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Lymphoblastoid interferon alfa treatment in chronic hepatitis C

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Abstract

Interferon is becoming the standard treatment in adults for chronic hepatitis C. Twenty one children with histologically proved chronic hepatitis C (10 boys, range 2·5–13 years), who were otherwise healthy, were enrolled in a randomised controlled study to test their response to interferon alfa. Eleven children were treated with lymphoblastoid interferon alfa (3 million units/m^2) for 12 months; 10 children received no treatment. All had raised transaminases and positive antihepatitis C virus (HCV) antibodies and HCV-RNA.

Alanine aminotransferase (ALT) serum levels became normal in five (45%) treated patients after a mean of three weeks (range 1–6 weeks) and no relapse had occurred by the end of follow up (30th month). Only one (10%) untreated patient had normal ALT serum levels from the 11th until the 30th month. Disappearance of serum HCV-RNA, persisting throughout the follow up period, was observed in the six children (five treated) whose ALT became normal. Biopsy specimens in treated patients showed a significant improvement in Knodell's score (median (SD) basal 9·0 (2·2); final 2·0 (0·4)). Interferon treatment was well tolerated in all. This study confirms the efficacy of interferon in children with chronic hepatitis C, not only by restoring normal ALT serum levels, but also viral clearance and histological amelioration of liver inflammation. Contrary to reports in adults no biochemical and virological relapses occurred in responder children.

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Hepatitis C virus (HCV) infection is characterised by a high chronicity rate (about 90% of infected subjects) and by propensity to cirrhosis and hepatocellular carcinoma.[1–5] Although children with chronic hepatitis C do not have severe clinical and histological findings, spontaneous remission is uncommon.[6 7] It is not known whether the minor histological lesions in children are related to the shorter duration of disease or age related host response to infection.[6] Because of its antiviral and immunomodulatory properties, interferon is a promising therapeutic agent for chronic hepatitis C.[8] In adults, where the efficacy of interferon treatment has been well documented, short duration of disease, young age, absence of cirrhosis, and mild histological lesions are considered predictive factors in response to treatment.[9 10] On this basis, children with chronic hepatitis C should be ideal candidates for interferon treatment. The present study was undertaken to evaluate, in a randomised controlled trial, the efficacy of lymphoblastoid interferon alfa treatment in children affected by chronic hepatitis C without underlying chronic systemic diseases.

Patients and methods

Forty five consecutive patients with HCV infection, aged 1–14 years (27 boys, 18 girls), attending the liver unit of the department of paediatrics of the University 'Federico II' of Naples from January to July 1992, were considered for inclusion in the trial if they met the following criteria: age between 2 and 14 years; anti-HCV antibodies detected by a second generation enzyme linked immunosorbent assay (ELISA) and confirmed by a second generation recombinant immunoblot assay; raised alanine aminotransferase (ALT) serum levels for at least six months before enrolment; histological evidence of chronic hepatitis; absence of underlying chronic systemic diseases or other liver diseases; and no clinical or biochemical evidence of autoimmunity. Exclusion criteria included a previous course of antiviral or immunosuppressive treatment; advanced or decompensated cirrhosis; presence in the serum of hepatitis B surface antigen and/or antibodies to HIV.

Twenty one children (10 boys, median age 7·5 years, range 2·5–13 years) met the criteria and were enrolled in the study. All were symptom free and none had a history of acute symptomatic onset of liver disease. On physical examination, 15 (71%) had mild hepatomegaly. The study was approved by the hospital ethics committee, and written parental consent was obtained. Using computer generated randomisation tables the patients were assigned to two groups: 11 (group 1) received lymphoblastoid interferon alfa for 12 months and 10 (group 2) received no treatment. Enrolled patients' clinical state was evaluated monthly during the treatment period, and thereafter every three months for 18 months. At the same times, the following investigations were performed: full blood count, ALT and aspartate aminotransferase, bilirubin, γ-glutamyltransferase, albumin, total protein, prothrombin time, creatinine, and serological markers of HCV infection. HCV-RNA analysis was preformed by polymerase chain reaction at enrolment and after

six, 12, 18, 24, and 30 months. Genotyping was performed and interpreted according to the method of Okamoto *et al*[11] in all patients using paired serum samples taken at entry and after 30 months. Furthermore, in the treated patients, ALT serum levels and full blood count were monitored weekly for the first two months of treatment. As both HCV related chronic liver disease and interferon treatment may be associated with autoimmunity, cryoglobulinaemia, and thyroid diseases,[12–16] the children were tested, before enrolment and at intervals of six months, for antinuclear, anti-smooth muscle and antiliver-kidney microsomal autoantibodies, cryoglobulins, total thyroxine, free thyroxine, tri-iodothyronine, free tri-iodothyronine, and thyroid stimulating hormone.

LIVER BIOPSY STUDIES

All patients had a liver biopsy within the six months preceding entry. Follow up biopsy was performed 24 months after enrolment in treated patients only. For ethical reasons a second biopsy was not performed in untreated patients. All biopsy specimens were evaluated, according to the European classification of chronic hepatitis,[17] and graded with respect to degree of periportal, portal and lobular inflammation, and fibrosis according to the Knodell scoring system.[18] Improvement or deterioration were defined as a difference of at least two points between the total scores for two biopsy specimens from the same patient.

TREATMENT SCHEDULE AND DOSE MODIFICATIONS FOR SIDE EFFECTS

The dose of lymphoblastoid interferon alfa was 3 million units/m^2 of body surface subcutaneously three times a week for 12 months. The dose was halved when neutrophil count was less than $1{\cdot}5\times10^9$/l and/or platelet count was less than 80×10^9/l, and stopped when the neutrophil count was less than $1{\cdot}0\times10^9$/l and/or platelet count less than 50×10^9/l. Treatment was reinstituted in a stepwise fashion, beginning with a maximum of 50% of the last administered dose. Interferon alfa was suspended if transaminases were more than six times the upper reference limit or if there was no reduction of ALT serum levels after six months of treatment.

DEFINITIONS OF TREATMENT RESPONSE AND RELAPSE

A biochemical response to treatment was defined as ALT normalisation occurring during the treatment and persisting until the suspension of interferon alfa treatment. A virological response to treatment was defined as disappearance of serum HCV-RNA occurring during the treatment and persisting until the suspension of interferon alfa treatment. Patients with persistent or fluctuating hypertransaminasaemia were defined as non-responders. A biochemical relapse was defined as an increase in the ALT level to more than 1·5 times the upper normal value after a biochemical response, a virological relapse was defined as the reappearance of serum HCV-RNA after a virological response.

ASSAYS

Aminotransferase serum concentrations were evaluated by standard methods. Serological markers of HCV infection were detected with a second generation ELISA (ELISA-2, Ortho Diagnostic Systems) and reactive samples were further tested by a second generation recombinant assay for anti-HCV (RIBA-2, Ortho Diagnostic Systems). HCV-RNA was detected by a nested polymerase chain reaction (PCR) involving primers deduced from the 5′-noncoding region of the HCV genome.[19] HCV genotypes were identified by primer specific reverse transcription PCR based on the HCV core region.[11]

STATISTICAL ANALYSIS

Results were expressed as mean (SD), if not indicated differently. Statistical analysis was performed with Fisher's exact test, Wilcoxon signed rank test, Mann-Whitney test, and Student's *t* test, when appropriate. A p value $<0{\cdot}05$ was considered significant.

Results

At entry, treated and untreated patients were comparable for age, sex, route of infection, duration of disease, and clinical, biochemical, virological, and histological features (table 1). Although fluctuations of ALT were observed in all patients before enrolment, none had normal levels for longer than two months. Eight of the 11 treated patients received interferon treatment for 12 months; the mean total amount of interferon administered was 93·1% of the scheduled dose (range 81–100%). Treatment was discontinued during the first month in a girl because of ALT levels more than 18 times the upper reference limit and at the sixth month in two patients who showed no reduction in ALT. Biochemical response was observed in five (45%) treated patients and only in one (10%) untreated patient ($p=0{\cdot}0936$). The normalisation of ALT serum levels in the five treated patients occurred after a mean time of three weeks (range 1–6 weeks) and persisted until the end of follow up (30th month), while in the untreated patient it occurred at the 11th month and persisted until the 30th month. A virological response was observed in the five biochemical responders of group 1 and persisted throughout follow up. Among children in group 2, the only patient whose ALT became normal also showed clearance of serum HCV-RNA from the 18th until the 30th month. All enrolled patients showed persistently positive serum anti-HCV without changes in RIBA-2 pattern. In 16 patients whose serum HCV-RNA did not clear, HCV genotype was identical at entry and at the end of follow up. The figure shows the profile of

Table 1 Epidemiological, clinical, biochemical, and histological features of treated (group 1) and untreated (group 2) patients

	Treated (n=11)	*Untreated (n=10)*
Median (range) age (years)	6·2 (2·5–10·4)	7·7 (3·0–13·0)
No (%) boys	7 (63)	4 (40)
Route of infection; No (%)		
History of transfusion	5 (45)	7 (70)
Vertical transmission	3 (27)	1 (10)
Minor surgical procedure	2 (18)	2 (20)
No overt exposure	1 (9)	0
Mean (SD) duration of chronic hepatitis C (months)*	59·3 (33·9)	56·0 (32·3)
Mean (SD) ALT (U/l)	140·0 (102·0)	127·8 (100·3)
RIBA-2 pattern; No (%)†		
Anti-c22-3+	1 (9)	2 (20)
Anti-c22+/c100-3+/5-1-1+	1 (9)	1 (10)
Anti-c22+/c33c+/c100-3+/5-1-1+	9 (81)	7 (70)
Genotype; No (%)		
1a	2 (18)	3 (30)
1b	5 (45)	4 (40)
2	2 (18)	2 (20)
3	2 (18)	1 (10)
Histological features; No (%) except Knodell score		
Minimal hepatitis	2 (18)	0
Chronic persistent hepatitis	2 (18)	3 (30)
Chronic active hepatitis	7 (63)	7 (70)
Cirrhosis	0	0
Median (SD) Knodell score	7·0 (2·9)	7·4 (2·6)

*Duration of disease is expressed as the time elapsed since the presumed exposure to contaminated blood for patients infected parenterally, as the time since birth for patients infected vertically, and as the time since first observation of an abnormal ALT level for patients with no overt source of infection.
†RIBA-2 pattern: anti-c22+ indicates subjects positive for antibodies to c22 protein; anti-c22+/c100+/5-1-1+ indicates subjects positive for antibodies to c22, c100, and 5-1-1 proteins; anti-c22+/c33+/c100+5-1-1+ indicates subjects positive for c22, c33, c100, 5-1-1 proteins.

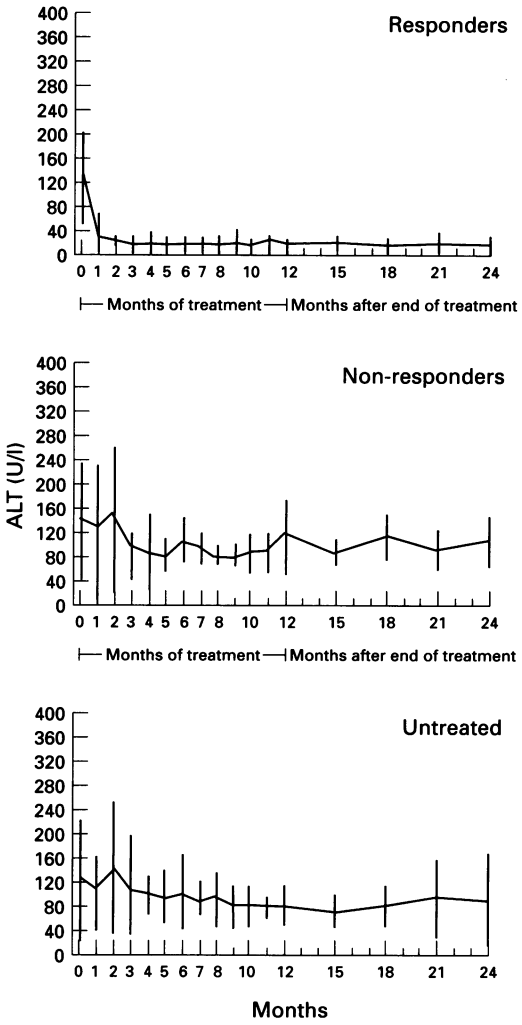
serum ALT. Other liver function tests were normal in all patients. All treated patients (whether responders or non-responders) showed a significant improvement in total Knodell's score (p=0·03), periportal necrosis (p=0·01), and portal inflammation (p=0·03) on follow up biopsy. Table 2 shows the changes in histological activity, separately for responders and non-responders. Table 3 shows that responders and non-responders did not differ in epidemiological, clinical, biochemical, virological, and histological features.

SIDE EFFECTS

Soon after the start of treatment, a transient influenza-like syndrome was observed in all patients; subsequently, anorexia, asthenia, irritability, headache, and abdominal pain were the most common observed side effects. However, interferon treatment was generally well tolerated and the quality of life did not deteriorate. Neutropenia requiring tapering of interferon dose was observed in five patients. There was no evidence of autoimmunity, cryoglobulinaemia, and thyroid disorders in any patient, including the one whose treatment was suspended because of raised transaminases.

Discussion

The natural history of childhood chronic hepatitis C is unknown because of a lack of longitudinal studies with long term follow up. Recently it has been reported that the disease is silent in most children[6 7] and, in the short term, few spontaneously remit.[7] The natural history in



Mean (SD) ALT serum levels (normal values <40 U/l) in treated (responders and non-responders) and in untreated patients during a 30 month period.

adults suggests that it may evolve insidiously to cirrhosis and hepatocellular carcinoma.[2 5 20] In adults interferon alfa may induce a sustained biochemical and virological response associated with amelioration of liver inflammation.[8] Little information is available about the efficacy of this treatment in childhood. Ruiz-Moreno *et al* in a pilot uncontrolled study demonstrated the efficacy of interferon alfa in children chronically infected by HCV,[21] but serological features of the studied patients were not representative of children affected by chronic hepatitis C. In fact, seven of the 12 studied patients were HCV-RNA positive but anti-HCV negative. This pattern is not typical of immunocompetent patients and indicates underlying immune defects.[22 23] Furthermore, in this study serum ALT in anti-HCV negative/HCV-RNA positive patients were characterised by significant peaks with respect to the basal values until the third month of treatment. This pattern of serum ALT has never been described in chronically HCV infected patients during interferon treatment.[24] Therefore, despite the small number of patients, our study represents the first controlled trial of interferon in children affected by chronic hepatitis C without underlying chronic systemic disease. Although differences in biochemical and virological response between treated

Table 2 Changes in liver histological activity; scores are median (SD)

Biopsy	*Total Knodell score*	*Periportal necrosis*	*Portal inflammation*	*Lobular inflammation*	*Fibrosis*
Responders					
Basal	9·0 (2·2)*	3·0 (1·3)*	3·0 (0·5)*	1·0 (0·8)	2·0 (0·5)
Final	2·0 (0·4)*	1·0 (1·2)*	1·0 (0)*	0 (0·4)	1·0 (0·5)
Non-responders					
Basal	5·0 (2·9)	1·0 (1·2)	2·0 (1)	1·0 (0)	1·5 (0·8)
Final	4·0 (3·4)	1·0 (1·1)	1·0 (1)	1·0 (0·6)	1·5 (1)

*p=0·04.

and untreated children were not statistically significant, interferon alfa treatment produced a very early and striking reduction of serum ALT in responders, sustained clearance of serum HCV-RNA and an impressive improvement in liver histology. Although long term outcome of these patients is uncertain, it seems reasonable to assume that – at least in responders – interferon treatment may reduce the rate of late complications of HCV infection.

Ideally, a significant effect on morbidity and mortality related to HCV infection would be the best determinant of a beneficial effect of interferon.[24] But because of the slowly progressive nature of HCV infection, using long term outcome as an end point for response is not practical. At present, aminotransferase serum levels and virological markers are used to judge the response to treatment.[24] Serum ALT alone may be misleading because of the fluctuations described in natural history of HCV infection,[6 7] and the marked variability of individual levels in enrolled patients. We believe it is important to consider the duration of the periods in which serum ALT remains normal. In our responders, normal ALT was sustained until the end of follow up. This has not been observed previously. In addition there was virological and histological response. Viral clearance is probably the major goal for interferon treatment and may explain also the lack of relapse observed in our patients during an 18 month follow up after discontinuation of treatment. In the present study we found neither discrepancy between biochemical and virological response nor a high relapse rate as observed in adults.[25 26] This sustained remission could be due to less heterogeneous viral populations, with a lower rate of mutants, depending on a shorter duration of disease but might be a function of the small number of studied patients. It has been suggested that better results are obtained by extending the duration of interferon treatment.[27] Our findings confirm the efficacy of schedules characterised by at least 12 months of treatment. Recent investigations have determined that HCV genotype was the most important factor in predicting interferon response.[28] Because of the small number of enrolled patients, we were not able to predict which factors would select HCV infected children candidates for treatment with interferon alfa; however, it is noteworthy that responders regained normal aminotransferase serum levels in the first two months. Therefore, if no such early response is observed treatment might be reasonably suspended.

Finally, in evaluating the efficacy of interferon treatment in childhood, the cost-benefit ratio has to be considered. Interferon is characterised by high costs in terms of drugs, in patient care, other medical expenses, and parental time lost from work. Furthermore, long term complications of interferon treatment are not clear. However, considering the frequency of viral clearance and histological activity improvement, lack of relapse, and of serious adverse effects, against the risk of cirrhosis and hepatocellular carcinoma, we consider interferon alfa should be considered in all children affected by chronic hepatitis C. Further studies are needed with larger number of patients and more careful evaluation of HCV-RNA in all known sites of virus replication to better define clearance and/or neutralisation of HCV by the host immune system.

Table 3 Epidemiological, clinical, biochemical and histological features of responders and non-responders at enrolment

	Responders (n=5)	*Non-responders (n=6)*
Median (range) age (years)	5·7 (2·5–10·4)	6·3 (6·1–10·3)
No (%) boys	4 (80)	3 (50)
Route of infection; No (%)		
History of transfusion	3 (60)	3 (50)
Vertical transmission	1 (20)	2 (33)
Minor surgical procedure	0	0
No overt exposure	1 (20)	1 (17)
No (%) with acute onset	0	0
Mean (SD) duration of chronic hepatitis C (months)*	54·8 (39·5)	63·2 (31·9)
No (%) with hepatomegaly	3 (60)	4 (67)
Mean (SD) ALT (U/l)	144·2 (44·7)	137·5 (138·3)
RIBA-2 pattern; No (%)†		
Anti-c22-3+	0	1 (17)
Anti-c22+/c100-3+/5-1-1+	1 (20)	0
Anti-c22+/c33c+/c100-3+/5-1-1+	4 (80)	5 (83)
Genotype; No (%)		
1a	1 (20)	1 (17)
1b	2 (40)	3 (50)
2	1 (20)	1 (17)
3	1 (20)	1 (17)
Histological features; No (%) except Knodell score		
Minimal hepatitis	0	2 (33)
Chronic persistent hepatitis	1 (20)	1 (17)
Chronic active hepatitis	4 (80)	3 (50)
Cirrhosis	0	0
Median (SD) Knodell score	9·0 (2·2)	5·0 (2·9)

*Duration of disease is expressed as the time elapsed since the presumed exposure to contaminated blood for patients infected parenterally, as the time since birth for patients infected vertically, and as the time since first observation of an abnormal ALT level for patients with no overt source of infection.
†RIBA-2 pattern: anti-c22+ indicates subjects positive for antibodies to c22 protein; anti-c22+/c100+/5-1-1+ indicates subjects positive for antibodies to c22, c100, and 5-1-1 proteins; anti-c22+/c33+/c100+/5-1-1+ indicates subjects positive for c22, c33, c100, 5-1-1 proteins.

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