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ORIGINAL PAPER

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Virus genotype 1b and long-term response to interferon alpha monotherapy in children with chronic hepatitis C

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Abstract Interferon alpha (IFN-α) remains the basic modality in the treatment of chronic hepatitis C in children, but the effects of therapy are still unsatisfactory. The aim of this study was to evaluate parameters linked to IFN-α response within a 2-year period. Human C virus (HCV) infected children ($n = 34$) were subdivided into IFN-treated ($n = 20$) and IFN-untreated ($n = 14$ control) groups. The IFN-treated group received a dosage 3 MU of IFN-α three times a week for 24 weeks. Liver biopsy was performed in all IFN-treated children and the HCV genotype was determined before the start of the study. Patients were sequentially screened for alanine transaminase (ALT) activity and tested for the presence of HCV-RNA in serum. All patients had either mild persistent or moderate active hepatitis, which was diagnosed from the liver biopsy. In the IFN-treated group ALT normalisation was observed by the end of treatment in 9/20 patients, but after 6 months 10 patients (50%) had sustained ALT normalisation and in 4 of them the virus was eliminated. They continued to show these features up to the end of the observation period (2 years). Eighteen out of 24 children tested had 1b genotype of virus. Out of 10 responders, all patients who were clear of HCV had the 1b genotype. The median age of responders (6.0, range 3.8–16) was significantly lower than non-responders (14.0, range 4–15) In the control group none of the children were clear of HCV-RNA. *Conclusion*: The negative predictive effect of HCV genotype 1b in the course of IFN-α treatment may be not valid in children and other features have to be taken into account in the assessment of the efficacy of therapy.

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Abbreviations *HCV* human C virus · *RNA* ribonucleic acid · *PCR* polymerase chain reaction · *ALT* alanine aminotransferase · *RBC* red blood cells · *HBsAg* hepatitis B surface antigen · *ELISA* enzyme linked immunosorbent assay · *CMV* cytomegaly virus · *IFN* interferon · *MU* mega units · *ETR* end of treatment response · *SR ALT* sustained biochemical (ALT) response · *HAI* histological activity index · *R* responders · *NR* non-responders · *U/l* units per litter · *CPH* chronic persistent hepatitis · *CAH* chronic active hepatitis · *APC* antigen presenting cells · *MHC* major histocompatibility complex · *ALL* acute lymphoblastic leukaemia · *CML* chronic myelogenous leukaemia

Introduction

Of the known types of virus mediated liver diseases hepatitis C constitutes a greatest challenge in medicine, particularly in paediatrics. According to Heintges and Wands [12], in the late 7th decade the percentage of human C virus (HCV) antibody positive blood donors ranged from 0 to1 in developed countries and 10 to 20 in developing ones. The frequency of HCV carriers in Eastern Europe is estimated at above 2 per 100,000. In Poland HCV infection is estimated at 2–4% in the general population, but the percentage of infected children seems to be lower [1]. There are thought to be approximately half a million Polish HCV+ paediatric patients. The percentage of HCV infected children in developed countries is estimated at 0.1 to 0.4 [24].

The origin of the infection of the HCV virus is almost always parenteral, occasionally perinatal, and often impossible to trace. The transmission of the virus from mother to child is most commonly seen during labour or at the time of delivery, is more frequent in the case of

high maternal viral load and the rate of transmission is in the range 3–7.8% [3, 26]. Most paediatric patients come from hospital wards after being chronically treated for cancer [6], renal insufficiency, after endoscopic procedures and those presenting with acquired and inborn immune deficits. Most infected children develop chronic hepatitis, but with a relatively mild course of the disease. The latter is usually linked to immaturity of the immune system in the first years of life [27]. Spontaneous resolution of the illness occurs in less than 10–20% of the children.

The incubation period of HCV infection varies from 6 to 12 weeks, with an average of 7 weeks. Serum HCV ribonucleic acid (RNA) can, however, be identified by a polymerase chain reaction (PCR) as early as 7–21 days following virus exposure [10]. A characteristic feature of the HCV virus infection is a symptom-free course of the disease in nearly 70% of cases, especially in the early stage of disease. In about 80% of infected patients the disease progresses to chronic hepatitis C. Clinical observations in adult patients indicate that after 20–30 years of virus "harbour," infected individuals present with liver cirrhosis in about 15–20% of cases and about 10% of patients will progress to primary liver carcinoma [11, 18].

The HCV virus belonging to the *Flaviviridae* family is a single stranded RNA virus with an average length of 9.4 kilobases, and known for the rapid replication rate with a half-life time of a few hours. The inherent feature of the HCV virus genome is a high error rate in RNA replication [19]. This is known as a quasispecies nature. This genetic instability results in the formation of genotype subtypes and pseudotypes of virus; some are known to prevail in certain areas of the world [8]. The most common HCV genotype in Poland is 1b, but not in all regions [5]. The current therapy for chronic hepatitis C in children is based either on interferon alpha (IFN-α) monotherapy or on the combination of IFN with nucleoside analogue ribavirin [9, 14, 20]. The use of IFN alone appears to produce fewer side effects and lower costs [15]. The response to the therapy is apparently linked to the of duration of the HCV infection [7].

The aim of this study was to assess the response to IFN alpha in a cohort of HCV infected children and to compare the effect of therapy on different viral genotypes.

Materials and methods

Thirty-four children took part in this study (13 girls and 21 boys, aged from 5 to 16 years, mean age 10.62 ± 4.62 years, median (range) 12.33 (3.8–16). The patients were hospitalised in the Department between 1994 and 2001.

The following criteria were observed in selecting patients for treatment:

1. Duration of HCV infection not less than 6 months
2. Presence of HCV RNA in the patient's serum
3. Increased alanine aminotransferase (ALT) activity
4. Features of chronic hepatitis in the histology of the liver biopsy
5. In all patients the anti-HCV antibody had been demonstrated.

From hospital records and data obtained from parents it was found that 8 out of 34 children had malignant disease, 6 out of 34 were subjected to surgical procedure, 7 had other diseases including patient who had been transfused with packed red blood cells (RBC) and platelets.

The presence of the anti-HCV antibody was demonstrated by the commercial enzyme linked immunosorbent assay (ELISA) Kit (Organon Teknika). HCV RNA was demonstrated by the RT-PCR test in all patients before IFN treatment. For exclusion of other aetiology of chronic hepatitis, all patients underwent serological tests for hepatitis B surface antigen (HBsAg), anti-cytomegaly virus (CMV) (IgM and IgG antibody) and toxoplasmosis (IgM and IgG). In all patients the genotyping of HCV was performed using InnoLipa HCV. ALT determination was carried out using the kinetic optimisation method before, during and after treatment. The normal values ranged from 0 to 40 U/l. In all patients routine haematological and biochemical testing was carried out including coagulation study, total protein, blood urea and creatinine. Treated patients were given 3 MU of IFN-α 2b (Intron A) three times a week for 6 months. Patients were divided into two subgroups. Twenty children were treated with IFN-α, while 14 untreated patients constituted the control group.

The mean duration of the HCV infection determined by positive antibody test up to the start of treatment was 2.44 ± 1.37 years (range 1–8 years). The total observation period of each patient was 2 years. All patients in the treated group were subjected to liver needle aspiration biopsy prior to IFN-α monotherapy. The tissues obtained were evaluated using the revised Knodell system [17].

ALT activity was checked in all patients before treatment, in 12th week of treatment, at the end of IFN therapy, and 6 months and 1 year after the termination of the treatment.

On the basis of ALT activity and/or virus clearance three types of biochemical/viral response were discerned:

1. End of treatment response (ETR) transaminase activity directly after termination of the treatment
2. Sustained biochemical response (SR ALT)—normalisation of ALT 6–12 months after termination of the treatment
3. Sustained response HCV RNA clearance of the virus after 6 months.

Eleven patients were non-responders in biochemical terms. Four of them were breakthrough cases, i.e. after an initial fall in ALT activity the patients showed an increase in this enzyme from the 12th week onwards.

Results

Study group ($n = 20$)

When treatment response was analysed in the 12th week, 10 patients out of 20 had normal ALT values.

End of treatment response

Biochemical responders included 9 patients (45%). They were divided into two subgroups:

1 – Initial responders continued to have normal ALT ($n = 7$)
2 – Initial non-responders started to respond by the 16th week of IFN treatment ($n = 2$)

The non-responders comprised 11 patients (55%). Four of them were breakthrough cases.

Table 1 Data of children from IFN-α untreated group ($n = 14$)

Age at diagnosis	Duration of illness	Gender	Association with malignancy	Genotype	ALT after 2 years
> 5 years: $n = 4$ (2 female, 2 male)	> 2 years: $n = 6$	6 female, 8 male	$n = 4$ (2 female, 2 male)	1b: $n = 6$ (3 female, 3 male)	
5–10 years: $n = 5$ (2 female, 3 male)	2–3 years: $n = 8$			1a: $n = 7$ (3 female, 4 male)	N (in 5)
< 10 years: $n = 5$ (2 female, 3 male)				3a: $n = 1$ (1 female)	< N (in 9)

Table 2 Liver histology in responders and non-responders

Treated patients group	Moderate chronic active hepatitis (%)	Mild chronic persistent hepatitis (%)
Responders	5/10 (50)	5/10 (50)
Non-responders	7/10 (70)	3/10 (30)

Table 3 Human C virus (HCV) genotype of responders (*R*) and non-responders (*NR*) for interferon alpha (IFN-α) treatment

Group	1b (%)	1a (%)	3a (%)	1b (%)	1a (%)	3a (%)
2R	6/10 (60)	4/10 (40)		6/14 (43)	7/14 (50)	1/14 (7)
NR	6/10 (60)	3/10 (30)	1/20 (5)			

Sustained biochemical response

Ten patients (50%) had normal ALT values after 6 months and also 12 months after the termination of IFN therapy . Two patients had raised aminotransferase activity during and after treatment but during the subsequent period they showed normalisation of ALT activity.

Sustained response

Four patients out of 20 were clear of HCV RNA (20%) after 6 months as judged from the RT-PCR data.

Control group ($n = 14$)

Five patients had normal ALT values from the beginning up to the end of the study. Nine patients had either slightly increased or fluctuating ALT values during the observation period. Out of the 14 patients in the control group none were clear of HCV RNA (Table 1).

Liver histology

Liver biopsies were assessed using the criteria of the Knodell scoring system. Determination of the histological activity index (HAI) allowed each patient to be given a particular histological diagnosis (Table 2). It was found that patients could be defined either as having:

n = 8 (HAI: 4–8)
n = 12 (HAI: 9–12)

Genotypes of treated and untreated patients

Genotyping of HCV RNA from patients studied has revealed that the following three genotypes were demonstrated (Table 3):

When the genotype of virus was compared between responders (SR) and non-responders within the IFN treated group of patients there was no major difference between the two groups.

The detailed data of children in the IFN treated group are shown in Table 4. It can be seen that of the patients who showed a complete response with viral elimination, 3 had moderate CAH, while 1 presented with mild CPH. All were free from malignant disease.

Clinical data

The mean age of the children who responded to therapy and had normal ALT levels 2 years after the termination of IFN treatment was 9.06 ± 4.9 years. The respective value of those classified as non-responders was higher: 12.4 ± 3.3 years ($p = 0.05$). The median value for responders was 6.0, range 3.85–16, while for non-responders it was 14.0, range 4–15.

When the mean duration of HCV infection prior to IFN therapy was compared in responders and non-responders, it was found that in responders it was slightly shorter (2.61 years) than in the non-responders (2.9 years). There were no major differences between responders and non-responders with regard to gender and incidence of malignancy (Table 4).

Interferon was generally well tolerated by most patients and therapy was not discontinued in any of the patients. Observed side effects of mild intensity included transient thrombocytopenia and leucopenia. In 1 patient hair loss was noted.

Discussion

The results of this study show that sustained late response to IFN-α treatment is ambiguous in HCV infected children, in spite of the fact that all the children studied in the current trial were treated with the same

Table 4 Combined data of IFN-α treated children. *PCR* polymerase chain reaction, *ALT* alanine aminotransferase, *CAH* chronic active hepatitis, *CPH* chronic persistent hepatitis, *CML* chronic myelogenous leukaemia

Patient's number, gender	Age (years) at start of therapy	Duration of illness prior to IFNα (years)	Genotype	Past history of malignancy	Liver histology	PCR 6 months after therapy	ALT after 2 years
2, female	6	3	1b	No	Moderate CAH	Negative	N
4, female	13	3	1b	No	Moderate CAH	Negative	N
8, male	16	2	1b	No	Mild CPH	Negative	N
9, male	5	4.77	1a	No	Moderate CAH	Positive	N
10, male	8	2	1b	No	Mild CAH	Negative	N
11, female	15.25	1.25	1b	CML	Mild CPH	Positive	N
14, male	5	1	1a	No	Mild CAH	Positive	N
17, male	14	3	1a	Lymphoma	Moderate CAH	Positive	N
18, female	3.83	2.25	1b	No	Mild CPH	Positive	N
19, male	4.5	3.92	1a	No	Moderate	Positive	N
1, female	14	1	1b	No	Moderate CAH	Positive	H
3, male	15	4	1b	No	Mild CPH	Positive	H
5, male	12	8	1b	No	Moderate CAH	Positive	H
6, female	13.75	1.75	1a	No	Mild CPH	Positive	H
7, male	4	3	1b	No	Moderate CAH	Positive	H
12, female	15	1.33	1b	No	Moderate CAH	Positive	H
13, male	12.33	1.92	1a	ALL	Moderate CAH	Positive	H
15, male	14.17	1.66	1a	Nephroblastoma	Moderate CAH	Positive	H
16, male	14.25	3	3a	No	Mild CPH	Positive	H
20, male	10	3	1b	No	Moderate CAH	Positive	H

ALT N <40 U/l; ALT H >100 U/l

IFN dose and all had a relatively mild course of the disease. Two years after termination of IFN therapy, they could be stratified into three types of response: those in whom ALT activity normalised but the virus was not eliminated ($n=6$); those in whom ALT activity was normalised and who were clear of the virus ($n=4$); and those in whom the virus was not eliminated and ALT activity was raised ($n=10$).

The explanation of the above-mentioned facts is far from clear, but in terms of immunogenetics two options should be considered:

1. Links between the major histocompatibility complex (MHC) of the patient and their antiviral immune response to IFN therapy. It is well known that the quality of the presentation of epitopes of viral antigens to T lymphocytes depends on genetic make-up of antigen presenting cells (APC) and in particular on the structure of the peptide binding groove formed by amino acids of the MHC molecule of the infected individual. Polymorphism of the alleles of the MHC has a major impact on the coded MHC molecule and thus on the cell mediated anti viral immune response [22]. Unfortunately, the MHC of the IFN treated children was not determined in the current study.
2. The effect of the HCV genotype. The genotype of the virus was determined in both IFN treated and untreated children. Most (18 out of 34) belonged to the 1b genotype. Both responders and non-responders in the IFN treated group shared this genotype equally (6/10). Interestingly, however, all the patients in whom the virus was eliminated also had the 1b genotype. This is at odds with the generally accepted view that patients harbouring the HCV 1b genotype have a worse prognosis because of the more severe damage to the hepatocytes [13]. The opinion is also held that the 1b genotype is associated with a worse response to treatment [4]. Our results suggest that this is not always the case. One possible explanation of this is that it may be due to the age of patients and the initial extent of liver damage. The conviction about the poor follow-ups of patients infected with the 1b variant of HCV was based on experience with adult patients, whereas our study involved children aged 3–16 years. It is known that paediatric patients may even eliminate HCV without specific treatment [25]. Besides, relatively mild liver damage, as evidenced by the histology of the biopsy specimens, may result in a fairly good response to IFN-α treatment in spite of the HCV 1b genotype. The competent immune system of paediatric patients may also contribute to the final result [21]. A sustained ALT response was seen in 50% of cases in our group of patients , while in adults the corresponding percentage is 8–9% [23].

It is remarkable that 3 of the 4 patients in whom the virus was eliminated had moderate chronic hepatitis. Some authors have suggested that sustained response is more common in those patients who show more advanced liver lesions [2], but this is not the general opinion. Liver fibrosis is apparently an unfavourable factor for predicting response to interferon treatment [16].

Sustained biochemical response was seen in children aged 3.8–16 years (mean 9.06 ± 4.9), while the mean age of non-responders was 12.4 (± 3.3 years). Likewise, the mean duration of the HCV infection was shorter in responders than in non-responders, albeit not significantly. These findings support the notion that young age

of the patient and the short duration of the HCV infection are apparently good predictive factors for therapy [7].

To conclude, our results suggest that sustained response to interferon-α therapy in HCV infected children is hard to predict and even the HCV 1b genotype does not exclude virus elimination in the long-term follow-up of patients.

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