Effect of interferon alfa and ribavirin treatment on hepatitis C virus RNA in serum and peripheral blood mononuclear cells in children with chronic hepatitis C

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Abstract

The correlations between the severity of hepatic lesions, age, gender, HBV co-infection and negativisation of HCV-RNA from serum and peripheral blood mononuclear cells (PBMC) after treatment of chronic hepatitis C (CHC) were analysed. 41 children (11 F/ 30 M), aged 5-16 years (mean 10 ± 2.8), were treated with IFN-α and ribavirin for 12 months. Sustained negativisation of HCV-RNA from serum was achieved in 25 patients (61%), in 3 (7%) it reappeared after treatment, and in 13 (32%) it was ineffective. Clearance of HCV did not correlate with age (p = 0.65), sex (p = 0.13), past HBV infection (n = 22 anti-HBc +) (p = 0.24), maximum pre-treatment ALT activity (p = 0.06), grade of inflammation (p = 0.33) or stage of fibrosis (p = 0.9) in liver biopsy. It was achieved in 6/16 children previously resistant to IFN-α monotherapy and in 19/25 naïve (p = 0.017). HCV-RNA was detected in PBMC in 9/24 (37%) seronegative children and in 1/21 (5%) in comparative group of seronegative adults ; p = 0.004. Persistence of HCV-RNA in PBMC after combined treatment occurred in 5/10 (50%) patients resistant to previous IFN-α monotherapy, 6/35 (20%) of them cleared HCV from PBMC (p = 0.04). Conclusions : Age and gender, infection route, history of HBV infection or severity of histopathologic liver lesions had no influence on the efficacy of treatment with IFN-α and ribavirin. Clearance of HCV from serum and from PBMC occurs less frequently in patients previously resistant to IFN-α. Children with CHC require longitudinal observation after successful antiviral treatment as in 37% of those considered to be free from the virus by ordinary measures, HCV-RNA was found in PBMC. (Acta gastroenterol. belg., **2006**, 69, **187-190**).

Key words : hepatitis C, HCV, treatment, interferon alfa, ribavirin, peripheral blood mononuclear cells.

Introduction

Chronic hepatitis in children is characterised by different course and prognosis from the same disease in adults (1,2,3). Vertical hepatitis C virus (HCV) transmission from a carrier mother is asymptomatic, with normal or only slightly elevated alanine aminotransferase (ALT) activities and periodically undetectable HCV-RNA in serum (4). Infection by transfusion of blood or blood products led, according to Vogt *et al*. (5) to spontaneous elimination of HCV within ten or more years in 50% of children infected via this route. The two aforementioned cohort studies confirm previous reports concerning hepatitis C in children from various clinical centres all over the world : in Italy (6), Taiwan (7), and Australia (8). All these studies emphasise the clinically asymptomatic course of chronic hepatitis C in children, normal or only slightly elevated aminotransferase activities, high spontaneous serum HCV-RNA negativization rates. Also histopathologic investigations of livers in HCV-infected children demonstrate low activity of inflammatory changes as well as small extent of liver fibrosis (6,9,10), whereas the percentage of adults with hepatitis C related cirrhosis reaches even 40% (11). According to current views, it is generally accepted that the clinical and biochemical course is milder and the prognosis more favourable in children than in adults infected with HCV (12). However, in each case of diagnosis of hepatitis C infection liver biopsy should be mandatory, as cases of hepatitis C related cirrhosis are seen even in small children (10,13,14). The analysis carried out in the Clinic of Infectious Diseases, Medical University of Lodz, indicated that the percentage of children with hepatitis C demonstrating grade of fibrosis S3 (piecemeal necrosis with architectural distortion) and S4 (cirrhosis of the liver) reached 15% (13). The need for and regimes of use of antiviral drugs in children are disputable not only because of mild course of the disease, but in view of very limited efficacy of such treatment in adults. There is a consensus among specialists that any attempts of such treatment should be undertaken only after detailed hepatological diagnostics and within the framework of controlled clinical studies (15,16,17).

Aim of the study

The aim of the study was to analyse the frequency and prognostic factors of favourable outcome of combined treatment with interferon alfa (IFN-α) and ribavirin in children with chronic hepatitis C.

Patients and methods

Between the years 1999 and 2003, 41 children with chronic hepatitis C were treated in the Clinic of Infectious Diseases and Hepatology, Medical University of Lodz, with combined antiviral therapy regimen.

Diagnostic techniques for HCV-RNA detection

HCV-RNA was detected in serum by reverse transcription polymerase chain reaction (RT-PCR) (Cobas, Amplicor HCV Monitor, version 2.0, Roche). For

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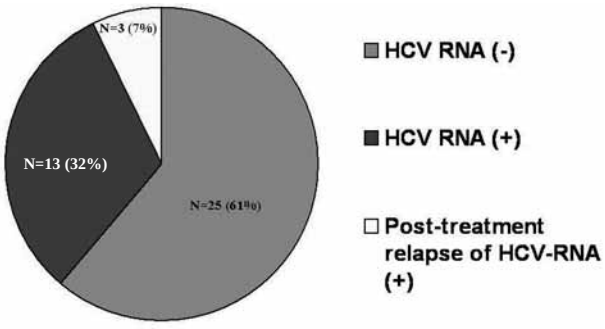

Fig. 1. — Efficacy of treatment with interferon alfa and ribavirin in 41 children.

detection of HCV-RNA PBMC were isolated from 5 ml of fresh blood by centrifugation in density gradient Histopaque1077 (Sigma) in room temperature at 400 G. The serum equivalent of peripheral blood mononuclear cells (PBMC) was collected and stored in – 20°C. RNA was isolated from cells using guanidine tiocyanate extraction. HCV RNA was detected simultaneously in RNA isolated from serum and PBMC by one tube RT and PCR as described by Dulak *et al.* (20) followed by nested PCR reaction based on amplification of cDNA from 5'region of viral genome. Reactions were carried out in UNO II (Biometra, Germany).

Inclusion/exclusion criteria

HCV infection had to be documented for at least 6 months prior the biopsy and the commencement of treatment. Thick-needle liver biopsy was performed and assessed using 0 to 4 score scale presented by Desmet and Scheuer with the inflammatory activity index (G) and fibrosis grade (S), (18,19).

The children with positive HBsAg (hepatitis B virus surface antigen) indicating HBV (hepatitis B virus) and HCV co-infection were excluded from this evaluation. Past hepatitis B was established on the basis of anti-HBc (anti-hepatitis B core) IgG present in blood serum. Both HBsAg and anti-HBc IgG were determined by ELISA (Organon Teknika, Boxtel, NL).

Detailed history including blood transfusions and administration of blood products was collected. The characteristics of children included in the study are presented in table I.

The treatment of chronic hepatitis C involved administration of IFN-α 3 MIU (s.c.) t.i.w. and ribavirin (p.o.) at 15 mg/kg b.w. daily doses, for 12 months. Alanine and aspartate aminotransferase (ALT and AST) activities were assessed before the commencement of treatment, then at 8-week intervals during the treatment period, 2-4 weeks after the completion of treatment and six months later. HCV-RNA in serum was determined before treatment, in the 4th to 6th month of treatment, immediately after its completion and six months later. The expected result of treatment was elimination of HCV-RNA from serum and normalisation of ALT activity, sustained also in the last post-treatment tests and six months after completion of the therapy. In children who achieved this, persistence of HCV-RNA was assessed in PBMC.

Table I. — **Characteristics of 41 children with chronic hepatitis C treated with interferon-alfa and ribavirin**

Duration of HCV infection (years)		
	Range from-to	2-14
	Mean ± SD	7 ± 3
HBV infection (anti-HBc +)		
	Confirmed	22
	Excluded	19
Probable infection route		
	Transfusion of blood preparations	16
Liver biopunctate histopathology results		
	S1-S2	11
	S3-S4	30
Maximum noted ALT activity (U/l)		
	Range from-to	40-442
	Mean ± SD	131 ± 96
Antiviral treatment prior interferon alfa + ribavirin		
Interferon alfa monotherapy		16
"Naive" patients		25

The results of HCV-RNA persistence in children were compared with similar group of 21 adults with chronic hepatitis C, seronegative after antiviral treatment, with no underlying chronic diseases. The groups of children and adults were similar in the respect of male/female ratio, liver histopathology, treatment regimes, ALT activities before treatment as shown in table II.

The study was approved by the local Study Review Board. In all cases written consent was obtained from the children's parents or legal representatives.

Statistical analysis was carried out using Statgraphics plus for Windows software package by means of Student-t test. The value of $p \leq 0.05$ was regarded as statistically significant.

Results

Forty one children (11 girls and 30 boys), aged 5-16 years (mean 10 ± 2.8 years), were treated with combined therapy.

The treatment was effective in 25 (61%) children. In 3 (7%) HCV-RNA disappeared from serum in the course of treatment, but reappeared after its completion, whereas in 13 (32%) no effect was obtained. The efficacy of treatment was independent of age ($p = 0.65$) and gender ($p = 0.13$). Transfusion of blood or blood products in 16 (39%) was a probable infection route, it did not affect the outcome of treatment. Past HBV infection (anti-HBc +, HBsAg-) was detected in 22 children, but it did not have any effect on the results of the therapy ($p = 0.24$). No correlation was found between the severity of inflammatory changes (G) or fibrosis grade (S) in liver

Table II. — **Comparison of children and adults groups, seronegative after IFN-α + ribavirin treatment, checked for HCV-RNA persistence in blood peripheral mononuclear cells**

	Adults (N = 21)		Children (N = 24)
Age (years) ; range Mean ± SD	22 – 51 40 ± 8	P < 0.001	6 – 17 12 ± 2
Male/female ratio Time after treatment (years) Median ALT before treatment (U/l) Mean ± SD	10/11 0,5-5 1 52-125 91 ± 32	P = 0.5 P = 0.005 P = 0.6	13/11 1-3 2 20-260 74 ± 67
Past ineffective IFN-α monotherapy	3 (14%)	P = 0.1	6 (25%)
Histopathology : S1-S2 S3-S4	 16 5	 P = 0.4	 18 6

Table III. — **Comparison of chronic hepatitis C patients, who were seronegative after antiviral treatment, according persistence of HCV-RNA in PBMC**

	HCV-RNA in PBMC (-) N = 35		HCV-RNA in PBMC (+) N = 10
Male/female ratio ALT before treatment (U/l) Mean ± SD	16/19 20-125 62 ± 34	P = 0.3 P = 0.03	6/4 45-260 109 ± 77
Past ineffective IFN-α monotherapy	6 (20%)	P = 0.04	5 (50%)

biopsy specimens and the effect of treatment (p = 0.33 and p = 0.9, respectively). The highest pre-treatment serum ALT activity value noted had no effect on the final outcome of the combined therapy (p = 0.06).

Sixteen children had been previously treated with IFN-α monotherapy, but only in 6 of them (37%) the treatment led to HCV-RNA elimination as a result of following combined therapy, whereas sustained HCV-RNA clearance was obtained in 19 out of 25 (76%) so-called "naïve" (previously untreated) patients. The difference was statistically significant (p = 0.017).

Twenty-four seronegative children have been investigated for HCV- RNA in PBMC, 9/24 (37%) were found positive ; in comparative group of seronegative adults 1/21 (5%) was HCV-RNA in PBMC positive ; p = 0.004.

To find clinical features influencing HCV-RNA persistence in PBMC we have stratified all 45 patients (24 children and 21 adults) in two groups : 10 positive and 35 negative. This comparison is presented in table III. Apart from the young age, positive results of HCV-RNA in PBMC were correlated with lack of response to previous IFN-α treatment : 6/35 (20%) of such patients were found HCV-RNA in PBMC negative, while 5/10 (50%) were positive ; p = 0.04. Patients with higher ALT before the commencement of treatment were more likely to remain HCV-RNA in PBMC positive (p = 0.03).

Discussion

Treatment of chronic hepatitis C with interferon alpha and ribavirin was introduced in 1996 (21) and has become the standard method in adults (22). Serum HCV-RNA negativation was obtained in 30 to 50% of patients. Attempts of such treatment have also been undertaken in children, however, because of small group sizes, they were performed in the course of uncontrolled clinical trials (23,24), with efficacy slightly better than in adults (up to 60%) (25). Combined therapy in our analysis was less effective in children previously treated with interferon alfa monotherapy. A similar correlation was found in adult patients (26,27). It results from the fact that the disease is most probably caused by a HCV strain resistant to antiviral drugs. Viral genotype (infection with genotype 1b results in worse treatment response), HCV viremia level > 2×10^6 copies/ml and duration of HCV infection influenced treatment results (27,28). In recent years pegylated interferon (PEG-IFN) was found to be more effective than the standard one and it was approved in combination with ribavirin for the treatment of adults (29,30). Trials concerning the use of PEG-IFN in children are still in progress (16).

Elimination of HCV-RNA from serum is an expected effect of treatment (1,2,31), but the virus is able to persist in cells (hepatocytes, peripheral blood mononuclear cells) after seroconversion. Assessment of HCV-RNA in peripheral blood leukocytes (PBMC) may enhance the sensitivity of diagnosis of treatment response (32,33). Negativation of HCV-RNA from serum is in children more frequent than in adults but our data point out that many of these young seronegative patients may be carriers of viral RNA in PBMC. The relationship between positive HCV-RNA in serum or in the hepatocytes and the liver injury is well documented (34), but it is not known if persistence of HCV-RNA in other cells has any influence on the long-term outcome of patients with chronic hepatitis C (35). Analysis by Laskus *et al.* (36) revealed the possibility of reinfection of the transplanted liver by

PBMC-derived HCV, proving that as long as hepatitis C virus is not eliminated completely from an organism, recurrence of active disease is possible.

Conclusions

1. Elimination of HCV-RNA from serum and normalisation of ALT activity was achieved in 61% of children with chronic hepatitis C treated with IFN-α and ribavirin, which is similar to other studies.
2. Age and gender, infection route, history of HBV infection or severity of histopathologic changes in the liver bioptate had no influence on the efficacy of treatment with IFN-α and ribavirin.
3. Lack of response to IFN-α monotherapy does not exclude the chance of positive results of combined therapy. However, the effectiveness of such treatment is significantly lower in patients previously treated with interferon monotherapy than in untreated ones (37% and 76%, respectively ; p = 0.017). In seronegative children from this group HCV-RNA can be found in PBMC more frequently than in previously untreated ones (50% versus 20% ; p = 0.04).
4. In 37% of children who were seronegative after treatment, HCV-RNA persisted in PBMC. This phenomenon was significantly more frequent in children than in adults (37% versus 5% ; p = 0.004).
5. Children with chronic hepatitis C should be longitudinally monitored for persistence of HCV-RNA after successful antiviral treatment.

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