ORIGINAL ARTICLE

Keio Multicenter Trial in High-dose Interferon- α 2b Treatment for Chronic Hepatitis C

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Abstract. The efficacy of two different high-dose treatment of IFN- α 2b was evaluated in this study. Serum hepatitis C virus (HCV) RNA levels were semi-quantified by simplified reverse transcription-polymerase chain reaction. Seventy-one patients with chronic hepatitis C received 10 million units of IFN- α 2b daily for 2 weeks or trice a week for 24 weeks. Alanine aminotransferase (ALT) levels overall normalized in 78.1% and 51.6% of the cases at the end of the therapy and 6 months after that, respectively. HCV RNA disappeared in 71.9% and 35.7% of the patients at the end of the therapy and 6 months after that, respectively. There was no significant difference between the 2 different regimes. The efficacy of the treatment was fair in cases in which the pretreatment level of the viral amount was low. The results of this study indicate that daily administration of IFN in the first 2 weeks during 6-month course does not increase the efficacy of the therapy in such a high-dose treatment regime. (Keio J Med 45 (3): 161–167, September 1996)

Key words: chronic hepatitis C, interferon, prediction, dose study

Introduction

It is well known that the majority of both transfusion-transmitted and sporadic non-A, non-B hepatitis is associated with hepatitis C virus (HCV) infection.^{1,2} The natural history of chronic hepatitis (CH)-C shows that the disease is progressive and often severe, suggesting that antiviral therapy should be used to eradicate the virus and halt the progression of CH,³ although this is still controversial.⁴ Recent randomized trial of interferon (IFN)- α which demonstrated the association between its use and a decreased incidence of hepatocellular carcinoma⁵ encourages the similar trial on the patients with CH-C.

Although IFN is known to be effective in the treatment of CH-C, not only in producing histological improvement⁶

but also in the elimination of HCV from serum,⁷ the efficacy of the therapy still remains to be improved. Therefore, some pretreatment factors predictive of a response, such as the absence of cirrhosis, low serum alanine aminotransferase (ALT) concentrations, serum HCV RNA levels, and the absence of HCV genotype 1b, have been suggested.^{8–10} Prediction of eventual response based on the response to the first 1 month's IFN therapy has also been reported.¹¹ However, the optimal dose and administration schedule have not been established.¹² In Japan, since IFN administration to patients with CH-C is limited to 6 months under the usual health insurance coverage, in most institutions the practice of administering IFN daily for 2 weeks followed by 3 times per week until 6 months has been adopted. However, it has not been clarified whether or not this treatment is superior to

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Table 1 The Members of the Keio Interferon- α 2b Study Group

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that in which IFN is administered 3 times a week for 6 months without the initial daily administration for 2 weeks.

Keio multicenter trial in IFN- α 2b therapy for CH-C has been performed since October 1992. The members of the Keio Interferon- α 2b Study Group are listed in Table 1. The aims of this study were (1) to clarify the impact on efficacy of the first 2 weeks daily administration of IFN in a high-dose treatment, and (2) to determine if there was any correlation between the response to the IFN therapy and the decrease of viral load occurring during the first 2 days of the therapy.

Subjects and Methods

Patients and treatment

Seventy-one CH-C patients with histologically proven chronic hepatitis and serologically positive for anti-HCV (second-generation assay) were enrolled. Patients' serum ALT levels had been persistently elevated for more than 1 year and they were negative for hepatitis B surface antigen and anti-smooth muscle antibodies. Before enrollment, patients with decompensated liver cirrhosis, alcoholic liver disease, hemochromatosis, Wilson's disease or alpha-1-antitrypsin deficiency were excluded.

All sera were proven positive for HCV RNA using the polymerase chain reaction (PCR) method.¹³ Needle biopsy of the liver was performed before IFN administration and 3 pathologists examined biopsy specimens independently without previous knowledge of the cases. Pathological findings were classified into chronic persistent hepatitis (CPH), chronic active hepatitis (CAH) according to the European classification.¹⁴ CH without piecemeal necrosis is diagnosed as CPH and CH with piecemeal necrosis is diagnosed as CAH. CAH with mild to moderate activity without the fibrotic formation of P-P bridging is diagnosed as CAH 2A. CAH with severe activity and with the fibrotic formation of P-P bridging is diagnosed as CAH 2B. The pre-treatment ALT levels were estimated by calculating the mean of a series of 4 serum ALT levels in blood samples taken at 4-week intervals before enrollment.

IFN treatment regimen

Before the start of the study, informed consent was obtained from all the patients and 10 million units (MU) of recombinant IFN- α 2b (Intron A; Schering-Plough) was administered to each patient intramuscularly. Before and 2 days after this administration, patients' sera were obtained. They were kept at -80°C until HCV-RNA was assayed. These patients were randomly assigned to two groups using the sealed envelopes method. One group received 10 million units (MU) of recombinant IFN- α 2b intramuscularly daily for 2 weeks followed by 3 times per week for 22 weeks (group A). The other group received 10 MU of the same IFN intramuscularly 3 times per week for 24 weeks (group B). Patients' profiles are summarized in Table 2.

Measurement of viral level

The viral level in the serum was semi-quantified by a simplified reverse transcription (RT)-PCR method as described previously.¹⁵ Oligonucleotide primers were designed from the nucleotide sequence of the 5'-noncoding region of the HCV-J.¹⁶ RNA was extracted from sera as described by Chomezynski and Sacchi,¹⁷ and the RNA pellet from 100 μL of the serum was dissolved in 100 μL of distilled water. One microliter of the RNA solution was subjected to RT according to the manufacturer's instruction (cDNA Synthesis System Plus; Amersham International, Plc, Amersham, UK). PCR was cycled 40 times, with each cycle including denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes; and extension at 72°C for 2 minutes in a GeneAmp DNA amplification kit (Perkin-Elmer Cetus, CT, USA). The PCR products were separated on a composite gel of 0.5% NuSieve and 0.5% SeaKem agarose (FMC Bio-Products, MD, USA), and were

Table 2 Base-line Characteristics of Patients with CH-C Treated with IFN- α 2b

	Group	A	B	Total
Sex	M	23	24	47
	F	10	7	17
Age	21–30	3	0	3
	31–40	5	6	11
	41–50	5	6	11
	51–60	10	16	26
	61–70	9	3	12
	71–	1	0	1
	mean	50.0	51.6	50.7
	SD	13.2	9.1	11.4
Liver Histology	CPH	3	5	8
	CAH2A	13	9	22
	CAH2B	17	17	34
ALT	0–35	6	4	10
	36–70	9	16	25
	71–100	12	6	18
	101–200	4	5	9
	201–	2	0	2
	mean	77.9	67.7	73.1
	SD	52.0	42.5	47.8
Transfusion	+	11	11	22
	–	16	16	32
	unknown	6	4	10
Alcohol	+	11	15	26
	–	21	12	33
	unknown	1	4	5
HCV-RNA	3+	23	23	46
	2+	1	2	3
	1+	9	6	15
Total		33	31	64

visualized by ethidium bromide staining with an ultra violet trans-illuminator. If the PCR products amplified from 1/100 of the original samples (1 μ L of the RNA solution) could be detected, the viral level in the sample was estimated as [3+]. When the PCR products amplified from the original serum (RNA was precipitated from 100 μ L solution, resuspended in 1 μ L of distilled water and subjected to RT-PCR) could be detected, whereas those from 1/100 of the original sample was not detected, the viral level was estimated as [2+]. In cases in which the PCR products from the original samples could not be detected, another PCR cycled 20 times under the same cycling conditions was performed using the same primers, and the samples produced a detectable band were designated as [+], and those without a detectable band were

Fig 1 Representative photographs of semi-quantification of the PCR products from sera of the patients with CH-C. With a sense primer (5'-CCATGGCGITAGTATGAGTG-3', HCV-J 83–102) and an anti-sense primer (5'-TAGACCGTGCACCATGAGCAC-3', HCV-J 329–349) (outer primers) cDNA synthesis was carried out. The cDNA was amplified by PCR using a sense primer (5'-AGAGCCATAGTGGTCTGCCA-3', HCV-J 134–154) and an antisense primer (5'-GTAGTGTGGGTCGCGAAAG-3', HCV-J 258–277) (inner primers). The amplified DNA was 144 bp in length (arrow). Molecular marker used was ϕ X174 RF DNA/Hae III fragments (1353 bp, 1078 bp, 872 bp, 603 bp, 310 bp, 271 bp, 234 bp, 194 bp, 118 bp, and 72 bp; lane 1). Twelve samples (started from 1 μ L of the RNA solution) were electrophoresed (lane 2–13) and visualized by ethidium bromide staining (a; lane 14, water; negative control). Samples No. 1–5, 7, 10, and 12 were positively stained and were semi-quantitatively estimated as (3+). As for the samples which were negatively stained, 100 μ L of the RNA solution was precipitated and resuspended in 1 μ L of distilled water and applied to RT-PCR followed by electrophoresis (b; lane 1, molecular markers; lane 2, positive control; lanes 3–6, samples No. 6, 8, 9, and 11, respectively; lane 7, water; negative control). Samples No. 11 was positively stained and were semi-quantitatively estimated as (2+). The samples 6, 8, and 9, which were negatively stained, were applied to the second PCR and electrophoresed (c; lane 1–3, sample No. 6, 8, and 9, respectively). Only sample No. 9 was positively stained and estimated as (+). The samples No. 6 and 8 were estimated as (–).

as [–] (Fig 1). One of the serum sample was amplified by multicyclic RT-PCR and cDNA copies were quantified as described previously.¹⁸ The stepwise dilution of this PCR product was made and electrophoresed on the composite gel and its staining was compared with those of 1st PCR products (Fig 2).

The viral levels in blood samples, taken before and 2 days after a single intramuscular administration of 10,000,000 U of IFN- α 2b were measured.

Fig 2 The correlation between the amount of 1st PCR products and copies of cDNA. The quantification of copy number of cDNA was performed by the multicyclic RT-PCR method as described in the text. The standard of cDNA copies and 1st PCR products were compared each other on the composite gel after electrophoresis with ethidium bromide staining.

The efficacy of IFN therapy

Biochemical and hematological tests were performed monthly. Serum ALT levels were determined by a conventional method with an autoanalyzer, and the results were expressed as IU/L. A value above 40 IU/L was regarded as an elevated level, as the mean plus 2 standard deviations (SD) for healthy subjects at our institution is 40 IU/L. The efficacy of IFN therapy was evaluated at the end of the therapy and 6 months after that.

Statistical evaluation

The comparison of variables (two tailed) in pre-treatment patient data profiles and the comparison of serum ALT levels, white blood cell (WBC) counts, and platelet (PLT) counts between groups was done using Student's *t*-test or chi-square test with continuity correction. The change in serum ALT levels in each group was evaluated by paired *t*-test (two tailed). In terms of serum ALT levels, WBC counts, and PLT counts, analysis was performed after obtaining a normal distribution by logarithmic transformation.

Results

Laboratory data

Among seven patients who did not complete the

study, 2 cases were dropped out because of the adverse events, 3 were lost to follow-up, 2 declined to undergo randomization. There were no significant differences in sex, age, liver histology, pre-treatment ALT levels, past history of transfusion, history of alcoholic intake, or HCV RNA levels (Table 2). Serum ALT levels in both groups A and B dropped soon after the start of therapy (Fig 2). Serum ALT levels during treatment were significantly lower than the pretreatment levels ($p < 0.05$ by paired *t*-test). Serum ALT levels at weeks 28, 40, 44, and 48 in the group A were also significantly lower ($p < 0.05$). In our previous study,¹⁹ serum ALT levels in patients with CH-C without IFN treatment did not decrease for 24 weeks. WBC counts decreased soon after the start of therapy and persisted during the therapy ($p < 0.05$) in both groups, but recovered soon after the end of therapy (Fig 3). PLT counts were also decreased soon after the start of therapy ($p < 0.05$) in both groups, but recovered soon after the end of therapy and were increased significantly at weeks 40, 44, and 48 in the group A, and at weeks 28, 36, 40, 44, and 48 in the group B ($p < 0.05$) (Fig 3).

Efficacy of the treatments

The normalization of ALT levels overall was obtained in 50 of 64 cases (78.1%) at the end of therapy and in 33 of 64 cases (51.6%) 6 months after the end of therapy. The rates of normalization of ALT levels in each group at the end of therapy and 6 months later were 75.8% and 54.5% in group A respectively, and 80.6% and 48.4% in group B, respectively. There was no significant difference between the two groups. The negativity rates of HCV RNA in each group at the end of the therapy and 6 months later were 71.9% and 35.7% in group A respectively, and 78.6% and 35.7% in group B, respectively. There was no significant difference between the groups.

The decrease in HCV RNA levels during the first 2 days and the efficacy of the therapy

The decrease in the viral level during the first 2 days and the efficacy of the therapy (disappearance rate of serum HCV RNA at the end of the therapy and 6 months after that) were compared (Fig 4). It is difficult to evaluate the correlation, because the patient number was small. It seems, however, that the efficacy of the IFN therapy was fair in the cases in which the pretreatment level of the viral amount was low (+ or 2+). On the other hand, when the pretreatment viral level was high (3+), the decrease in the viral level during the first 2 days was significant, suggesting the effectiveness of the therapy.

Fig 3 Changes in serum ALT levels, WBC counts, and platelets counts, according to treatment group. * $p < 0.05$ in group A vs base-line level. # $p < 0.05$ in group B vs base-line level. IFN indicates the duration of IFN treatment.

Fig 4 The decrease rate in viral level during the first 2 days and the efficacy of IFN treatment. Semi-quantitative HCV RNA levels before and 2 days after 1 million units of IFN- $\alpha 2b$ administration are indicated in the left column. The HCV RNA disappearance rates at the end of the therapy are shown in the middle column and those 6 months after the end of therapy are shown in the right column.

Table 3 Treatment-Related Adverse Events, Number of Patient and Percentage

Adverse Events	n (Totally 66) (%)
All Patients with Any Adverse Experiences	57 (86.4%)
Fever	57 (86.4%)
Hair Loss	5 (7.6%)
Headache	4 (6.1%)
Thirsty	3 (4.5%)
General Malaise	3 (4.5%)
Appetite Loss	2 (3.0%)
Arthralgia	2 (3.0%)
Depression	2 (3.0%)
Abdominal Pain	1 (1.5%)
Myalgia	1 (1.5%)
Hyperthyroidism*	1 (1.5%)
Dizziness	1 (1.5%)
Feeling of Ear Closed	1 (1.5%)
Eruption	1 (1.5%)
Toxicoderma*	1 (1.5%)
Retinal Bleeding	1 (1.5%)

Seventy-one patients were enrolled in this study. Three patients were lost to follow-up and 2 declined to undergo randomization. Among 66 patients, 2 cases (indicated as * in this Table) were dropped out because of the adverse events.

Side effects

Safety was evaluated in 66 patients. The treatment-related adverse events experienced in this study are shown in Table 3. Treatment was discontinued because of side effects in two patients in group A. The reasons for discontinuing therapy were hyperthyroidism and toxicoderma. Each of these adverse effects resolved after discontinuing the treatment. No significant differences were observed among the two treatment groups. The most frequent adverse event was flu-like syndrome, including fever, headache, general malaise, anorexia, arthralgia, and myalgia. These syndromes resolved either during or after treatment and could be reduced by the use of non-steroidal anti-inflammatory drugs.

Discussion

A standard 6 month course of treatment with IFN- $\alpha 2b$; 3 MU three times weekly, having used in most European and American studies, leads to ALT normalization in about 50% of patients with chronic HCV infection. However, relapses occur most of the patients and then a higher dose has been considered to improve treatment results. There have been several studies to determine the optimal dose regime of IFN- $\alpha 2b$. Marcellin *et al*²⁰ compared the efficacy of 2 different doses. They studied on 60 patients and demonstrated

that a dose of 3 MU was superior to 1 MU of IFN given three times weekly for 24 weeks in inducing improvements in serum ALT levels and liver histology. Hakozaiki *et al*²¹ compared the efficacy of three different doses. They administered 3, 6, or 10 MU of IFN- α 2b three times weekly for 24 weeks and the percentage of HCV RNA clearance 12 months after treatment cessation was significantly higher in doses of 6 and 10 MU than a dose of 3 MU. The efficacy was rather higher in a dose of 6 MU regime than that of 10 MU regime but there was no significance.

The total dose of IFN administered in this study was very high and the efficacy of this protocol is fairly good in accord with the previous reports.^{22–24} One of the methods to increase the total dose of IFN is to extend the duration of the therapy. Diodati *et al*,²⁵ Poynard *et al*,⁷ and Reichard *et al*²⁶ already reported encouraging results. In Japan, however, 6 months' therapy is thought to be standard. One of the aims of the present study was to determine the efficacy of the first 2 weeks daily administration of IFN. In the present study, there was no significant difference between groups A and B. Serum ALT levels in group A were significantly lower than pretreatment level at 40, 44 and 48 weeks. ALT normalization rate and RNA disappearance rate were higher at the end of the therapy in the group A, however, the difference was not significant. These results combined with other report¹⁹ indicate that the efficacy of the first 2 weeks daily administration of IFN during 6 months' therapy does not make a significant difference in such high-dose treatment. During 6 months' IFN therapy, if the total dose is higher than a certain efficient level, we think that the first 2 weeks daily administration of IFN may not be more beneficial than when IFN is administered only 3 times a week throughout the treatment period.

We compared viral levels before and 2 days after one IFN administration. In patients whose sera contained higher levels of HCV RNA, the decrease of viral level was greater, the HCV RNA disappearance rate seemed to be higher. Kleter *et al*¹¹ reported that patients with a reduction in the serum ALT or HCV RNA levels within the first month after the treatment were more likely to obtain a sustained response than patients who did not show such changes. If we can evaluate the sensitivity of HCV against IFN by measuring the decrease of the viral level in the first 2 days, it may be very useful in predicting efficacy and obtaining cost benefit value. The quantitative assay of HCV RNA is necessary for evaluating this result.

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