variables were compared by Student's t test. The sign test was used to assess change in histological scores (histological activity scores and paired assessment).

RESULTS

The three groups of children were comparable with respect to gender, age, mean known duration of HBsAg, number with HBsAg+ mothers, results of liver biochemical tests, HBV DNA serum concentration and histological activity (Table 1). Patients were examined at 1 mo intervals during therapy and every 3 mo thereafter until the end of the study (15 mo). At each visit a clinical examination was performed and blood samples were taken for blood cell counts, biochemical liver function tests (ALT, prothrombin time, serum albumin, total bilirubin levels, etc.) and HBV markers (HBsAg, HBeAg, and HBV DNA).

After 3 mo, HBV DNA was undetectable in five children (42%) in group I, four (33%) in group II and only two (17%) of the controls. At the end of the therapy, HBV DNA was negative in seven (58%) patients from group I, five (42%) from group II and two (17%) of the controls. At the end of treatment and at final follow-up, the rate of loss of HBV DNA was significantly higher in group I in comparison with the controls ($p < 0.05$). The differences between group II and the controls, as well as between groups I and II, were not statistically significant. The mean time to loss of HBV DNA was 17 ± 9 wk (range = 8 to 28 wk) in group I, 6.4 ± 2.2 wk (range = 4 to 20 wk) in group II and 6 ± 2.8 wk (range = 4 to 8 wk) in group III, which were not significantly different. No further patients lost HBV DNA during follow-up (15 mo). All patients who remained HBV DNA+ were considered to be nonresponders.

The clearance of HBV DNA with therapy was followed by a loss of serum HBeAg in all but one patient, occurring between 12 and 48 wk (34 ± 12 wk) after the beginning of the study in the treated patients and between 20 and 24 wk (26 ± 2.8 wk) in the controls. HBeAg clearance was sustained and was followed by development of anti-HBe in all patients. All patients were still HBsAg+ at the end of the trial.

TABLE 2. Changes in ALT

Response	ALT (IU/L)		No. patients with normal ALT (final)
	Initial	Final	
HBV DNA clearance (N = 12)	161.7 ± 69.5[a]	52.3 ± 34.5[b]	10 (83%)
Without HBV DNA clearance (N = 12)	126.2 ± 40.2	195.6 ± 50.3	0
Controls	114.5 ± 30.3	120.5 ± 35.2	1 (8%)[c]

[a]Values are given as mean ± S.E.M. Normal ALT ≤ 45 IU/L.
[b]$p < 0.01$ in comparison with initial value.
[c]This patient lost serum HBV DNA spontaneously.

All children were asymptomatic at the start of the trial, and after 15 mo they remained clinically well. The children who cleared HBV DNA from serum had a statistically significant decrease in serum ALT values (Table 2).

A second biopsy was performed at 15 mo in 34 of 36 patients (two refused). The histological activity index decreased in both treatment groups (from 7.7 ± 2 to 5.8 ± 3.3 in group I and from 8 ± 2 to 5.3 ± 3.3 in group II), but these changes did not reach statistical significance. There was no change in histological activity index in the controls (basal = 7 ± 3, final = 7 ± 4). The total histological activity decreased significantly in the patients who lost HBV DNA (7.6 ± 2.1 vs. 3.9 ± 2.4; $p < 0.05$) but not in those patients who remained HBV DNA+ (8 ± 1.8 vs. 7 ± 3). Scoring of the liver biopsy specimens in the patients who cleared serum HBV DNA showed significant improvement with respect to the degree of portal inflammation (basal = 3.1 ± 0.3 vs. final = 1.5 ± 0.9; $p < 0.01$) and lobular cytolysis (basal = 1.8 ± 0.8 vs. final = 0.8 ± 0.9; $p < 0.05$). Piecemeal necrosis also decreased, but the change did not reach statistical significance (1.8 ± 1.4 vs. 0.8 ± 1.2). No changes in the degree of liver fibrosis were observed.

The presence and state of HBV DNA in liver was assessed by Southern-blot hybridization in the initial biopsies of all 36 children. Intermediate replicative forms of HBV DNA with bands at 3.2 kb (linear HBV DNA), 2 kb (covalently closed circular HBV DNA) and 1.3 kb (single-stranded HBV DNA) were observed (Fig. 1). HBV DNA was evaluated in 34 biopsy specimens after 15 mo. Again, HBV DNA intermediate replicative forms were detected in the 22 liver biopsy specimens from children who still had HBV DNA in serum. In contrast, no HBV DNA was found in the final biopsy specimens from children without serum HBV DNA.

The predictive factors of a response to interferon-α were analyzed retrospectively. Gender, age, known duration of HBsAg, pretreatment ALT and HBV DNA and results of histological examination were not significantly associated with favorable antiviral response. Patients with sustained clearance of serum HBV DNA tended to be HBsAg chronic carriers for a shorter period and to have higher initial serum ALT levels than those without a loss of HBV DNA, but the differences were not statistically significant (Table 3).

TABLE 3. Predictive factors of response to recombinant interferon-α therapy

Analyzed parameters	Responders (12)	Nonresponders (12)
Sex (M/F)	8/4	7/5
Age (yr)[a]	7.8 ± 3.0	8.2 ± 4.2
Duration of HBsAg (mo)[a]	24 ± 18	39 ± 32
Mothers with HBsAg+	6/12	5/12
Initial ALT (IU/L) values[a]	184.4 ± 110	143.8 ± 82
Initial serum HBV DNA (ng/ml)[a]	0.6 ± 0.8	0.7 ± 0.9
Histological activity index	7.6 ± 2.1	8 ± 1.8

[a]Values are given as mean ± S.E.M.

No differences in frequency or severity of side effects were observed between the two treatment groups. Of the 24 treated children, 22 (92%) had an influenza-like syndrome consisting of fever, chills, malaise, myalgias, anorexia and headache at the start of therapy. After the second week of therapy, these symptoms disappeared and no further complications arose. Only seven children (29%) continued to have low-grade fever throughout the course of treatment. Other common side effects included hair loss (8%) and fatigue (25%). In two children (one in group I and one in group II) a decrease in leukocytes (2,200 to 3,000/mm^3) took place during the fourth and fifth month of therapy, but the doses of interferon-α were not modified and leukocytes later increased spontaneously. No child had weight loss, and all grew normally during therapy.

DISCUSSION

The efficacy of interferon-α in treatment of adults with chronic hepatitis B has been well established (6, 7, 12). The efficacy of this agent in treating children is less well accepted. We have studied the effectiveness of interferon-α in 36 white children with chronic hepatitis B. Children treated with 10 MU of interferon-α three times weekly for 24 wk had a significantly higher clearance of HBV DNA as compared with the control group. This group also had a higher rate of clearance of HBeAg than the control group. These results demonstrated that interferon-α is as effective in white children as in adults with chronic hepatitis B (6, 7, 13). These results are different from those obtained in Chinese children (8), which may be explained by the different
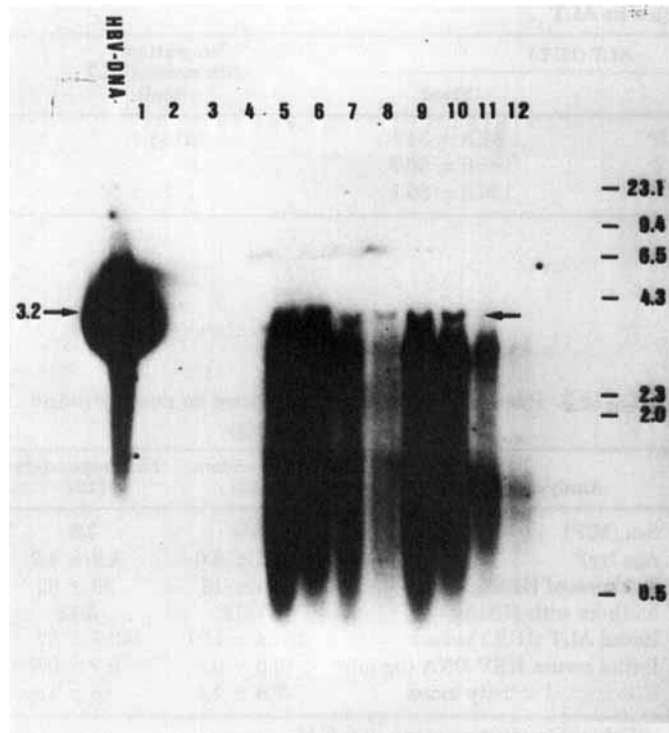
FIG. 1. (Lane 1) HBV DNA isolated from the recombinant plasmid pBH20-HBV. (Lanes 2 and 3) Negative controls. (Lane 4) Liver sample from a patient without serum HBV DNA (responder). (Lanes 5 to 12) Replicative intermediates of viral DNA from patients with serum HBV DNA.

characteristics of the children included in both trials. The Chinese children largely had inactive liver disease with normal ALT levels, whereas the children in this study had abnormal ALT levels and active liver disease (proven histologically). Previous studies have shown that response to treatment correlates with disease activity, which could therefore explain the differences in these two trials (14, 15).

A significant decrease in ALT values and significant improvement in the liver histological findings occurred in treated patients who cleared serum HBV DNA but not in the nonresponders or control children. Histological improvement consisted of significant regression of portal inflammation and lobular necrosis but no change in fibrosis. Thus the natural history of chronic HBV infection appeared to be improved in those treated children who lost HBV DNA.

The pattern of HBV DNA expression in liver cells from the initial and final biopsy specimens of the children showed that the lack of detectable HBV DNA in serum was accompanied by a lack of replicative intermediates of HBV DNA in liver. All patients who cleared HBV DNA from serum did not have HBV DNA detectable in the biopsy specimen taken after treatment, whereas the nonresponders all had intermediate replicative forms of HBV DNA. It should be stressed, however, that Southern hybridization may not be adequately sensitive

to detect small amounts of HBV DNA that may persist after loss of HBeAg in integrated forms.

The lack of detection of integrated HBV DNA in the biopsy specimens of these children is a striking result because many of them were infected perinatally or during early childhood. Thus HBV DNA integration seems to be an uncommon event in children with chronic hepatitis B (16). However, all the children, including those who cleared serum HBV DNA, remained HBsAg+. One explanation could be that HBV DNA is still in the liver, undetected by the Southern hybridization technique. Future studies using more sensitive techniques such as polymerase chain reaction need to be performed on liver tissue and serum (17). Another explanation for the lack of HBV DNA detected in livers from these HBsAg+ children is that HBsAg may eventually be cleared as was recently reported in adults studied 3 to 6 yr after a favorable response to interferon-α (18).

Two different doses of interferon-α were used (10 MU/m² and 5 MU/m²). The response rate was slightly higher with 10 MU/m², but the difference was not statistically significant. Nevertheless, it seems prudent to recommend the higher dose regimen. Indeed, even at this higher dose, interferon-α was well tolerated.

In summary, interferon-α treatment of chronic hepatitis B in children was associated with a sustained clearance of the serological markers of HBV replication in 50% of patients. Loss of HBV DNA and HBeAg was followed by a decrease of serum ALT levels to normal and histological improvement, with a regression of lobular hepatocellular necrosis and portal inflammation.

REFERENCES

1. Nordentfelt E, Kjellen L. Dane particles, DNA polymerase and e antigen in two distinct categories of hepatitis B antigen carriers. Intervirology 1975;5:225-232.
2. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981;94:744-748.
3. Bortolotti F, Calzia R, Cadrobbi P, Giacchini R, Piravegna B, Armigliato M, Piscopo R, et al. Liver cirrhosis associated with chronic hepatitis B virus infection in childhood. J Pediatr 1986;108:224-227.
4. Ruiz-Moreno M, Camps T, Garcia Aguado J, Porres JC, Oliva H, Bartolomé J, Carreño V. A serological and histological follow-up of chronic hepatitis B infection. Arch Dis Child 1989;64:1165-1169.
5. Wu TC, Tong MJ, Hwang B, Lee SD, Hu MM. Primary hepatocellular carcinoma and hepatitis B infection during childhood. HEPATOLOGY 1987;7:46-48.
6. Alexander G, Williams R. Antiviral treatment in chronic infection with hepatitis B virus. BMJ 1986;292:915-917.
7. Davis GL, Hoofnagle JH. Interferon in viral hepatitis: role in pathogenesis and treatment. HEPATOLOGY 1986;6:1038-1041.
8. Lai CL, Lok ASF, Lin HJ, Wu PC, Yeoh EK, Yeung CY. Placebo-controlled trial of recombinant alpha 2-interferon in Chinese HBsAg-carrier children. Lancet 1977;2:877-880.
9. Ruiz-Moreno M, Jimenez J, Porres JC, Bartolomé J, Moreno A, Carreño V. A controlled trial of recombinant interferon-alpha in Caucasian children with chronic hepatitis B. Digestion 1990;45:26-33.
10. Berninger M, Hammer M, Hoyer B, Gerin J. An assay for the detection of the DNA genome of hepatitis B virus in serum. J Med Virol 1982;9:57-68.

11. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. HEPATOLOGY 1981;1:431-435.
12. Dusheiko G, Di Bisceglie A, Bowyer S, Sachs E, Ritchie M, Schoub B, Kew M. Recombinant leukocyte interferon treatment of chronic hepatitis B. HEPATOLOGY 1985;5:556-560.
13. Lok ASF, Weller IVD, Karayiannis P, Brown D, Fowler MJF, Monjardino J, Thomas HC, et al. Thrice weekly lymphoblastoid interferon is effective in inhibiting hepatitis B virus replication. Liver 1984;4:45-49.
14. Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to interferon therapy? A statistical analysis of predictive factors. HEPATOLOGY 1989;10:761-763.
15. Mora I, Porres JC, Bartolomé J, Quiroga JA, Gutiez J, Hernández-Guío C, Bas C, et al. Changes of hepatitis B virus (HBV) markers during prolonged recombinant interferon alpha-2A treatment of chronic HBV infection. J Hepatol 1987;4:29-36.
16. Bartolomé J, Moraleda G, Ruiz-Moreno M, Porres JC, Carreño V. Hepatitis B virus DNA patterns in the liver of children with chronic hepatitis B. J Med Virol 1990;31:195-199.
17. Kaneko S, Miller RH, Di Bisceglie A, Feinstone SM, Hoofnagle JH, Purcell RH. Detection of hepatitis B virus DNA in serum by polymerase chain reaction. Gastroenterology 1990;99:799-804.
18. Korenman JC, Baker BL, Waggoner JG, Di Bisceglie AM, Hoofnagle JH. In chronic hepatitis B loss of serum hepatitis B viral DNA (HBV-DNA) detectable by polymerase chain reaction (PCR) usually occurs at the time of loss of hepatitis B surface antigen (HBsAg) [Abstract]. HEPATOLOGY 1990;12:846.