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RECOMBINANT INTERFERON-α-2A WITH OR WITHOUT STEROID PRETREATMENT IN CHILDREN WITH CHRONIC HEPATITIS B*

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SUMMARY: Özen H, Koçak N, Gürakan F, Yüce A. (Division of Gastroenterology, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Recombinant interferon-α-2A with or without steroid pretreatment in children with chronic hepatitis. Turk J Pediatr 1998; 40: 503-514.

Interferon is the most promising therapeutic agent for the treatment of chronic viral hepatitis. The results of studies suggest that corticosteroid pretreatment may improve the response rate. Twenty-nine children with chronic hepatitis B (CHB) were randomly assigned to receive recombinant interferon alpha (rIFN-α) alone (Group 1.5 million units/m² body surface, 3 times a week for 24 weeks) or to receive oral prednisone (Group 2.2 mg/kg/day for 3 weeks, discontinued by tapering the dose within 1 week) followed by rIFN-α (same dose as above). Tests for liver function and hepatitis B virus (HBV) markers including HBV-DNA were done periodically. Overall, 10 patients (34.5%) cleared hepatitis Be antigen and 13 (44.8%) HBV-DNA. Anti-HBe seroconversion was observed in nine patients (31%). Only three patients (10.3%) cleared hepatitis B surface antigen and seroconverted to anti-HBs. No response was obtained in 11 patients (37.9%). There was no statistically significant difference between the two treatment groups regarding response rate. Baseline transaminases levels and HBV-DNA concentrations were predictive parameters for HBeAg clearance. It is concluded that prednisone pretreatment does not have a beneficial effect in children with CHB. *Key words: children, chronic hepatitis B, recombinant interferon-α, prednisone.*

Clinical studies have clearly documented that both adults and children with chronic hepatitis B (CHB) and persistent hepatitis B virus (HBV) replication are at increased risk of progression to cirrhosis and hepatoma[1-4]. Disappearance of HBV replication takes place together with amelioration of liver disease. Because of its antiviral and immunomodulatory properties, interferon (IFN) is the most promising therapeutic agent for the treatment of various chronic viral infections, including chronic viral hepatitis[5]. Recently, it has been demonstrated that recombinant interferon alpha (rIFN-α) treatment of chronic hepatitis B is effective in both adults and children, with the percentage of patients responding around 30 to 40 percent[6-8].

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Initial reports of IFN treatment in children with chronic HBV infection have demonstrated a variable response rate (measured by clearance of viral DNA and hepatitis Be antigen [HBeAg], and normalization of serum alanine aminotransferase levels), between no response to 58 percent[9-13]. Complete recovery (with loss of HBsAg) is usually noted in less than 20 percent of treated individuals; similar response rates have been reported in the relatively small number of children evaluated to date[14]. Nevertheless, a significant number of patients do not respond to IFN-α therapy alone, and an alternative approach to treatment is desirable. Many drugs, including levamisole[15,16]. zidovudine[17], acyclovir[18], vidarabine[19] and interleukin-2[20], have been prescribed together with IFN to achieve a higher response rate. Corticosteroids alone have not been shown to be beneficial in treating hepatitis B virus related chronic active hepatitis. In fact, some studies have shown a deleterious effect[21,22]. However it has been shown that long or short courses of corticosteroids may result in a decline of viral replication or even successful treatment due to an immunologic rebound that follows corticosteroid withdrawal[23,24]. A severe defect in suppressor-cell activity in hepatitis B surface antigen positive chronic active hepatitis has been shown in comparison with healthy subjects[25]. The results of studies suggest that a combination therapy, in which corticosteroid is followed by antiviral drugs, is superior to corticosteroid withdrawal alone.

The aim of this study was to evaluate the possible benefit of rIFN-α administered either alone or after prednisone to children with chronic HBV infection.

Material and Methods

Patients with elevated aminotransferases concentrations (>40 IU/L) and HBeAg and HBV-DNA positivity for at least six months were included in the study. All patients had chronic hepatitis (24 chronic persistent hepatitis [CPH] and 5 chronic active hepatitis [CAH]), histologically proven by liver biopsy within one month prior to entry in the study. None of the children had decompensated liver disease or evidence of other forms of liver disease. Criteria for exclusion were antiviral therapy during the preceding 12 months, leukopenia ($<3 \times 10^9$/L), thrombocytopenia ($<100 \times 10^9$/L) or an elevated serum creatinine level, cirrhosis, and other causes of liver disease or malignancy.

Children were randomly assigned to two groups. Patients in Group 1 received five million units (MU) of human recombinant interferon alpha 2A (rIFN-α-2A) (Roferon-A, Roche) per square meter of body surface, three times a week for 24 weeks by subcutaneous injection. Patients in Group 2 received oral prednisone (2 mg/kg/day, maximum 60 mg/day) for three weeks, which was discontinued by tapering the dose within one week. After one week of rest, patients received

rIFN-α-2A as described above. Paracetamol was administered to alleviate fever and myalgia during the first weeks, if needed. If the platelet count decreased to less than $100x10^9$/L and neutrophil count to less than $1.2x10^9$/L, the next doses were withheld until the abnormality improved.

A blood sample was analyzed monthly during the treatment (24 weeks) and every three months thereafter for liver function tests, hematological parameters, and HBV markers. HBV-DNA was determined at the end of the treatment and every six months thereafter. Hepatitis B surface antigen (HBsAg), HBeAg, antibodies to HBsAg (anti-HBs), HBeAg (anti-HBe), hepatitis delta infection, hepatitis C infection, and human immunodeficiency virus were determined by commercially available radioimmunoassay kits. Liver function tests were analyzed automatically by sequential multiple autoanalysis (Hitachi 911 Automatic Analyzer), at 37 °C, with standards supplied by the manufacturer. HBV-DNA in serum was tested by dot-blot hybridization according to the method described previously[26].

A complete response was recorded if a patient had (1) cleared both HBsAg and HBeAg, (2) both anti-HBs and anti-HBe, (3) undetectable circulating levels of HBV-DNA, and (4) normal ALT and AST activity, by 12 months after initiation of therapy (6 months after the end of the treatment). A partial response was recorded when a patient had (1) cleared HBeAg with or without development of anti-HBe (2) undetectable circulating levels of HBV-DNA, and (3) normal or decreased ALT and AST activity. A minimal response was recorded when a patient had any response towards normalization of any of the efficacy parameters measured. Nonresponse was considered as persistence of HBeAg, HBsAg, detectable circulating HBV-DNA, and elevated/unchanged ALT or AST activity. In the presentation of data, responders include complete, partial and minimal responders unless otherwise noted.

All family members, whenever available, were tested for HBV markers, including HBsAg, HBeAg, anti-HBe and anti-HBc IgG. When a family member was positive for HBV (positivity for HBsAg and/or anti-HBs) the patients was considered as infected horizontally. When the patients was infected during the first year of life, the patient was considered as infected vertically if the mother was HBsAg positive.

The results were expressed as mean ± SD and a two-tailed "p" value less than 0.05 was considered as significant. Comparisons of measurable data between the two groups were done using Mann-Whitney U test. Repeated parameters in the same group were tested using Wilcoxon paired signed-ranks test or Friedman two-way ANOVA. Proportions were compared using chi-square test or Fisher's exact chi-square test when expected frequencies were less than five[27].

Results

Twenty-nine patients, aged 100.7 ± 35.5 months (range 52 to 160), were entered in this study. Sixteen of them (55.2%) were assigned to monotherapy (Group 1) and 13 of them (44.8%) to combination therapy (Group 2). Twenty-four patients (82.8%) had CPH and five CAH. All of them completed the 12 months follow-up period. The mean follow-up was 17.2 ± 3.3 months in Group 1 and 15.8 ± 3.1 months in Group 2.

The two groups were comparable with respect to age, sex, duration of HBsAg, baseline levels of ALT and of HBV-DNA and histological features (Table I). Although it was not statistically significant, the number of patients with baseline ALT values more than three times the upper limit of normal (>120 IU/L) was higher in Group 1, and baseline HBV-DNA concentrations were higher in Group 2.

Table II shows the change in serological events and laboratory parameters at the end of the treatment and 12 months after randomization. Mean concentrations of ALT decreased significantly in both groups. HBV-DNA concentrations also decreased but this decline was not statistically significant. Clearance of HBeAg

Table I: Clinical, Serological and Biochemical Characteristics of Patients in Two Treatment Groups

	Group 1 (n=16)	Group 2 (n=3)	p
Age (mos)	98.1 ± 32.8*	101.2 ± 36.1	.73
Sex (M/F)	13/3	11/2	1.0
Duration of HBsAg positivity (mos)	19.6 ± 14.0	19.2 ± 11.6	.93
HBsAg positive family member (%)	4 (25.0)	9 (69.2)	.45
Liver histology (CAH/CPH)	3/13	2/11	1.0
HBV-DNA (pg/ml)	48.5 ± 70.6	156.9 ± 212.9	.11
HBV-DNA>100 pg/ml (%)	2 (12.5)	5 (38.5)	.19
ALT (IU/L)	175.3 ± 120.9	177.6 ± 147.4	.66
ALT>3 times normal (>120 IU/L) (%)	8 (50.0)	5 (38.5)	.81
AST (IU/ml)	119.6 ± 59.9	131.8 ± 92.2	.91
Serum protein (g/dl)	7.2 ± 0.8	7.2 ± 0.5	1.0
Serum albumin (g/dl)	4.7 ± 0.4	4.7 ± 0.2	.58
Leukocyte (/mm^3)	6968 ± 1428	6746 ± 1633	.52
Hemoglobin (g/dl)	12.9 ± 0.9	12.8 ± 0.6	.96
Thrombocyte (/mm^3)	334938 ± 53971	259386 ± 74455	.32

* Plus-minus values are mean ± SD.

Tablo II: Biochemical and Serological Parameters at Baseline, Six Months and Twelve Months After Treatment

	Group 1 (n = 16)				Group 2 (n = 13)			
	Basseline#	6 months	12 months	p*	Basseline#	6 months	12 months	p*
ALT (IU/L)	175.3 ± 120.9	98.5 ± 69.6	70.1 ± 65.2	.013	177.6 ± 147.4	77.6 ± 62.7	62.1 ± 31.0	.013
AST (IU/L)	119.0 ± 59.9	80.5 ± 41.6	75.4 ± 62.5	.20	131.8 ± 92.2	81.9 ± 5.9	58.4 ± 15.8	.049
HBV-DNA (pg/ml)	48.5 ± 70.6	40.2 ± 74.3	40.3 ± 70.6	.22	156.9 ± 212.9	117.5 ± 215.2	146.8 ± 269.8	.31
Serum protein (g/dl)	7.2 ± 0.8	7.9 ± 0.6	7.8 ± 0.7	.001	7.2 ± 0.5	7.6 ± 0.4	7.5 ± 0.7	.01
Serum albumin	4.7 ± 0.4	4.9 ± 0.3	4.7 ± 0.3	.006	4.7 ± 0.2	4.7 ± 0.3	4.6 ± 0.2	.27
Leukocyte (/mm³)	6968 ± 1428	6413 ± 1718	6490 ± 973	.62	6746 ± 1633	6415 ± 692	7062 ± 2180	.37
Hemoglobin (g/dl)	12.9 ± 0.9	12.4 ± 1.1	13.4 ± 0.8	.025	12.8 ± 0.6	12.3 ± 0.9	12.7 ± 0.9	.054
Clearance of HbeAg (%)a	—	6 (37.5)	7 (43.8)		—	2 (15.4)	3 (23.1)	
Clearance of HBV-DNA (%)a	—	8 (50.0)		—	4 (30.8)	4 (30.8)		
Seroconversion to anti-HBe (%)a	—	3 (18.8)	6 (37.5)		—	1 (7.7)	3 (23.1)	
Seroconversion to anti-HBsa	—	—	3 (18.8)		—	—	—	
Responsea								
Complete response			3 (18.8)				—	
Partial response			2 (12.5)				2 (15.4)	
Minimal response			6 (37.5)				5 (38.4)	
No response			5 (31.3)				6 (46.2)	

Plus-minus values are means ± SD
* Friedman two-way ANOVA
a p>0 05 (Group 1 vs. Group 2)

and HBV-DNA occurred in most of the patients during the treatment period (80% and 92.3%, respectively), whereas the majority of anti-HBe seroconversion (5/9, 55.6%) occurred during the second six months. In one patient in Group 1, HBV-DNA clearance occurred at the 24th month. Although statistically significant differences were observed in serum protein and albumin concentrations and hemoglobin leves, no patient had abnormal levels. The mean durations of HBeAg clearance and seroconversion to anti-HBe were 4.1 ± 2.9 months and 6.8 ± 3.3 months in Group 1, and 5.5 ± 3.1 months and 6.3 ± 3.8 months in Group 2, respectively ($p>0.05$ Group 1 vs. Group 2). There was also no significant difference for HBV-DNA clearance duration between Group 1 and Group 2, 5.7 ± 2.8 and 8.6 ± 8.7 months, respectively. Seroconversion to anti-HBe occurred at 3.0 ± 2.4 months and 1.0 ± 1.7 months after HBeAg clearance in Groups 1 and 2, respectively ($p>0.05$). Seroconversion to anti-HBs occurred at 5.3 ± 3.2 months after HBeAg clearance and almost at the same time as HBsAg clearance (Table III). All patients except one in Group 1, who cleared HBeAg, seroconverted to anti-HBe.

Table III: Predictive Epidemiological, Biochemical and Serological Parameters for HBeAg Clearance

Features	Clearance of HBeAg (n=10)	No clearance of (n=19)	p
Sex			1.0
Male	8 (33.3)*	16 (66.7)	
Female	2 (40.0)	3 (60.0)	
Age (mos) (range)	95.6 ± 27.2# (56-126)	101.5 ± 37.2 (5-160)	.69
Treatment			.43
Group 1	7 (43.8)	9 (56.2)	
Group 2	3 (23.1)	10 (76.9)	
Liver histology			.31
CPH	7 (29.2)	17 (80.8)	
CAH	3 (60.0)	2 (40.0)	
HBsAg Family member positive			1.0
Present	4 (30.8)	9 (69.2)	
Absent	6 (37.5)	10 (62.5)	
Clearance of HBV-DNA			.21
Yes	7 (50.0)	7 (50.0)	
No	2 (18.2)	9 (81.8)	
Duration of HBsAg positivity (mos)	20.2 ± 14.1	19.0 ± 12.4	.94
Baseline			
ALT (IU/L)	241.3 ± 142.7	142.2 ± 113.7	0.37
AST (IU/L)	165.4 ± 85.6	103.8 ± 60.6	.037
HBV-DNA (pg/ml)	27.6 ± 85.6	133.6 ± 186.2	.028
ALT>120 IU/L	7/13	6/13	.064
HBV-DNA>100 pg/ml	0/7	7/7	0.62

* Values in parentheses are percentages
\# Plus-minus values are means ± SD

The final evaluation of efficacy 12 months after randomization revealed a complete response in three patients (18.8%) treated with IFN-α-2A alone and in none of 13 patients receiving combination therapy. Partial or minimal response was observed in eight patient (50%) in Group 1 and in seven patients (53.8%) in Groups 2. The ratio of nonresponders was 31.2 percent in Group 1 and 46.2 percent in Group 2 patients (p=0.41) (Table II). The higher response rate was observed in treated patients with low HBV-DNA concentrations (<100 pg/ml) and higher ALT levels (>3 times the upper normal value, >120 IU/L) at baseline, although statistically insignificant (p=0.062 and 0.064, respectively). All patients showed transient ALT elevation before seroconversion to anti-HBe. Age, sex, duration of hepatitis, histological conditions and baseline levels of ALT, AST and HBV-DNA were analyzed as predictive factors of a response to therapy (Table III). Only pretreatment ALT, AST and HBV-DNA concentrations were predictive factors of response rate. Neither HBV reactivation nor reappearance of previously cleared markers such as HBV-DNA or HBeAg occurred in any patient.

The source of HBV infection appeared to be horizontal in all patients except who underwent an exchange transfusion during the neonatal period because of hyperbilirubinemia. Although at least one family member was HBsAg positive in only 13 families (44.8%), seropositivity for HBV (HBsAg or anti HBs) was observed in all families. Only one child in Group 1 had a clinical history of acute hepatitis, and she had complete response. In the rest of the patients, the infection was discovered incidentally on routine screening.

All patients tolerated the treatment well without major side effects which resulted in discontinuation of therapy. There was no difference in side effects between the two groups. Fewer was seen in 20 out of 29 patients (68.9%) within two weeks. Asthenia was observed in five patients (17.2%), myalgia and abdominal pain in four (13.8%), convulsion, headache, weight loss, vomiting and anorexia in two (6.9%), and hair loss, change in mood, anxiety, leukopenia and hypothyroidism in 1 (3.4%). Two children with convulsion had a history of previous febrile convulsions and each had one episode of afebrile convulsion during the treatment. None of the side effects caused discontinuation of therapy.

Discussion

As a result of its success in several studies, alpha interferon is the only recognized therapy for chronic viral hepatitis. Clearance of HBV-DNA, HBeAg, and normalization of ALT are observed in approximately 30 to 45 percent of patients[14]. To enhance its effect, many drugs have been prescribed together with IFN to achieve a higher response rate[15-20]. Corticosteroids are one of these drugs. Although there are several reports on adult patients[28-35], reports on children are

rare. Some studies with adults showed beneficial effects[28,31,35]. whereas the majority showed no benefit[30,32-34] or even a harmful effect[29].

Reports on children also revealed controversial results. The response rate changed from 0 to 78 percent[9,36], but study designs and the dose and duration of the treatment were not similar. Children infected vertically and at an early age are less likely to have response[9]. In their first study, Utili et al.[37] reported that prednisone priming followed by rIFN-α-2A was effective in six of nine (66.7%) treated children in reducing HBV replication and disease activity. In their second study[38], similar to our study, they evaluated the efficacy of sequential treatment with prednisone and interferon on 43 children with chronic hepatitis B (34 CPH, 9 CAH). They prescribed either a one-month course of prednisone (0.6 to 0.3 mg/kg/day) followed by IFN-α-2A (3 MU/m², thrice weekly, for 12 months; 22 patients) or no treatment (21 patients). At the end of the study (20 months), clearance of HBV-DNA and HBeAg seroconversion were observed in nine (41%) of the patients treated and in two (9.5%) of the untreated controls (p=0.02). Although the IFN dose was lower than in our study, the cumulative dose was similar in the two studies. However, they did not compare the IFN regimens alone and with prednisone. Two of the treated patients (9.1%) who lost HBeAg also cleared HBsAg, and 13 (59%) patients had stable normal levels of ALT on their last examination.

The overall clearance rate of HBsAg was 10.3 percent in our study and all seroconverted patients had stable ALT levels at the end of the study. Gregorio et al.[39] conducted a multicenter-controlled trial with 95 HBV-DNA/HBeAg positive children. On follow-up between 12 and 18 months (median, 15 months) after treatment, the loss of HBeAg with anti-HBe seroconversion was more common in patients pretreated with steroids (35%) or placebo (40%) as against controls (13%). The anti-HBe seroconversion rate in our study was 37.5 percent in the combination therapy group and 23.1 percent in the IFN alone group. No statistically significant difference could be found between the two treatment groups in both studies. Giacchino et al.[40] compared lymphoblastoid interferon alone and with prednisolone, but they prescribed IFN for only 12 weeks. The influence of prednisolone priming was not statistically significant. HBeAg clearance was similar in both groups (44% after prednisolone/interferon and 53% after interferon alone, 48% overall). In one study[29], it was shown that pretreatment with prednisolone before IFN therapy might cause clinical rebound and hepatic decompensation. We did not observe hepatic decompensation or clinical rebound.

There is almost complete agreement on predictive parameters of response. A number of smaller studies have suggested that response to treatment is more likely to occur in patients with higher levels of transaminases, with recent onset,

a history of acute hepatitis and low levels of HBV-DNA. Krogsgaard et al.[41] collected data from 751 patients from 10 controlled trials on alpha interferon (lymphoblastoid or recombinant) treatment for chronic hepatitis B. When disappearance of HBeAg was considered as the major end point, the results of survival analysis showed that the HBeAg disappearance rate with or without interferon treatment was higher in patients with high aminotransferases levels, with a history of acute hepatitis and in male heterosexual patients disregarding HIV status. The relative treatment effect of alpha interferon was independent of the tested pretreatment host variables, but dependent on the total interferon dose. Although all patients seem to have the same relative benefit, the absolute benefit of alpha interferon treatment seems to be greatest in patients with high transaminases levels and with a history of acute hepatitis. Only one patient in our study had a history of acute hepatitis and she seroconverted to anti-HBs. Mean concentration of ALT was also higher in the responder than in the nonresponder in our study. Interferon dose was the same in all patients; we could not determine the effect of the cumulative IFN dose. Baseline HBV-DNA concentration was also reported as a predictor of response. All seroconverted patients had a baseline HBV-DNA level of <100 pg/ml in the study. In Utili et al.'s study[13], similar to our study, HBeAg clearance was observed in 75 percent of patients with a baseline HBV-DNA level lower than 100 pg/ml and in none with a level above 100 pg/ml. A greater degree of portal tract inflammation on pretrial biopsy is also a predictive factor[39]. However, there are studies that show the response does not correlate with the pretreatment serum transaminases levels, HBV-DNA concentrations or degree of histological activity[40].

Ethnic origin may also have an effect. In a recent study of Polish children with HBV infection treated with prednisone and IFN[36], there was a similarly high response rate (7/9, with 6 complete response) to IFN treatment in children younger than three years of age. In another study[9], HBV-infected Asian children showed no response, but the majority of the children did not have elevated ALT and were probably symptom-free carriers.

Interferon is better tolerated by children than adults[40,42]. A flu-like syndrome is the most frequent side effect and has been reported in 87 percent of children[43]. A flu-like syndrome with fever was seen in 68.9 percent of our patients. Other side effects were also similar in our study and that of Ruiz Moreno et al.[43], but we observed leukopenia in only one patient whereas they found it in 34.8 percent. In a study of 11,241 consecutive patients with chronic viral hepatitis who underwent interferon alpha treatment[42], five patients (0.04%) died during IFN therapy due to liver failure or complications arising from sepsis. Life-threatening side effects were observed in eight (0.07%) patients: two cases attempted suicide because of depression and six patients had severe bone marrow suppression.

All these symptoms completely disappeared after IFN withdrawal. All side effects also disappeared in our study after IFN withdrawal. The incidence of thyroid disease was reported as 0.6 percent and it was 3.4 percent in our study. Fatal or life-threatening side effects were not related to actual total dose or duration of IFN alpha, while the majority of patients with de novo non-hepatic morbidity received medium-to-high doses. Only 443 (4%) of the patients were younger than 20 years of age and none of them had non-hepatic morbidity.

The results show that IFN is well tolerated by children and is useful in the treatment of CHB. We conclude that it is unnecessary to add prednisone to IFN treatment as prescribed in this study because no beneficial effect could be demonstrated.

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