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Treatment of chronic non-A non-B hepatitis with human interferon β: a preliminary study

K. Kiyosawa, T. Sodeyama, Y. Nakano, H. Yoda, E. Tanaka, T. Hayata, K. Tsuchiya, M. Yousuf and S. Furuta

Department of Internal Medicine, Sinshu University School of Medicine, Matsumoto, Japan

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Summary

Twenty-four patients with chronic non-A non-B hepatitis were randomly assigned to receive either human fibroblast interferon (HuIFN-β) at doses of 1 or 3 million international units (MIU) per day for 4 or 12 weeks (12 patients) or to receive no therapy (12 patients), and were then compared with 5 patients with chronic type B hepatitis who were treated with HuIFN-β. Elevated serum aminotransferase levels decreased more rapidly during the treatment of chronic non-A non-B hepatitis than of chronic hepatitis B. Variations in serum aminotransferases were not observed in any of the untreated chronic non-A non-B hepatitis patients. In 3 of the 9 patients with chronic non-A non-B hepatitis who responded to HuIFN-β therapy, serum aminotransferase levels remained normal 15, 21 and 31 months after therapy was discontinued; liver biopsy specimens obtained after therapy from 2 patients showed marked histological improvement. In the six other patients aminotransferase activity levels became again elevated following cessation of interferon therapy. No response to HuIFN-β was seen in the remaining 3 patients with chronic non-A non-B hepatitis.

Chronic non-A non-B hepatitis; Chronic type B hepatitis; Human interferon-β

Introduction

Spontaneous resolution of non-A non-B chronic hepatitis (NANB-CH) is rare and approximately 30% of cases of NANB-CH evolve into cirrhosis of the liver,

Correspondence to: Kendo Kiyosawa, 2nd Department of Internal Medicine, Shinshu University School of Medicine, 3-1-1, Asahi, Matsumoto, Nagano-ken 390, Japan

and 15% develop hepatocellular carcinoma 10 to 15 years after onset of the clinical course (Kiyosawa et al., 1984; Gibo et al., 1986). In Japan, there has been an increase in the number of patients with hepatocellular carcinoma who have no serological evidence of hepatitis B virus (HBV) infection (Okuda et al., 1987). As non-A non-B hepatitis virus (NANBV) is thought to be the etiologic agent of NANB-CH, it is warranted to attempt antiviral therapy of NANB-CH. For the treatment of type B chronic hepatitis (B-CH), various antiviral therapies have been pursued, and some favorable, though not fully satisfactory, effects have been achieved (Greenberg et al., 1976; Scullard et al., 1979; Davis et al., 1986). Hoofnagle et al. (1986) and Thomson et al. (1987) have tried alpha-interferon in the treatment of NANB-CH and obtained favorable results in some cases. Furthermore, Jacyna et al. (1989) reported the efficacy of low doses of alpha-interferon against NANB-CH. In the present study, we tried human fibroblast interferon (HuIFN-β) therapy for NANB-CH, and compared the results with those obtained in untreated NANB-CH patients and HuIFN-β-treated patients with B-CH.

Patients and Methods

Patients

A total of 24 patients with NANB-CH entered into the trial and were randomly allocated to either HuIFN-β treatment (12 patients) or no treatment (12 patients). There were 16 men and 8 women aged from 21 to 63 years. Four had a history of blood transfusion, but none of them had a history of alcoholism, drug abuse, homosexuality or hemophilia. Abnormal levels of serum glutamic pyruvic transaminase (GPT) had persisted for 6 months to 10 years (mean: 3.8 years), before the trial was started. All patients were negative for hepatitis B surface antigen (HBsAg), hepatitis B virus DNA (HBV-DNA), anti-nuclear antibody, anti-smooth muscle antibody, and anti-liver cell membrane antibody, which were determined by an immunofluorescent technique described previously (Kiyosawa et al., 1986). Histological diagnosis of liver biopsy specimens revealed chronic active hepatitis (CAH) in 14 patients, chronic persistent hepatitis (CPH) in 6 and CAH with bridging necrosis in 4. Five patients with B-CH (4 men and 1 woman, aged from 36 to 55) also received HuIFN-β. All of them were positive for HBsAg, hepatitis B e antigen (HBeAg) and HBV DNA polymerase activity (HBV-DNA-p), and they had been followed for more than 6 months before treatment was started. All patients were negative for antibody to hepatitis D virus (anti-HD). The histological diagnosis was CAH in 3 patients and CPH in 2. The trial was carried out with the informed consent of all subjects. The characteristics of the patients are shown in Table 1.

TABLE 1

Patient characteristics before treatment

Characteristic	Type non-A, non-B		Type B treated
	Treated	Non-treated	
Number	12	12	5
Age (yr) mean	48.4	49.1	40.3
Age (yr) range	21–61	23–60	36–55
Sex male:female	8:4	8:4	4:1
History of blood transfusion	2	2	0
Period of GPT elevation			
mean	3.8 yr	4.3 yr	2.4 yr
range	6 mo–10 yr	6 mo–11 yr	1–5 yr
Histological appearance			
chronic persistent hepatitis	3	3	2
chronic active hepatitis	7	7	3
chronic active hepatitis with bridging necrosis	2	2	0
GOT mean±SD (Karmen units)	252±140	263±129	202±96
GPT mean±SD (Karmen units)	271±115	283±152	225±104
HBsAg +	0	0	5
HBeAg +	0	0	5
HBV DNA polymerase +	0	0	5
Autoantibodies			
anti-nuclear antibody	0	0	
anti-smooth muscle antibody	0	0	
anti-liver cell membrane antibody	0	0	
HuIFN-β treatment			
protocol A[a]	5	0	5
protocol B[b]	7	0	0

HuIFN-β treatment: [a]protocol A: 3 million international units/day for 4 weeks, [b]protocol B: 1 million international units/day, 3 days/week, for 12 weeks.

Interferon therapy

HuIFN-β (Toray Co., Tokyo) was administered intravenously according to 2 different procedures (protocols A and B). In protocol A, HuIFN-β was given at a dose of 3 million international units (MIU) every day for 28 days (a total of 84 MIU). In one patient with NANB-CH, the dose of HuIFN-β had to be decreased during therapy because of neutropenia. This protocol was followed by 5 patients with NANB-CH and 5 with B-CH. All patients in this protocol were hospitalized during treatment. In protocol B, HuIFN-β at a dose of 1 MIU was given to 7 patients with NANB-CH once a day, 3 times weekly for 12 weeks. These patients were hospitalized during the first 4 weeks of treatment. Of the 12 control patients with NANB-CH, 5 were matched with the 5 patients treated according to protocol

A and 7 were matched with the 7 patients treated according to protocol B. All control patients were followed as outpatients.

Follow-up study

Conventional liver function tests, including determination of GPT (normal <35 Karmen Units), glutamic oxaloacetic transaminase (GOT) (normal <35 Karmen Units), gamma glutamyl transpeptidase and total bilirubin levels, were carried out every week during therapy and every 2 to 4 weeks after therapy for one year. In patients given HuIFN-β for 28 consecutive days (protocol A), leucocyte counts and platelets counts were determined every week during therapy. HBsAg and HBeAg were determined by an enzyme immunoassay using Enzygnost HBs and HBe kits (Behringwerke, Marburg, F.R.G.). Anti-HD was determined by solid-phase radioimmunoassay (Abbott Laboratories, North Chicago, IL). Follow-up liver biopsy was performed after therapy in 4 patients with NANB-CH. To monitor the side effects, patients were observed for the occurrence of symptoms such as fever, exanthema, generalised malaise, myalgia and arthralgia. The peripheral blood cell counts and the presence of proteinuria were also monitored.

Statistical analysis

Analysis of variance was performed by the two-way layout method with duplication and Student's *t*-test was used for comparison of the biochemical and histological changes.

Results

Changes in serum GPT levels in treated and untreated patients with NANB-CH

One of the 5 patients with NANB-CH who were treated according to protocol A showed no response either during or after administration of HuIFN-β, whereas a marked decrease in GPT was found during therapy in the remaining 4. Of these 4 patients, 2 showed a marked elevation and one a moderate elevation in GPT immediately after discontinuing therapy. In the other patient, the GPT level has remained normal for 21 months after therapy was stopped. Of the 7 patients treated according to protocol B, GPT levels were not affected in 2 patients, whereas a marked decrease in GPT levels occurred in the other 5 patients. In 3 of these 5, the GPT returned to the pretreatment level shortly after discontinuing therapy. In 1 of the remaining 2 patients, the GPT level has remained normal for 31 months after therapy was stopped. In the other patients, the GPT level is still normal at present (15 months after treatment), in spite of a mild increase in GPT level just after therapy. Thus, taking protocol A and protocol B together, prolonged normalization of GPT for more than 15 months after therapy occurred in 3 patients (good responders), a decrease in GPT only during therapy occurred in 6 patients

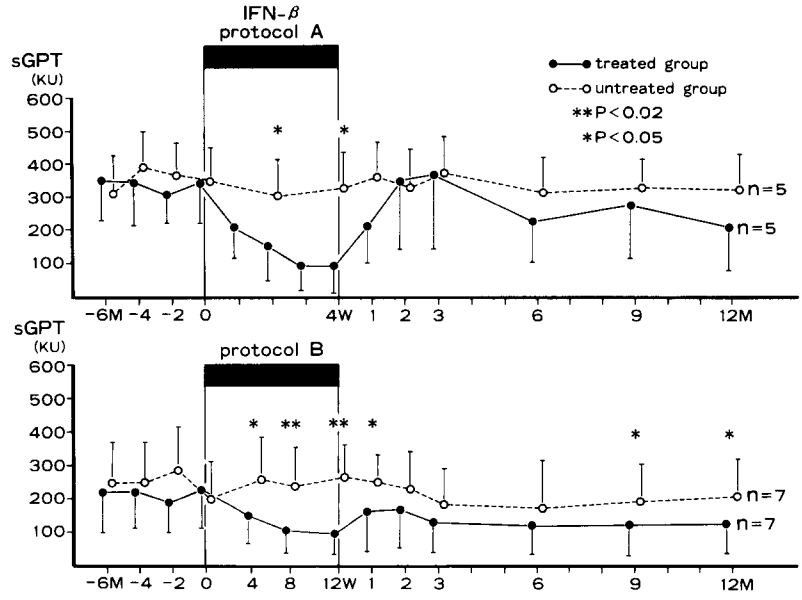

Fig. 1. Comparison of changes in serum glutamic pyruvic transaminase (GPT) levels in patients with NANB-CH treated or not with HuIFN-β. Protocol A: HuIFN-β was given to 5 patients at a dose of 3 million international units (MIU) per day for 28 days (total dose 84 MIU). In 1 case the dose of HuIFN-β was decreased from 3 MIU to 1 MIU during therapy because of leucopenia. Protocol B: HuIFN-β was given to 7 patients at a dose of 1 MIU per day, 3 times weekly for 12 weeks.

(transient responders), and no response was seen during or after therapy in 3 patients (non-responders). Of the untreated patients with NANB-CH none showed a normalization of serum transaminase levels during the follow-up period. Fig. 1 shows the changes in mean values of serum GPT in 5 patients with NANB-CH treated according to protocol A and in 7 patients with NANB-CH treated according to protocol B in comparison with the untreated control patients. The mean serum GPT levels in patients treated according to protocols A and B decreased gradually during interferon therapy, and the levels in treated patients were significantly lower than in controls.

Comparison of changes in GPT levels between NANB-CH and B-CH patients treated with HuIFN-β

Fig. 2 shows the changes in the mean GPT level in 5 patients with B-CH and 5 patients with NANB-CH treated according to protocol A. The mean GPT level rose slightly 1 week after the beginning of therapy and decreased thereafter in patients with B-CH, whereas there was an initial sharp decrease and then a rebound phenomenon with a peak occurring 3 months after the end of therapy in patients with NANB-CH. The GPT level remained abnormal in all 5 patients with B-CH both during and after therapy.

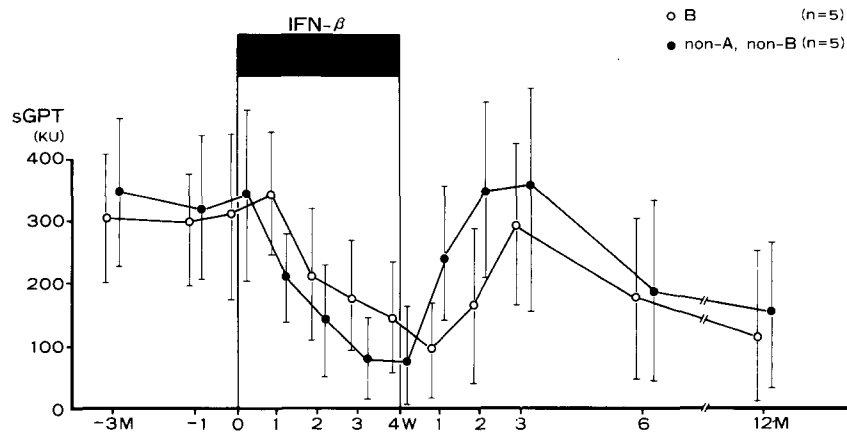

Fig. 2. Comparison of changes in serum glutamic pyruvic transaminase (GPT) levels after HuIFN-β therapy (protocol A) for patients with NANB-CH or B-CH.

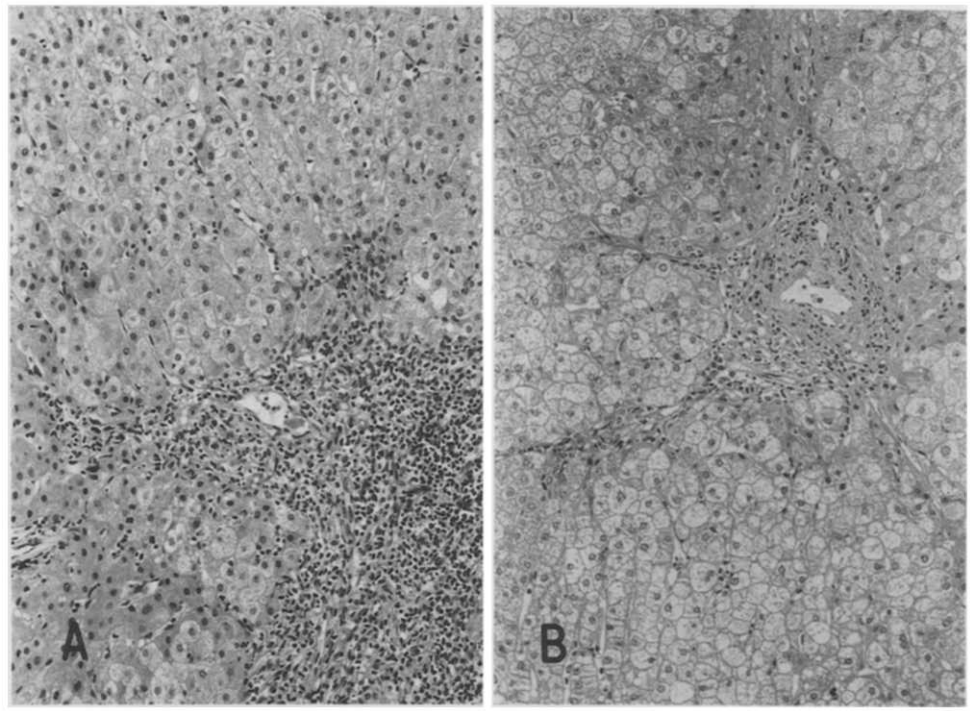

Fig. 3. Histological appearance of the liver of case R.M. (36, female) who was treated by protocol A. A: Chronic active hepatitis (3 months before therapy). B: slight periportal fibrosis with non-specific reactive hepatitis (6 months after therapy) (hematoxyline-eosin stain 168×).

Histological changes in patients with NANB-CH treated with HuIFN-β

Follow-up biopsy was performed after HuIFN-β therapy in 4 patients. Two of them showed normal GPT levels after treatment, 1 patient showed transient normalization of GPT during therapy and in 1 patient the GPT level did not change. Two patients who had continuing normal GPT levels, following liver biopsy at 6 and 14 months after discontinuing HuIFN-β therapy, showed a change to slight periportal fibrosis with nonspecific reactive hepatitis. The histological picture of 1 (case R.M., 36, female) is presented in Fig. 3. The histological picture of case K.U. (21, male), who was treated twice by protocol A at an interval of 11 months and had a normal GPT level only during HuIFN-β therapy, is presented in Fig. 4. Fig. 4A and C (taken just before treatment) show CAH with severe intralobular inflammation while Fig. 4B and D (taken just after treatment) show non-specific reactive hepatitis. In 1 patient who did not respond liver histology after interferon treatment showed CAH with bridging necrosis.

Pretreatment factors associated with response to interferon treatment

On the basis of their responses to interferon therapy (as assessed by serum transaminase level changes) patients could be divided into 3 groups (good responders, transient responders and non-responders). The pretreatment factors for each group are summarized in Table 2. There were no significant differences in age, sex, history of transfusion, GPT level before treatment and period of GPT elevation. All 3 patients with CPH fell into the good responders group and 2 of 3 non-responders showed CAH with bridging necrosis.

Side effects

Fever over 38°C developed in all patients with both types of hepatitis, general malaise in 10 patients with NANB-CH and 5 patients with B-CH, and myalgia in 1 with NANB-CH and 2 with B-CH. Despite these complications, all patients except 1 with NANB-CH completed the designed schedule of treatment. This one patient had a leucocyte count below $1000/mm^3$ and so the dose of IFN-β was reduced from 3 MIU to 1 MIU half-way through the course. There were no side effects such as serious thrombocytopenia, exanthema, arthralgia or proteinuria in any of the subjects.

Discussion

HuIFN-β therapy for B-CH has been investigated in several studies. It causes only moderate side effects, such as fever, headache, myalgia and granulocytopenia (Desmyter et al., 1976; Dolen et al., 1979; Oda et al., 1982). In the present study, we treated 12 patients with NANB-CH with a low dose of HuIFN-β. Concomitantly with the administration of HuIFN-β, the mean serum GPT level markedly

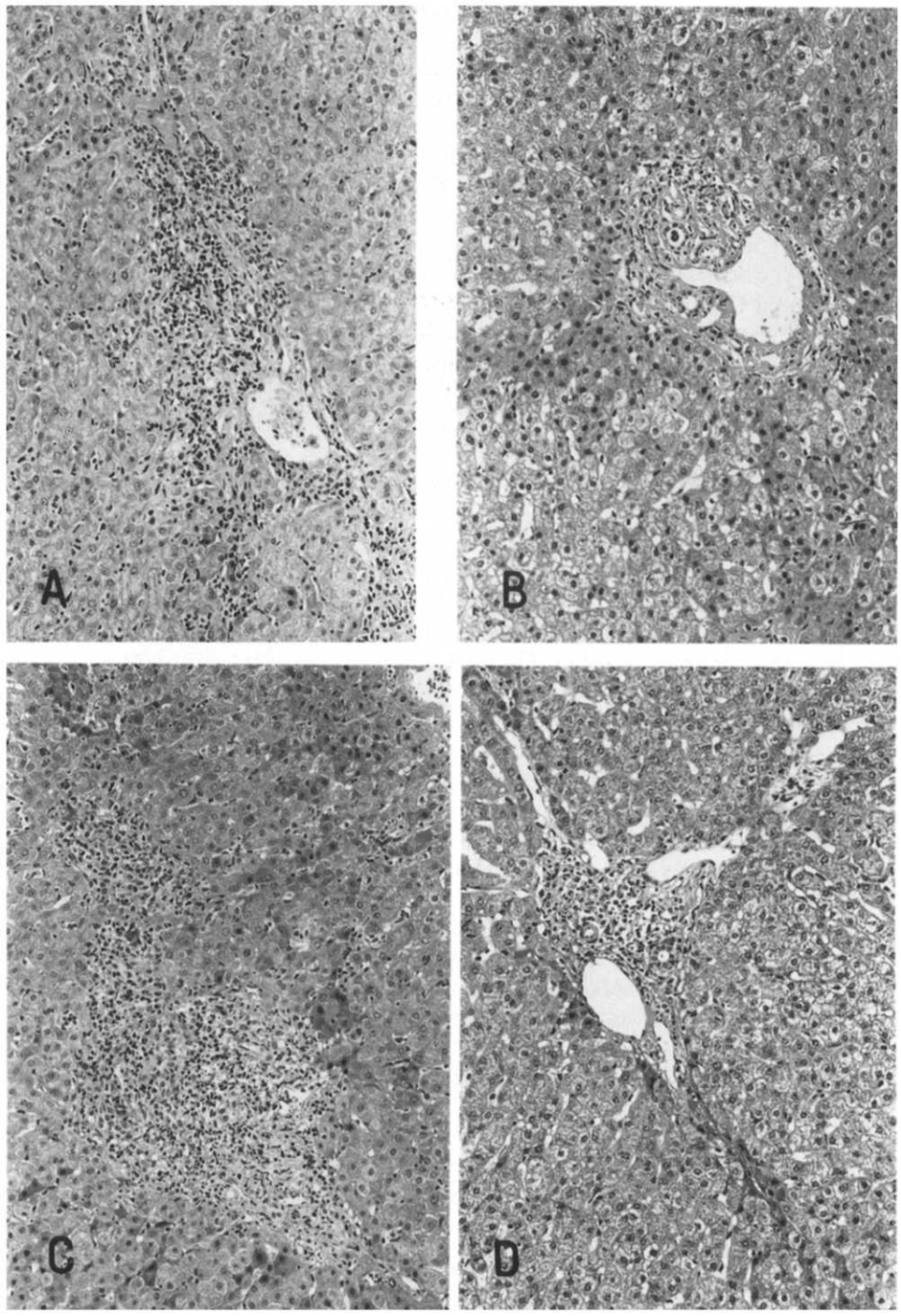

Fig. 4. Histological appearance of the liver of case K.U. (21, male). Biopsies A and C were obtained just before the first and the second rounds of interferon therapy, and both show chronic active hepatitis with severe intralobular inflammation. B and D were obtained just after each round of therapy, and show non-specific reactive hepatitis (hematoxylin-eosin stain 168×).

TABLE 2

Pretreatment factors associated with response to HuIFN-β

Factor	Good responders $N=3$	Transient responders $N=6$	Non-responders $N=3$
Age (yr)[b]	46.0±10.0	49.5±14.6	48.0±11.3
Sex M:F	2:1	5:1	2:1
History of transfusion	1	1	0
GTP level before treatment[b] (Karmen units)[a]	238.0±127.9	276.3±168.7	295.3±130.6
Period of GPT elevation (yr)[b]	2.7±1.1	4.8±3.8	3.1±2.3
Liver histology before treatment			
chronic persistent hepatitis	3	0	0
chronic active hepatitis	0	6	1
chronic active hepatitis with bridging necrosis	0	0	2

[a]Serum glutamic pyruvic transaminase (GPT): normally <35 Karmen units.
[b]No significant difference among the 3 groups by Student's *t*-test (mean ± SD).

decreased in the treated patients compared with the control patients. The therapy was considered to be effective in 3 patients (good responders), transiently effective in 6 patients (transient responders) and ineffective in 3 patients (non-responders), based on the changes in serum transaminase levels. The 3 good responders still have normal GPT levels at present, 15 to 31 months after termination of therapy. In 2 of the 3 patients, a change to a non-active disease state was demonstrated histologically. This occurred in parallel with the normalization of the serum transaminase levels. Histological examination carried out just after the end of therapy in 4 patients at a time when GPT levels were normal, revealed a marked decrease of inflammation in the intralobular and portal areas. The same phenomenon was observed following treatment with recombinant leucocyte (alpha) interferon (Omata et al., 1989). These findings suggest that HuIFN-β therapy is effective in a number of patients with NANB-CH despite the low doses used. However, the fact that 3 patients did not respond to interferon therapy might indicate that the dose of HuIFN-β used is too low to eradicate NANBV. Also, the severity of the liver damage may interfere with the action of IFN, and autoimmune hepatitis cannot be excluded completely despite the negative autoantibody results. Recently, the Chiron group have isolated a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome (Choo et al., 1989). They have also developed a specific assay for a blood-borne non-A non-B hepatitis virus in which a polypeptide synthesized in recombinant yeast clones of the hepatitis C virus (HCV) was used to capture circulating viral antibodies (Kuo et al., 1989). Thus we need to clarify the association between the efficacy of HuIFN-β therapy and the anti-HCV level. This and the other problems mentioned above remain to be elucidated by future studies.

It is known that in B-CH pretreatment factors affect the response to interferon treatment. Dusheiko et al. (1986) have reported that B-CH patients with mark-

edly elevated pretreatment serum transaminase levels and histological evidence of severe chronic hepatitis showed clearance of both HBeAg and HBsAg. In the patients with NANB-CH in this study, though the numbers were too small for accurate analysis, there were no apparent differences in age, sex, pretreatment serum GPT level and period of GPT elevation between the good responders, transient responders and non-responders. However, 2 of 3 non-responders showed CAH with bridging necrosis.

No valid conclusions can be drawn as to the relative value of treating NANB-CH according to protocols A or B, since the number of subjects was too small. However, the GPT rebound after therapy was less pronounced in protocol B than in protocol A. GPT levels remained completely normal in 2 of 7 patients following protocol B, whereas the corresponding rate for protocol A was 1 out of 5. In addition, the total dose was lower in protocol B than in protocol A. Recently, Jacyna et al. (1989) have reported that intermittent therapy with continuous low-dose alpha-interferon reduced aspartate aminotransferase activity to normal in most patients with NANB-CH. Thus intermittent administration of low-dose interferon for a prolonged time period may be the first step in optimizing the conditions for interferon therapy.

Side effects including fever, general malaise and myalgia, were noted but few patients complained of such symptoms 3 weeks after termination of therapy. In 1 patient the peripheral leucocyte count decreased, but later improved following a reduction in the dose of HuIFN-β. In none of the patients therapy had to be stopped because of side effects.

In conclusion, therapy with relatively low doses of HuIFN-β is able to significantly decrease the GPT level in some patients with NANB-CH. No severe side effects were observed. However, the optimal dosage and treatment regimen need to be further assessed in a greater number of subjects.

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