USE OF RECOMBINANT ALPHA-2b-INTERFERON IN COMBINATION WITH ANTIOXIDANTS IN THE FORM OF RECTAL SUPPOSITORIES (VIFERON) IN CHILDREN WITH CHRONIC HEPATITIDES B AND C

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Received March 18, 1998; accepted January 11, 2000

Summary. – A new antiviral and immunomodulating preparation Viferon, produced as rectal suppositories containing recombinant alpha-2b-interferon (IFN) and antioxidants, was used in complex therapy of viral chronic hepatitides B and C (ChHB and ChHC) in children. Results of our investigation showed a high efficiency of Viferon. Viferon was found to suppress replication of hepatotropic viruses and to decrease activity of the pathologic process in the liver of children with ChHB and ChHC. After a Viferon treatment with daily doses of (1–2) x 10^6 IU of IFN (3.0 x 10^6 IU/m^2) primary remission was registered in 78% of patients with ChHB and in 44% of patients with ChHC, while lasting remission was found in 82% of ChHB and in 33% of ChHC patients. Thus, a more marked effect was observed with ChHB, in which 3.0 x 10^6 IU/m^2 was the optimal daily dose for children. Increasing the dose to 5.0 x 10^6 IU/m^2 did not result in rise of the percentage of the remissions. Side effects, which are characteristic for injection of IFN preparations, were never found even after a longterm treatment. Absence of induction of neutralizing antibodies was observed after administration of alpha-2b-IFN, an integral part of Viferon. In pediatrics, the method of rectal administration has advantages over parenteral delivery due to its convenience, non-traumatic character and possibility of use for prolonged periods.

Key words: chronic viral hepatitis; treatment; interferon; Viferon; suppository

Introduction

Both native and recombinant IFN preparations are used in clinical practice for treatment of patients with viral ChH. There are important reasons for use of IFNs in this pathology: active prolonged replication of hepatotropic viruses, deficit and unbalance of cellular immunity, functional defectiveness of mononuclear phagocytes, and insufficiency of the IFN system (Dudley *et al.*, 1972; Rizzetto *et al.*, 1985; Uchaikin *et al.*, 1998).

In the treatment of viral ChH in adults, IFN prepatations are used for long periods (for 6 and more months), parenterally, and in high individual doses (3–6) x 10^6 IU) (Hoofnagle *et al.*,1987; Ryff,1997; Sherlock *et al.*, 1985; Thomas *et al.*, 1991; Wong *et al.*, 1993). In pediatrics, IFN preparations are used very seldom (Ruis Moreno *et al.*,1990;

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Abbreviations: 5-H = ectoferment 5-nucleotidase; ALT = alanine aminotransferase; anti-HBcAg = antibodies to HB core antigen; anti-HBeAg = antibodies to HB e antigen; anti-HBsAg = antibodies to HB s antigen; anti-HDV = antibodies to HD virus; anti-HCV = antibodies to HC virus; AST = asparagine aminotransferase; CD4 = T-helpers; CD8 = T-suppressors; ChH = chronic hepatitis; ChHB = chronic hepatitis B; ChHC = chronic hepatitis C; ChHD = chronic hepatitis D; CPE = cytopathic effect; ELISA = enzyme-linked immunosorbent assay; HB = hepatitis B; HC = hepatitis C; HD = hepatitis D; IFN = interferon; PCR = polymerase chain reaction; PHAT = passive hemagglutination test

Socal *et al.*, 1993; Uchaikin *et al.*, 1993, 1998a,b), which is usually explained by side effects of high doses of IFN. These data served as a basis for search of new forms of IFN preparations and new modes of their administration in the treatment of viral ChH in children. In the therapy of viral ChH, we used a rectal suppository, Viferon containing recombinat alpha-2b-IFN and antioxidants.

Materials and Methods

Viferon, a rectal suppository, contained 0.5, 1.0 or 3.0 x 10^6 IU of a recombinant alpha-2b-IFN and antioxidants, vitamins E and C in therapeutic effective doses, which prolong the action of IFN (Solovyov and Malinovskaya, 1989). Good absorption of the recombinant alpha-2b-IFN from rectal suppositories into blood was proved in rabbits, volunteers and newborns with pneumonia and sepsis. In this way high concentrations of IFN are reached, which are identical to those after parenteral administration (Malinovskaya *et al.*, 1990).

Clinical characteristics of children with ChHB and ChHC. A hundred children were under observation; their age ranged between 6 months and 14 years. Diagnosis of ChH was confirmed in most children by liver biopsies. ChHB and ChHC were found in 41 and 59 children, respectively, by serological tests for viruses of hepatides B, C and D, based on enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). The disease was at acute stage in all the children. Symptoms of intoxication and vegetative asthenia of various degree of manifestation were observed in most of them during clinical and laboratory examinations; there was evidence of hepatolienal and cytolytic syndroms in all the children: activity of alanine aminotransferase (ALT) and asparagine aminotransferase (AST) increased 5–10 times according to monthly determinations. The children were divided into experimental (68) and control (32) groups. Viferon rectal suppositories were used in complex therapy of viral ChHB and ChHC. The control group was selected randomly and included 20 children with ChHB and 12 children with ChHC. They were treated conventionally, only by cholagogs, vitamins and hepatoprotectors (Carsil, Silibore, etc.).

Schemes of Viferon therapy. In complex therapy of ChH 68 children (experimental group) were treated with Viferon 3 times per week. Forty-eight children (14 with ChHB and 34 with ChHC) received individual doses of 1.0 x 10^6 IU (under 7 years of age) or 2.0 x 10^6 IU (above 7 years) of IFN for 6 months in total doses of 8.8 x 10^7 IU and 16.6 x 10^7 IU of IFN, respectively (3.0 x 10^6 IU/m^2). Three children with ChHB were treated with the same individual doses during 9 months. Twenty children (7 with ChHB and 13 with ChHC) received individual doses of 3.0 x 10^6 IU (under 7 years) and 6.0 x 10^6 IU (above 7 years) of IFN for 6 months in total doses of 16.6 x 10^7 IU and 33.2 x 10^7 IU of IFN, respectively (5.0 x 10^6 IU/m^2). Sera of the treated children were titrated for IFN antibodies.

Titration of neutralizing antibodies to IFN (IFN antibodies). Addition of a constant dose of IFN to serial dilutions of a serum is the most used method of titration of neutralizing antibodies ("method of constant dose of IFN"). Neutralizing titer is usually determined as inverse value of maximum dilution of a serum, which still neutralizes the constant dose of IFN, which is 10 IU (Kawade,1986). In titration of neutralizing antibodies we used a diploid line from cutaneous-muscle tissue of human embryo (M19) and mouse encephalomyocarditis (EMC) virus. A tested serum was serially diluted twofold in a 96-well microplate in 0.1 ml volume. Then 0.1 ml of Reaferon (8–16 U/ml, NPO Vektor, Koltsovo, Russia) was added to each serum dilution. After 1 hr of incubation an aliquot (0.1 ml) of the mixture was transferred to wells of a plate with a 24 hrs cell culture. After incubation for 24 hrs the cells were infected with 10 $TCID_{50}$ of EMC virus (0.1 ml per well) and were incubated again for 24 hrs. All incubations were performed at 37°C. The titer of IFN antibodies was determined on the basis of cytopathic effect of EMC virus.

Markers of hepatitis viruses. Replication of hepatitis B, C and D (HB, HC and HD) viruses was studied in dynamics in all patients. The following markers in blood serum were followed: HB e antigen (HBeAg), antibodies to HBeAg (anti-HBeAg), antibodies (IgM) to HB core antigen HbcAg (anti(IgM)-HBcAg), and antibodies to HD virus (anti-HDV) by ELISA (Abbott), HBs antigen (HBsAg) by a passive hemagglutinin test (PHAT, SPA Preparat, Russia), and HCV RNA by polymerase chain reaction (PCR). The latter was tested only in the case anti-HCV were present.

Indices of immune and functional status of mononuclear phagocytes. Immune status of the children was evaluated by determination of the level of T lymphocytes (CD3) and their subpopulations (CD4 and CD8) by a theophylline method (Jondal *et al.*, 1972; Shore *et al.*, 1978). Functional status of mononuclear phagocytes was evaluated according to the level of their migration (chemotactic) activity (Edelson and Cohn, 1976; Naidoo and Pratt, 1954) and concentration of the ectoferment 5-nucleotidae (5-H) in membranes of these cells by a "skin window" test according to Rebuck and Crowley (1955).

Activity of the complement system was determined according to the levels of C3 and C4 components using the method of Kolb (1979).

Results and Discussion

The children were treated with either a "low" (1–2) x 10^6 IU) or "high" dose (3–6) x 10^6 IU) of IFN in Viferon suppository. The patients' condition improved significantly after 6 or 9 months of the therapy: intoxication symptoms disappeared, liver and spleen sizes decreased, and activities of ALT and AST were reduced to normal values (Tables 1 and 2). There were no side effects in any of these patients.

Viferon therapy of children with ChHB or ChHC using the low IFN dose

Primary remission (according to the EUROHEP consensus) was registered in 11 of 14 (78%) patients, permanent remission in 3 of 8 (38%) patients and lasting remission in 9 of 11 (82%) patients after completion of the Viferon therapy of patients with ChHB for 6 months.

Table 1. Dynamics of some laboratory indices in children with ChHB treated with Viferon

Index	Healthy group	Experimental group			Control group (n=20)	
		Before treatment (n=24)	Six months after treatment			
			Low IFN dose[a] (n=14)	High INF dose[b] (n=7)	Before treatment	Six months after treatment
			Aminotransferases			
ALT (U)	>40	206.5±52.8	58.43±19.6*	62.25±21.2*	290.0±122.3	252.0±44.4
AST (U)	>40	228.0±67.3	68.0±7.8	57.25±16.7*	266.0±34.7	266.0±34.7
			T and B cell immunity			
CD3 (%)	64±7.5	38.0±2.9	42.5±3.8	44.3±2.9	36.3±1.9	46.2±4.4
CD4 (%)	38.6±8.7	27.3±7.9	29.9±5.0	26.5±7.3	29.23±7.3	28.2±2.3
CD8 (%)	18.5±5.0	11.3±3.8	11.0±3.9	11.1±5.5	11.1±4.0	10.9±5.8
B cells (%)	13.2±2.8	11.3±3.0	12.4±3.4	12.4±3.3	11.6±2.1	11.9±5.3
			Macrophages in 2nd phase AIR in the skin window test			
Chemotaxis (%)	75.0±1.1	23.2±3.1	47.7±10.1**	39.7±17.6	19.6±2.8	26.5±6.1
5-H activity[c]	20.0±5.4	4.3±2.3	8.0±1.2***	6.0±2.1	2.4±0.6	4.1±0.8
			Complement			
C3 (mg/100 ml)	64.0±9.0	42.4±6.6	60.8±2.1	62.1±4.3	41.8±6.8	44.4±7.0
C4 (mg/100 ml)	20.0±6.3	12.0±1.1	18.0±6.1	16.0±5.2	12.0±4.2	13.6±4.0
			HB virus markers			
HbsAg	All negat.	All posit.	All posit.	All posit.	All posit.	All posit.
HbeAg	All negat.	24 posit.	3 posit.	3 posit.	20 posit.	17 posit.
Anti-HbeAg	All negat.	All negat.	11 posit.	4 posit.	All negat.	3 posit.

[a](1–2) x 10^6 IU. [b](3–6) x 10^6 IU.
[c]No. of cells with positive staining.
AIR = aseptic inflammatory reaction; *p <0.001; **p <0.01; ***p <0.05.

With ChHC, primary remission was registered in 15 of 34 (44%) patients, permanent remission in 5 of 22 (23%) patients and lasting remission in 4 of 12 (33%) patients. Immediately after completion of the 9-month Viferon therapy a remission with seroconversion of HBeAg into anti-HBeAg was observed in all patients with ChHB.

In patients with ChHB or ChHC, who were treated with the low IFN dose, functional state of macrophages improved significantly (Tables 1 and 2). That was expressed in improvement of indices of their migration activity (chaemotaxis data). Also the functional state of membranes of these cells – 5-H activity, responsible for phagocitic activity of these cells (Jondal *et al.*, 1972), improved.

Accelerated seroconversion of HBeAg into anti-HBeAg was observed in the ChHB patients after Viferon therapy with the low dose of IFN during 6 months (Table 1). A similar effect of treatment of children with a recombinant alpha-IFN was reported by other authors too (Ruis Moreno *et al.*, 1990; Sokal *et al.*, 1993). The same tendency of inhibition of HB virus replication was found in adult patients treated with alpha-IFN (Wong *et al.*, 1993).

The Viferon therapy resulted also in disappearance of the HC virus RNA in 44% of the children treated for 6 months (Table 2).

Viferon therapy of children with ChHB or ChHC using the high IFN dose

In the group of children with ChHB or ChHC treated daily with the high IFN dose, primary remission was registered in 4 of 7 (57%) patients with ChHB and permanent remission in 1 of 3 (33%) patients. Among 13 patients with ChHC treated with the same IFN dose, primary remission was observed in 3 of 9 (33%) patients, permanent remission in 1 of 5 (20%) patients and lasting remission in 1 of 5 (20%) patients.

The increase of IFN dose from low to high enhanced the migration activity of macrophages in ChHC as well (Table 2). These results agree with the data on the ability of IFN to modulate the activity of macrophages reported by Black *et al.* (1987). Activated macrophages begin intensive production of IFN (Maeyer *et al.*, 1987),which is of principal importance for patients with ChHB or ChHC, who are considerably deficient in IFN. On the contrary, the increase of the IFN dose and of the duration of the treatment from 6 to 9 months did not result in significant improvement of functional status of macrophages in ChHB patients (Table 1).

Table 2. Dynamic s of some laboratory indices in children with ChHC treated with Viferon

Index	Healthy group	Experimental group			Control group (n=12)	
		Before treatment (n=47)	Six months after treatment			
			Low IFN dose[a] (n=34)	High INF dose[b] (n=13)	Before treatment	Six months after treatment
			Aminotransferases			
ALT (U)	>40	514.0±23.7	51.8±6.5*	52.25±4.2*	252.5±20.0	239.0±59.0
AST (U)	>40	213.5±86.2	42.3±6.6*	44.8±5.4***	210.0±50.0	175.0±41.0
			T and B cell immunity			
CD3 (%)	64±7.5	38.0±5.2	44.3±4.0	41.7±3.0	37.4±7.3	44.3±4.0
CD4 (%)	38.6±8.7	30.3±9.3	27.2±4.5	28.5±3.5	32.3±8.8	27.2±4.5
CD8 (%)	18.5±5.0	13.0±4.6	14.8±1.0	15.6±0.8	12.5±6.7	14.8±1.0
B cells (%)	13.2±2.8	17.3±3.2	15.3±0.54	14.4±0.3	15.7±3.3	15.3±0.54
			Macrophages in 2nd phase AIR in the skin window test			
Chemotaxis (%)	75.0±1.1	16.5±3.4	41.2±13.8**	37.8±8.6*	16.4±3.6	17.8±1.9
5-H activity[c]	20.0±5.4	4.0±0.74	7.0±1.2***	6.5±0.7	1.3±0.6	1.6±0.6
			Complement			
C3 (mg/100 ml)	64.0±9.0	36.6±4.3	48.9±7.0	50.4±5.9	38.6±4.5	40.2±5.0
C4 (mg/100 ml)	20.0±6.3	11.1±3.4	17.4±5.9	15.9±8.6	11.4±3.5	12.5±3.9
			HB virus markers			
RNA HCV	All negat.	All posit.	15 negat.	3 negat.	All posit.	All posit.
Anti-HCV	All negat.	All. posit.	All posit.	All posit.	All posit.	All posit.

[a](1–2) x 10^6 IU. [b](3–6) x 10^6 IU.
[c]No. of cells with positive staining.
AIR = aseptic inflammatory reaction; *p <0.001; **p <0.01; ***p <0.05.

An accelerated seroconversion of HbeAg into anti-HbeAg was observed in the ChHB patients after the Viferon therapy with the high dose of IFN (Table 1). On the other hand, increasing the IFN dose did not reduce incidence of HCV RNA; it was still of 23%.

Status of the humoral and cell immunity in children with ChHB or ChHC after Viferon therapy

There were no changes in the Tand B cell immunity during the Viferon therapy and further catamnestic observations in patients of all groups were independent on etiology of ChH and duration of the therapy.

We found that the Viferon therapy did not result in increase of the level of T lymphocytes and their subpopulations. However, there are data (Yoo *et al.*, 1989) on enhancement of immune mechanisms *in situ* due to treatment with alpha-IFN: number of natural killers, total contents of T cells and their subpopulations, T helpers and T suppressors (cytotoxic) in the infiltrates of portal tracts increased in the liver of ChHB patients. Also, expression (representation) of histocompatibility antigens in lymphocytes, which leads to activation of these cells was enhanced. In patients with ChHB or ChHC the treatment with alpha-IFN may decrease activity of the pathologic process and cause remission of ChH due to combined effect of activated immunological mechanisms and antiviral activity. However, the IFN therapy did not result in a positive clinical outcome in all patients.

Significant changes in levels of C3 and C4 components of complement were not found as well.

There is discussion in the literature on the possibility of appearance of antibodies to IFN in patients after treatment with a recombinant IFN. In particular, some authors (Porres *et al.*, 1989) have found IFN antibodies in serum of patients with ChHB after a completed treatment with a recombinant alpha-IFN. At the same time others (Ruis Moreno *et al.*,1990) did not find such antibodies after the same treatment of children with ChHB. According to our results production of neutralizing antibodies to the recombinant IFN present in Viferon was not observed in any patient during and after the treatment.

Immunological status of children of the control group after conventional treatment

Independing on etiology of ChH, active ChH and suppression of the functional status of macrophages, which was expressed by inhibited chemotaxis, reduced indices of

5-N activity as well as C3 and C4 components of complement were observed in the children of the control group during and after conventional therapy during the same periods of observation. The primary and permanent stability was not registered in any of 20 patients with ChHB or 12 patients with ChHC.

The results of our study show high efficiency of Viferon suppositories in complex therapy of viral ChHB or ChHC, absence of any side effects and of production of antibodies, which would neutralize antiviral activity of the recombinant alpha-2b-IFN present in Viferon suppositories. This preparation was found to suppress clearly replication of hepatotropic viruses and to reduce the activity of the pathologic process in the liver of children with ChHB or ChHC. Primary remission was registered in 78% of ChHB and 44% of ChHC patients after the Viferon therapy for 6 months with daily dose of 1–2 x 10^6 IU of IFN (3.0 x 10^6 IU/m^2).

Increasing the dose of IFN to 3–6 x 10^6 IU did not result in higher percentage of remissions. There were no side effects after Viferon administration in any patient. The method of rectal delivery has important advantages over parenteral administration in pediatrics due to its convenience, non-traumatic character and possibility to use it for prolonged periods.

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