

lamivudine-treated patients before such treatment was provided. YMDD mutant viruses were detected only in patients with breakthrough. The same results were obtained by the two detection methods. Although PCR-ELMA was the method employed by Kobayashi et al.,¹¹ in our hands, it produced results contradicting those that they reported. The reason for the discrepancy is unknown at present.

Of note, all patients in whom YMDD motif mutations were detected before treatment were ASC and positive for anti-HBe antibody, as stated in a previous study.¹¹ These patients were relatively old and they may have had mutant viruses after prolonged host immune pressure. Therefore, we investigated ASC who were confirmed to have had stable normal ALT levels for a longer period than those reported in the above study.¹¹ We also investigated patients with hepatitis B who lost HBsAg during follow-up, who were thought to have been under heavy host immune pressure and were followed-up for a longer period. In our study, none of these patients had any YMDD motif mutations. However, Kirishima et al., using a highly sensitive method of peptide nucleic acid PCR clamping,¹⁷ recently reported that YMDD motif mutations were detected in patients chronically infected with HBV who were not treated with lamivudine. In their study, all patients with YMDD motif mutation were also positive for anti-HBe antibody. It is possible that YMDD motif mutations in these patients may emerge during HBeAg seroconversion and host immune pressure. The method described by Kirishima et al.¹⁷ is highly sensitive and can detect the presence of even a few mutant viruses. It is not clear whether YMDD mutant viruses detected by this method before treatment have mutations in other regions of the HBV DNA and whether such viruses really increase enough *in vivo* to exist. However, there are no reports of patients infected with preexisting lamivudine-resistant viruses who were resistant to lamivudine treatment or who rapidly showed clinical evidence of HBV breakthrough. One study using PCR-RFLP showed that it took at least 2 weeks after the commencement of lamivudine treatment before the first resistant virus was detected.¹⁸ Although this report showed early emergence of lamivudine resistance during treatment, YMDD mutant viruses were not detected before treatment. When few YMDD mutant viruses exist before lamivudine treatment in patients infected with HBV, HBV DNA levels can be increased by the presence of lamivudine-resistance viruses during an early period after the commencement of lamivudine therapy. However, our results showed that HBV DNA levels had diminished in all patients after 3 months of lamivudine therapy. Based on these results, we consider that if lamivudine treatment is judged to be appropriate for a patient with hepatitis B, the treatment may be started

without taking into account the possible presence of YMDD mutant viruses. Nevertheless, it is necessary to monitor for the possible emergence of YMDD mutant viruses, at least periodically, once lamivudine treatment is employed.

In conclusion, our results showed that YMDD motif mutations were not detected in patients who were not treated with lamivudine. However, using a method with a high sensitivity for detecting polymerase gene mutation, we were able to identify a few patients with YMDD motif mutation. In these patients, it was not clear whether these mutant viruses had emerged and caused breakthrough hepatitis. We believe that lamivudine therapy could be started without taking into account the possible presence of YMDD mutant viruses.

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Adequate timing of ribavirin reduction in patients with hemolysis during combination therapy of interferon and ribavirin for chronic hepatitis C

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Background. Hemolytic anemia is one of the major adverse events of the combination therapy of interferon and ribavirin. Because of ribavirin-related hemolytic anemia, dose reduction is a common event in this therapy. In this clinical retrospective cohort study we have examined the suitable timing of ribavirin reduction in patients with hemolysis during combination therapy. **Methods.** Thirty-seven of 160 patients who had HCV-genotype 1b, had high virus load, and received 24-week combination therapy developed anemia with hemoglobin level <10 g/dl or anemia-related signs during therapy. After that, these 37 patients were reduced one tablet of ribavirin (200 mg) per day. After reduction of ribavirin, 27 of 37 patients could continue combination therapy for a total of 24 weeks (group A). However, 10 of 37 patients with reduction of ribavirin could not continue combination therapy because their <8.5 g/dl hemoglobin values decreased to or anemia-related severe side effects occurred (group B). We assessed the final efficacy and safety after reduction of ribavirin in groups A and B. **Results.** A sustained virological response (SVR) was 29.6% (8/27) in group A and 10% (1/10) in group B, respectively. A 34.4% (12/27) of SVR + biological response in group A was higher than 10% (1/10) in group B ($P = 0.051$), with slight significance. With respect to hemoglobin level at the time of ribavirin reduction, a rate of continuation of therapy in patients with ≥ 10 g/dl hemoglobin was higher than that in patients with <10 g/dl ($P = 0.036$). **Conclusions.** Reduction of ribavirin at hemoglobin level ≥ 10 g/dl is suitable in terms of efficacy and side effects.

Key words: chronic hepatitis C, interferon, ribavirin, HCV-RNA, hemolytic anemia

Introduction

The addition of nucleotide analogue ribavirin to interferon (IFN) in the treatment of patients with chronic hepatitis C has significantly improved sustained virological response (SVR) rates.^{1–7} With the advent of combination therapy, the proportion of patients treated with IFN monotherapy has much diminished. The combination therapy of interferon and ribavirin is now in widespread use. However, the number of side effects in combination therapy is increased compared to IFN monotherapy.^{1,2,8} Hemolytic anemia is one of the major adverse events of the combination therapy of interferon and ribavirin. This side effect has been ascribed to the accumulation of ribavirin triphosphate in the erythrocytes.⁹ The incidence of hemolytic anemia has been reported to be 7%–9% by Poynard et al.^{1,2} and 67% by Van Vlierberghe et al.¹⁰ Because of ribavirin-related hemolytic anemia, dose reduction is common. However, there has been no study that tried to assess when the reduction of ribavirin should begin. In this clinical retrospective cohort study, we have examined the suitable timing of ribavirin reduction in patients with hemolysis during combination therapy for chronic hepatitis C.

To reduce the effect of various virus-related factors, we selected in this study patients with chronic hepatitis C genotype 1b and high pretreatment viral load who specifically showed poor response to IFN therapy relative to that of patients with other genotypes or lower viral load.

Methods

Patients

A total of 260 patients were diagnosed with chronic hepatitis C virus (HCV) infection and subsequently received a combination course of interferon alpha-2b (IFN- α -2b) and ribavirin at the study hospital between October 1999 and March 2003. Of these, 165 patients fulfilled the following criteria: (1) laparoscopy and liver biopsy taken within 3 months of initiation of IFN and ribavirin therapy, which showed histopathological features of chronic active hepatitis; (2) serum HCV-RNA level >100 KIU/ml by quantitative polymerase chain reaction (PCR) assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems, Pheasanton CA, USA) before IFN therapy; (3) HCV-genotype 1b; (4) average alanine aminotransferase (ALT) elevation greater than 1.5 times the upper normal limits (ALT normal range, 12–50 IU) for more than 6 months before IFN therapy; (5) no treatment with corticosteroid, immunosuppressive agents, or antiviral agents within 6 months of commencement of IFN therapy; (6) negative for hepatitis B surface antigens (HBsAg), hepatitis B virus DNA (HBV-DNA), antinuclear antibodies (ANA), and antimitochondrial antibodies (AMA) in the serum, as determined by radioimmunoassay and spot hybridization; and (7) leukocyte count $>3000/\text{mm}^3$, platelet count $>80000/\text{mm}^3$, and serum bilirubin <2.0 mg/ml before the initial period of IFN therapy. These 165 patients were given IFN- α 2b (Intron-A; Schering-Plough, Kenilworth, NJ, USA) subcutaneously at a dosage of 6 million units (MU) daily for 2 weeks, followed 3 times weekly for 22 weeks, and ribavirin (Rebetol; Schering-Plough) at an oral dose of 600 mg (for patients with body weight <60 kg) or 800 mg (for those with body weight ≥ 60 kg) daily for 24 weeks.

The dose of ribavirin was adjusted based on hemoglobin concentrations or anemia-related signs: the dose was reduced by one tablet of ribavirin (200 mg) per day when concentrations fell below 10 g/dl or the drop in hemoglobin (Hb) level exceeded 3.5 g/dl and/or patients had anemia-related signs: fatigue, pallor, and reduced exercise capacity. Ribavirin was discontinued when Hb values dropped below 8.5 g/dl and/or patients had severe anemia-related signs including orthostatic hypotension.

Fourteen patients discontinued the combination therapy because of adverse events other than hemolytic anemia. Of the remaining 151 patients, 37 patients developed anemia with Hb level <10 g/dl or anemia-related syndrome. After that, the dose of ribavirin was reduced by one tablet of ribavirin (200 mg) per day in these 37 patients. After reduction of ribavirin, 27 of 37 patients were able to continue the combination therapy

for a total of 24 weeks (group A). However, 10 of 37 patients with reduction of ribavirin could not continue combination therapy because their Hb level was <8.5 g/dl or anemia-related severe side effects occurred (group B). The remaining patients completed the combination therapy without severe side effects. In this present study, we assessed the final efficacy and safety after reduction of ribavirin. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions, to each patient, who later gave his/her informed consent for participation.

A sustained virological response (SVR) to the combination therapy was defined as undetectable serum HCV-RNA 24 weeks after termination of combination therapy, using the qualitative PCR assay (Amplicor HCV version 2.0; Roche Molecular Systems) with a low detection limit of 100 copies/ml. Biochemical response (BR) was defined as normalization of serum ALT but positive HCV-RNA 24 weeks after termination of IFN therapy. Nonresponse (NR) was defined as patients who did not show SVR or BR.

Blood testing

Blood samples were obtained just before and 24 weeks after combination treatment. The samples were stored at -80°C until analyzed. Using these blood samples, HCV-RNA levels before combination therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems);¹¹ HCV-RNA 24 weeks after combination therapy were analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/ml.¹² HCV genotype was examined by PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously.¹³ Serum ribavirin concentrations were determined by a validated high-performance liquid chromatography/tandem mass spectrometric assay using ^{13}C -ribavirin as an internal standard.^{14,15} The assay was validated with respect to linearity within a range of 50.1–5005 ng/ml, specificity, accuracy (within 15% for all runs), and precision (within 15% for all runs).

Liver histology

Liver biopsy specimens were obtained percutaneously under observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style; Kakinuma, Tokyo, Japan). Baseline liver histopathology of chronic hepatitis before IFN therapy was classified according to the extent of fibrosis, into three stages: mild (periportal expansion),

Table 1. Patient characteristics at baseline based on difference of continuation or discontinuation after reduction of ribavirin

Characteristic	Continuation group	Discontinuation group	P value
<i>n</i>	27	10	
Sex (male/female)	17/10	7/3	0.694
Age (years) ^a	55 (37–62)	54 (42–64)	0.382
Ribavirin dosage/body weight (mg/kg)	11.35 (9.17–13.27)	11.35 (7.34–13.29)	0.937
Stage of liver pathology (F1/F2/F3)	11/11/5	5/3/2	0.710
Activity of liver pathology (A1/A2)	20/7	7/3	0.672
AST (IU/l)	58 (28–171)	62 (29–155)	0.635
ALT (IU/l)	70 (24–288)	100 (28–274)	0.572
Hb (g/dl)	14.1 (12.3–16.4)	14.2 (12–15.6)	0.588
Platelet ($\times 10^4/\text{mm}^3$)	13.8 (7.5–32.5)	18.9 (10.7–48.0)	0.172

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; HCV, hepatitis C virus; F, fibrosis

^aData are median (range)

Table 2. Patient characteristics at and after reduction of ribavirin

Characteristic	Continuation group	Discontinuation group	P value
<i>n</i>	27	10	
Time treated with full dose of ribavirin (day)	55 (7–151)	46 (12–150)	0.473
Total treatment day	166 (161–176)	61 (21–150)	<0.001
Hb at reduction of ribavirin (g/dl) ^a	10.3 (8.5–12.3)	9.4 (8.8–11.5)	0.036
($<10 \geq 10$)	11/16	8/2	
Serum ribavirin level at reduction of ribavirin (ng/ml) ^a	3025 (1710–4333)	3160 (2090–4222)	0.564
Serum ribavirin level after reduction or discontinuation (ng/ml) ^a	2457 (1947–3675) ^b	250 (234–439) ^c	<0.001

^aData are median (range)

^bSerum ribavirin level 4 weeks after reduction of ribavirin

^cSerum ribavirin level 4 weeks after discontinuation of ribavirin

moderate (portoportal septa), and severe (portocentral linkage or bridging) fibrosis.¹⁶

Statistical analysis

Baseline characteristics and treatment differences between groups were analyzed using Fisher's exact test (two-tailed) or Wilcoxon rank sum test, as appropriate. Correlation between serum ribavirin level and average urinary pH or decrease of hemoglobin was analyzed by Pearson test. A *P* value <0.05 was selected to indicate statistical significance. The SPSS software package (SPSS Inc., Chicago, IL, USA) was used for analyses.

Results

Safety profile

Six of 10 patients who discontinued the combination therapy stopped because their Hb level was <8.5 g/dl; the remaining 4 of 10 stopped because of anemia-related side effects such as orthostatic hypotension.

Difference of background based on the presence or absence of discontinuation of combination therapy after reduction of ribavirin

Tables 1 and 2 show the difference of background based on the presence or absence of discontinuation of combination therapy after reduction of ribavirin. There were no significant differences in characteristics in patients of groups A and B before combination therapy (Table 1). Table 2 shows patient characteristics at and after reduction of ribavirin. With respect to Hb level at the time of ribavirin reduction, a rate of continuation of therapy in patients with ≥ 10 g/dl Hb was higher than that in patients with <10 g/dl (*P* = 0.036). That is, the discontinuation rate of combination therapy was more than 40% in patients with Hb level <10 g/dl at reduction of ribavirin (Fig. 1).

Next, serum ribavirin concentration [median (range)] 4 weeks after reduction of oral ribavirin 200 mg was 2457 (1296–3581) ng/ml in the patients who with reduction of ribavirin could continue the therapy for a total of 24 weeks. In contrast, the serum ribavirin concentration 4 weeks after discontinuation of combination therapy was 234 (128–439) ng/ml in patients who could not continue the therapy after reduction of ribavirin.

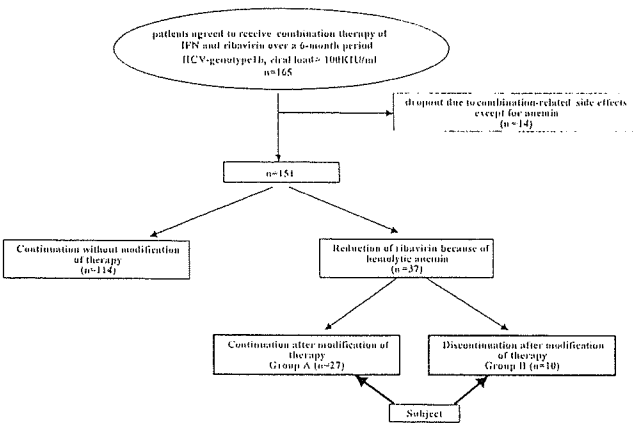


Fig. 1. Flowchart for this trial

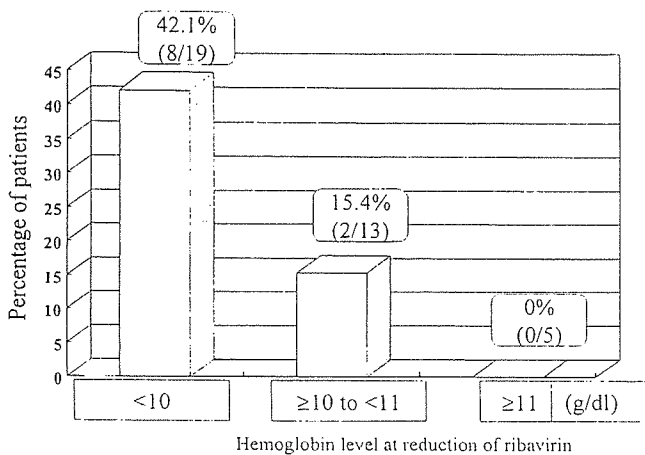


Fig. 2. Relationship between discontinuation of combination therapy and hemoglobin level at time of ribavirin reduction

Table 3. Sustained virological response (SVR) and biochemical response (BR) rates based on serum ribavirin level at 8 weeks after initiation of combination therapy

Group	Treatment	SVR ^a	BR ^a
A	Continuation after reduction of ribavirin	29.6% (8/27)	14.8% (4/27)
B	Discontinuation after reduction of ribavirin	10% (1/10)	0% (0/10)

^aData are percentages (number of patients who showed SVR/total number)

Efficacy of treatment based on the presence or absence of discontinuation and modification of combination therapy

Table 3 shows the efficacy of treatment based on the presence or absence of discontinuation and modification of combination therapy. When patients with reduction of ribavirin could continue the therapy for a total of

24 weeks, the efficacy of treatment was similar to that of the completion group without reduction and/or discontinuation of therapy. In contrast, when patients with reduction of ribavirin could not continue the therapy, the SVR rate of treatment was low.

Efficacy of treatment in patients without discontinuation and modification of combination therapy

In 114 patients of HCV-genotype 1b and high virus load without discontinuation and reduction of combination therapy, 22.8% (26/114) had SVR and 17.5% (20/114) had BR.

Discussion

Several investigators have reported that IFN and ribavirin combination therapy is effective in reducing and eliminating HCV-RNA levels compared with IFN monotherapy.¹⁻⁷ However, various studies have investigated the adverse events of hemolytic anemia. Hemolytic anemia is one of the major adverse events of the combination therapy of interferon-ribavirin. Hemolytic anemia often causes discontinuation and reduction of combination therapy. It is an important problem to decide the timing of ribavirin reduction in patients with hemolysis. In the present study, we assessed when the reduction of ribavirin should take place with respect to efficacy and side effect-related signs. We selected patients with chronic hepatitis C genotype 1b who had high pretreatment viral loads and specifically showed poor response to IFN monotherapy relative to that of patients with other genotypes or lower viral load. HCV-genotype 1 is the most common genotype in Japan¹⁷ as well as in many European¹³ and Western countries.¹⁸

In the present study, 37 of 160 (23.1%) patients had reduction and/or discontinuation of ribavirin. SVR in patients who had reduction of ribavirin and could continue the modified combination therapy was similar to that in patients without discontinuation and reduction of combination therapy. In general, the half-time of serum ribavirin is about 300h; therefore, serum ribavirin concentration 4 weeks after discontinuation of combination therapy is estimated as <500 ng/ml. In contrast, serum ribavirin concentration 4 weeks after reduction of ribavirin was 2000–3000 ng/ml. In our previous study, we reported that SVR was high in patients with high ribavirin concentrations at 4 or 8 weeks after the initiation of combination therapy.^{6,7} In patients with reduction of ribavirin, serum ribavirin levels had a tendency to show a high median level of ≥3000 ng/ml before reduction of ribavirin and to maintain 2000–

3000 ng/ml 4 weeks after reduction of ribavirin. From these results, to increase the eradication rate of serum HCV-RNA, it might be necessary for the serum ribavirin level to reach a high serum level ≥ 3000 ng/ml at 8 weeks after the initiation of combination therapy and to be maintained 2000–3000 ng/ml after that.

In patients who received reduction of ribavirin and could continue combination therapy for a total of 24 weeks, the SVR and BR rate was similar to that of patients without reduction and discontinuation. Thus, when ribavirin-related side effects of hemolytic anemia appear, it is reasonable to reduce the dose of ribavirin at an early stage of the side effects to prevent discontinuation of the combination therapy.

Recently, novel long-acting formulations of IFN known as pegylated IFN induced high SVR rates.¹⁹ Moreover, a recent report indicated that ribavirin in combination with pegylated IFN yields further improvements in SVR exceeding 50%.^{20,21} In combination therapy of pegylated IFN and ribavirin, it will be important to prevent discontinuation of therapy based on therapy-related side effects such as hemolytic anemia.

In conclusion, reduction of ribavirin at hemoglobin level >10 g/dl is suitable in terms of efficacy and side effects of combination therapy.

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Sustained Negativity for HCV-RNA over 24 or More Months by Long-Term Interferon Therapy Correlates with Eradication of HCV in Patients with Hepatitis C Virus Genotype 1b and High Viral Load

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Key Words

Chronic hepatitis C · Long-term interferon therapy ·
HCV-genotype 1b

Abstract

Objective: We assessed whether sustained negativity for HCV-RNA over 24 or more months by long-term interferon (IFN) therapy correlates with eradication of HCV in patients with hepatitis C virus genotype 1b and high viral load or not. **Methods:** The number of patients with HCV-genotype 1b and high viral load exceeding 1 Meq/ml who received 6 MU of natural IFN- α daily for 2–8 weeks, followed by three times/week for 16–22 weeks and negativity for HCV-RNA during IFN administration was 403. Forty-one of 403 patients received 6 MU of natural IFN- α three times/week for more than 18 months after the initial IFN therapy (long-term-IFN-group). Three hundred and two patients did not receive any IFN treatment for 6 months after the termination of the 6-month course (6-month-IFN-group). Sustained virological response (SVR) was defined as negative HCV-RNA at both 3 and 6 months after the completion of IFN therapy. **Results:** SVR

was noted in 73.2% (30/41) of long-term-IFN-group and 18.2% (55/302) of 6-month-IFN-group. Multivariate analysis showed that long-term IFN therapy was the most significant contributor to SVR ($p < 0.0001$). **Conclusion:** Sustained negativity of HCV-RNA for 24 or more months by long-term IFN therapy correlated with SVR in patients with genotype 1b and high viral load.

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Introduction

Chronic hepatitis C is a slowly progressive liver disease that could evolve into liver cirrhosis or hepatocellular carcinoma (HCC) [1–3]. It has been reported that clearance of hepatitis C virus (HCV) or normalization of serum alanine aminotransferase (ALT) after interferon (IFN) therapy contribute to the notably suppressed incidence of HCC caused by chronic HCV infection [4–14]. Previous studies have identified various factors that could predict the response to IFN, including a high response (e.g. low HCV RNA level, HCV genotype 2a, short duration of the disease, and absence of cirrhosis) and low response (e.g. high

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HCV RNA level and genotype 1b) [15–18]. In Japan, approximately 50–60% of patients with chronic HCV infection exhibit genotype 1b and have a high level of serum HCV-RNA (>1 mega equivalents/ml, Meq/ml) [19].

Recent studies demonstrated the positive effects of new treatments for HCV infection, such as the combination of IFN-ribavirin, IFN-alfacon-1 (consensus IFN), and pegylated-IFN [20–27]. In Japan, however, the HCV-RNA clearance rates by these new treatments were at most about 20–50% in patients with HCV-genotype 1b and a high virus load. Despite the low HCV clearance rate by IFN therapy in patients with genotype 1b and high level of serum HCV-RNA, the serum level of HCV-RNA is often negative when determined by reverse transcription nested polymerase chain reaction (RT-nested PCR) during IFN administration. However, many patients relapse after termination of IFN therapy. Recently, several centers reported an increase in the frequency of responders among patients on prolonged IFN therapy [28–32].

The present study was designed to further clarify this point, focusing specifically on the efficacy of prolonged IFN therapy in patients with genotype 1b and a high viral load. That is, we conducted this clinical trial to determine the significance of an additional long-term course of IFN in patients who were HCV-RNA negative during a 6-month course of IFN therapy. A retrospective study was used to examine the efficacy of prolonged IFN therapy in the present trial.

Methods

Patients and Treatment Protocol

A total of 403 patients with chronic hepatitis C satisfied the following conditions in our hospital from 1993 to 2000: (1) Who had HCV-genotype 1b and serum HCV-RNA levels greater than the ≥ 1 Meq/ml as determined by the branched DNA probe assay (version I or II) before IFN therapy. (2) Who had average ALT greater than the upper normal limit (ALT normal range, 12–50 IU) for more than 3 months before the initial course of IFN treatment. (3) Who had histological evidence of chronic hepatitis within 1 year before the IFN administration. (4) Who hadn't been given corticosteroids, immunosuppressants, or antiviral agents used within 6 months before IFN therapy. (5) Who received 6 MU of natural IFN- α (human lymphoblastoid IFN; Sumitomo pharmaceuticals, Tokyo, Japan) intramuscularly daily for 2–8 weeks, followed by three times/week for 16–22 weeks and had negative serum HCV-RNA level by RT-nested PCR [33] during the 6-month course of IFN therapy. (6) Who were negative for hepatitis B surface antigen (HBsAg) or hepatitis virus DNA (HBV-DNA) in the serum, as determined by radioimmunoassay and spot hybridization. (7) Who were negative for antinuclear antibodies (ANA) or antimitochondria antibodies

(AMA) in the serum as determined by immunofluorescence on rat liver and kidney.

Patients with the following conditions were excluded from the study: (1) HCC or severe liver failure; (2) pregnant women; (3) febrile patients with leukocyte counts $< 3 \times 10^3$ cells/ μ l and/or platelet count $< 1 \times 10^5$ / μ l; (4) patients with renal disorders; (5) patients with past history of hypersensitivity reactions to biological preparations such as vaccines.

Forty-one of these 403 patients received continuous treatment of 6 MU of natural IFN- α three times weekly for more than another 18 months after the initial 6-month IFN therapy (long-term IFN group). In long-term IFN group, the initial course of IFN treatment consisted of 6 MU of natural IFN- α given according to one of two schedules. In 31 patients, the daily natural IFN- α was administered for 8 weeks, followed by IFN three times a week for 16 weeks. In another ten patients, the daily natural IFN- α was administered for 2 weeks, followed by IFN three times a week for 22 weeks. Three hundred and two of 403 patients did not receive any IFN treatment for 6 months after the termination of the 6-month course (6-month IFN group). In 6-month IFN group, the initial course of IFN treatment consisted of 6 MU of natural IFN- α given according to one of three schedules. In 245 patients, the daily natural IFN- α was administered for 8 weeks, followed by IFN three times a week for 16 weeks. In another 6 patients, the daily natural IFN- α was administered for 4 weeks, followed by IFN three times a week for 20 weeks. In the third group of 51 patients, the daily natural IFN- α was administered for 2 weeks, followed by IFN three times a week for 22 weeks. The remaining 60 of 403 patients were continuously treated with IFN after the initial 6-month course of IFN treatment. However these patients did not receive continuous treatment of 6 MU of natural IFN- α three times weekly for more than another 18 months after the initial 6-month IFN therapy. The study protocol of this clinical trial is shown in figure 1. The physicians in charge explained the purpose and method of the clinical trial, as well as potential adverse reactions, to each patient and informed consent for participation was obtained from all patients. The clinical trial commenced in December 1993 and ended in May 2000. All patients were followed-up monthly for at least 6 months after cessation of IFN therapy, and blood samples were taken during each visit. The criterion of termination of long-term IFN therapy was defined as the attainment of constantly negative HCV-RNA for the period of more than 24 months during IFN therapy. Termination of long-term IFN therapy in the former group was decided by conference between the physician in charge and each patient.

Definition of Response to IFN Therapy

The presence or absence of HCV-RNA and improvement of serum ALT concentrations were evaluated both at 3 and 6 months after cessation of long-term IFN treatment using the following three grades: Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR at both 3 and 6 months after the completion of long-term IFN therapy. Biochemical response (BR) was defined as normalization of serum ALT in the presence of positive HCV-RNA by RT-nested PCR at both 3 and 6 months after cessation of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR or BR.

Blood Testing

Blood samples were obtained just before therapy and stored at -80°C until assayed. Serum ALT concentrations were measured at least once per month for 3 months prior to the initiation of long-term

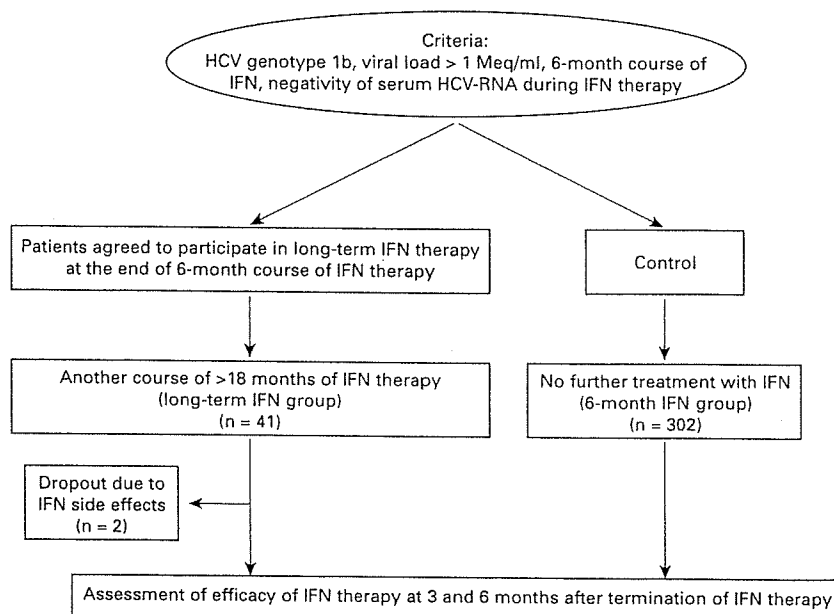


Fig. 1. Study protocol and treatment groups.

IFN therapy, one to four times per month during long-term IFN therapy, and once or twice per month thereafter. HCV-RNA levels before IFN therapy were analyzed at the same time by a branched DNA probe assay (b DNA probe assay, version 2.0, Chiron, Dai-ichi Kagaku, Tokyo) and the results were expressed in Meq/ml [34]. Blood samples obtained during and after long-term IFN therapy were also tested by the RT-nested PCR.

Histopathological Examination of Liver Biopsy

Liver biopsy specimens were obtained percutaneously or at laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo). The histopathological state was determined using the criteria of Desmet et al. [35]. Baseline liver histology of chronic hepatitis prior to IFN therapy was also classified into three stages based on the extent of fibrosis: mild (periportal expansion), moderate (portoportal septa), and severe (portocentral linkage or bridging fibrosis).

Statistical Analysis

The Fisher's exact test or Mann-Whitney U test was used for comparison of group frequencies as appropriate. The efficacy of IFN treatment was assessed by the intention to treat (ITT) analysis. As for Mann-Whitney U test A, a $p < 0.05$ was considered statistically significant. In the Fisher's exact test, a $p < 0.05$ by the two-tailed test was considered statistically significant. The associated factors for attainment of SVR after IFN therapy were examined by logistic regression analysis. Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, Ill., USA).

Results

Baseline Clinicopathological Features of Both Groups

Table 1 summarizes the profiles and laboratory data of patients on long-term IFN therapy and those on 6-month IFN therapy. Both groups had HCV-genotype 1b and serum HCV-RNA level exceeding 1 Meq/ml at entry into the study. There were no significant differences between the two groups with regard to age, sex, liver histology, serum HCV-RNA level, AST, and ALT levels. The rate of IFN retreatment on long-term IFN group was significantly higher ($p < 0.001$) compared with those of 6-month IFN therapy group.

Safety and Tolerability of Prolonged IFN Therapy

Of the 41 patients on prolonged IFN therapy, two (4.9%) stopped the treatment at 9 and 13 months after its commencement due to the appearance of natural IFN- α -related side effects. These included anorexia and general fatigue. These IFN- α -related adverse effects disappeared one month after cessation of long-term IFN therapy. The remaining 39 patients continued to be treated without IFN-related side effects. Four of these 39 patients showed reactivation of HCV-RNA at 6, 7, 10 and 13 months, respectively, on prolonged IFN therapy.

Table 1. Characteristics of patients at study entry

	Long-term IFN group	6-Month IFN group	p value
n	41	302	
Male/female	30/11	196/106	0.162
Age, years*	46 (24–64)	48 (21–67)	0.828
Liver histology staging (F1/F2/F3)	25/12/4	197/99/6	0.378
HCV-RNA, Meq/ml*	9.6 (1.1–54.6)	5.7 (1–64)	0.258
AST, IU/l*	58 (19–366)	59 (16–230)	0.705
ALT, IU/l*	86 (14–699)	96 (16–594)	0.280
History of IFN therapy naïve/retreatment	19/22	261/41	<0.0001
Period of IFN therapy in this study, months	26 (26–68)	6	<0.0001

* Data are expressed as median (range).

Table 2. Effects of long-term IFN therapy examined by the intention to treat analysis

	Outcome of IFN therapy		
	SVR	BR	NR
Long-term IFN group	30/41 (73.2%)	3/41 (7.3%)	8/41 (19.5%)
6-Month IFN group	55/302 (18.2%)	68/302 (22.5%)	179/302 (59.3%)

SVR = Sustained virological response; BR = biochemical response; NR = non-response.

Table 3. Analysis of predictors of sustained virological response (SVR) after IFN therapy

	SVR (n = 85)	Non-SVR (n = 258)	p value
Sex (male/female)	61/24	161/97	0.065
Age, years*	45 (27–64)	47 (21–67)	0.111
Liver histology staging (F1/F2/F3)	60/21/4	162/90/6	0.169
HCV-RNA, Meq/ml*	3.8 (1–29)	6.3 (1–64)	0.009
AST, IU/l*	65 (19–366)	60 (16–144)	0.109
ALT, IU/l*	105 (14–807)	90 (16–594)	0.060
Duration of IFN therapy (>24 months/6 months)	30/55	11/247	<0.0001

* Data are expressed as median (range).

Efficacy of Prolonged IFN Therapy

Table 2 compares the efficacy of IFN in long-term IFN therapy group to that of a 6-month course. The efficacy of IFN therapy was estimated based on ITT analysis. SVR was noted in 30 of the 41 (73.2%) patients on long-term IFN therapy and in 55 of the 302 (18.2%) of the 6-month course of IFN therapy.

Predictive Factors for Virological Response

A total of 85 patients were confirmed to show SVR at 6 months after the completion of IFN therapy. In the next step, we determined the predictive factors for SVR. The following factors were evaluated: age, sex, liver histology, viral load, transaminase, and type of protocol of IFN treatment (table 3). Univariate analysis showed that long-

Table 4. Factors associated with SVR after IFN therapy by multivariate analysis

Factors	Category	Odds ratio	95% CI	p value
Duration of IFN	long term 6 months	11.61	4.50–29.94	<0.0001
HCV-RNA	<5 Meq/ml ≥ 5 Meq/ml	2.88	1.32–5.87	0.0051

CI = Confidence interval.

term IFN therapy and low level of HCV-RNA were significant factors that contributed to SVR. Because the variables were mutually correlated; multivariate logistic regression analysis was performed with two statistically significant variables in the univariate analysis. As a result, the multivariate analysis showed that the period of IFN administration was the most important factor for attainment of SVR. That is, the risk ratio for SVR appearance in patients treated with more than 24 months (long-term treatment group) was 11.61 compared with patients treated with IFN for 6 months (table 4).

Discussion

Many investigators have reported that IFN therapy is effective in reducing serum levels of ALT, reducing/eliminating HCV-RNA level, improving liver histology and reducing the incidence of HCC in patients with chronic hepatitis C [4–14]. However, clearance of HCV-RNA was achieved in only 30–40% of patients who received a 6-month course of IFN therapy. Moreover, in patients with HCV-genotype 1b and a high viral load exceeding 1 Meq/ml by the DNA probe assay, the clearance of HCV-RNA was even lower (achieved in only 10%) [11, 18, 32]. However, genotype 1b is the predominant genotype in Japan [19], similar to many European [36] and Western countries [37]. Therefore, there is a pressing need to develop an effective strategy for the treatment of patients with genotype 1b and a high HCV-RNA viral load.

Recent reports indicated that increases in the total dosage and duration of IFN therapy enhance the therapeutic efficacy of such treatment [28–32]. However, due to the lack of data on the effects of long-term monotherapy, the optimal duration of IFN in patients who initially fail to respond to such treatment is yet to be determined. We examined here the efficacy of long-term IFN therapy in patients who showed HCV-RNA negativity during the

cycle of IFN therapy within a 6-month course. Our results showed that attainment of consistently negative serum HCV-RNA for a period of more than 24 months by long-term IFN therapy correlates significantly with SVR. In general, patients with genotype 1b and a high viral load are often negative for HCV-RNA in the serum during IFN therapy. However, the relapse rate of HCV-RNA after a 6-month IFN course is high [11, 18]. In the present study, only a few patients showed relapse of HCV-RNA among those who remained negative for HCV-RNA over more than 24 months during long-term IFN therapy. The above results indicated that patients with a high viral titer of HCV-genotype 1b who become HCV-RNA negative after initiation of IFN therapy are highly likely to show SVR following sustained negativity for HCV-RNA in response to long-term IFN therapy. However, since long-term IFN therapy can be associated with increased chance of development of adverse effects and is costly, selection of patients for long-term IFN therapy is extremely important.

The two-drug regimen of IFN and ribavirin enhances sustained viral response rates. Despite the increased efficacy, such combination therapy is also associated with serious adverse effects, particularly those associated with ribavirin, e.g. anemia, teratogenesis. Therefore, although the combination therapy of IFN and ribavirin is the first choice therapy in patients with genotype 1 and high viral load, viral eradication could be achieved at least in some patients with long-term IFN therapy.

In conclusion, we have demonstrated in the present study that attainment of persistent negativity for HCV-RNA for a period of more than 24 months during long-term IFN therapy correlates significantly with SVR.

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Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan

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Editorial on page 1019

Key words: interferon, HBV, HBeAg, genotype

Background. In Japan, there are few studies of long-term (more than 1 month) interferon (IFN) therapy for chronic hepatitis B (CHB). In this retrospective study, we investigated the efficacy and predictors of response to 6-month IFN therapy. **Methods.** We analyzed 66 Japanese patients with CHB who were treated with IFN for 6 months. They comprised patients who were hepatitis B e antigen (HBeAg)-positive ($n = 45$) and -negative ($n = 21$). One (2%), 8 (12%), and 51 (77%) patients were infected with hepatitis B virus (HBV) genotypes A, B, and C, respectively. Responders in patients positive for HBeAg were defined as those who showed normalization of serum alanine aminotransferase (ALT) level, HBeAg loss, and HBV DNA negativity at 6 months after completion of IFN therapy. In patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at the same 6-month time point. **Results.** Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Young patients, especially those with a high serum ALT level, were significantly more likely to respond to IFN therapy. Of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in clinical characteristics between responders and nonresponders among patients negative for HBeAg. Multivariate analyses identified HBeAg negativity and young age as independent factors associated with a positive response to 6-month IFN therapy. However, long-term follow-up of the treated patients showed a fall in the response rate. **Conclusions.** The response rate to 6-month IFN therapy among HBeAg-positive patients was low. However, young patients may require long-term IFN therapy.

Introduction

Hepatitis B virus (HBV) infection is a common disease that can lead to a chronic carrier state, and it is associated with the risk of development of progressive disease and hepatocellular carcinoma.¹ Interferon (IFN) and lamivudine are two currently approved treatments for chronic hepatitis B (CHB) in most countries.² IFN is associated with significant adverse effects, whereas long-term therapy with lamivudine may result in drug resistance. A metaanalysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN- α at doses of 5–10 million units (MU), administered at intervals ranging from daily to three times weekly, for 4–6 months.³ Loss of hepatitis B e antigen (HBeAg) occurred in 33% of the treated patients compared with 12% of controls. Loss of detectable HBV DNA and normalization of alanine aminotransferase (ALT) level were also more common in treated than control patients. The major pretreatment factors that correlated with a response were high ALT levels,^{4–6} low HBV DNA,^{4,5} female sex, and greater degrees of activity and fibrosis on liver biopsy.² However, the optimal duration of IFN therapy for CHB is not well established. Moreover, in the 1990s in Japan, the duration of IFN therapy was mainly 1 month, and the efficacy was limited.^{7–9}

Recently, HBV genotypes have been implicated in HBeAg seroconversion as well as response to antiviral treatment. Genotype A was found to be associated with a higher rate of IFN-induced HBeAg seroconversion than genotype D in a study of 64 German patients with HBeAg-positive CHB.¹⁰ Another study, of 58 Taiwanese patients who received IFN treatment for HBeAg-positive CHB, found that patients with genotype B had

a significantly higher rate of HBeAg loss compared with those with genotype C.¹¹ Moreover, Sugauchi et al.¹² proposed that genotype B could be provisionally classified into a Ba ("a" for Asia) subgroup and a Bj ("j" for Japan) subgroup, and that such virological differences could explain the clinical differences in various Asian countries. Our previous study indicated that, in Japan, the proportions of HBV infection associated with genotypes B and C were 9% and 88%, respectively.¹³ Our study also showed that the majority of genotype B patients were HBeAg-negative at the first examination and showed a mild degree of hepatic fibrosis, while genotype C infection was associated with progressive liver fibrosis.¹³ Therefore, mainly patients with genotype C of CHB have received antiviral treatment in Japan.

In Japan, there are few studies of long-term (more than 6 months) IFN therapy for CHB. The present study was designed to re-examine retrospectively the efficacy of 6-month IFN therapy and to determine the potential predictors of a positive response to IFN treatment in Japan.

Patients and methods

Patients

We retrospectively studied 66 Japanese adult patients (19 women and 47 men) who commenced IFN treatment between June 1988 and October 2002 at the Department of Gastroenterology of Toranomon Hospital (Table 1). All patients were followed up from the commencement of therapy at our hospital. They all were positive for hepatitis B surface antigen (HBsAg) in the serum for more than 6 months. Causes of hepatitis other than HBV were excluded, such as infection with hepatitis C virus, as well as autoimmune hepatitis. None of the patients had a history of drug abuse or alcoholic hepatitis, and none had received lamivudine therapy before the commencement of IFN.

Interferon therapy and assessment of response to therapy

Patients received 3 to 12 Mega Units (MU) of IFN- α or - β (Sumiferon; Sumitomo Pharmaceutical, Osaka, Japan; Canferon A; Takeda Chemical Industries, Osaka, Japan; Intron A; Schering-Plough, Osaka, Japan; or Feron; Toray, Tokyo, Japan). The regimen in 36 patients was two or three times a week for 6 months, while that applied for the remaining 30 patients was daily for 4 or 8 weeks, followed by three times a week for 20 or 16 weeks. The duration of treatment was 6 months (23–26 weeks) and the median total dose of IFN was 363 MU (Table 1). In patients with HBeAg, responders were defined as those patients who showed

Table 1. Characteristics of patients at commencement of interferon therapy

Demographic data	
Total number of patients	66
Sex (female/male)	19/47
Age (years) ^a	36 (21–61)
Family history of liver disease	37 (56%)
Previous interferon treatment	13 (20%)
Total dose of interferon (Mega Units) ^a	363 (120–1892)
Duration of treatment (weeks) ^a	24 (23–26)
Laboratory data	
Aspartate aminotransferase (IU/l) ^a	87 (30–755)
Alanine aminotransferase (IU/l) ^a	169 (47–802)
Bilirubin (mg/dl) ^a	0.8 (0.3–1.8)
Albumin (g/dl) ^a	3.9 (3.1–4.8)
Staging of liver history (F1/2/3/4/ND) ^b	37/15/6/2/6
Serum HBV DNA ^c (bDNA; Meq/ml) ^a	41.5 (0.5–4000)
HBeAg-positive	45 (68%)
HBV genotype (A/B/C/unknown)	1/8/51/6

^aData values are medians (ranges)

^bScores could range from 0 to 4; a score of 4 indicates liver cirrhosis. ND, not done

^cHBV DNA levels were measured by branched-chain DNA probe assay (bDNA). All HBV DNA values below the lower limit of detection ($<0.7 \times 10^6$ viral genomic equivalents/ml) were set to 0.5 and those over the upper limit of detection ($>3800 \times 10^6$ viral genomic equivalents/ml genomic equivalents) were set to 4000 for calculation purposes

normalization of serum ALT level (normal level, 6–50 IU/l), HBeAg loss, and HBV DNA negativity at 6 months after the completion of IFN therapy. On the other hand, in patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at 6 months after the completion of IFN therapy. All patients except for responders were considered nonresponders.

Blood tests and serum viral markers

Routine biochemical tests were performed, using standard procedures, before and at least once every month during therapy. Serial blood samples were taken from some patients before and during therapy and were stored at -80°C until used for measuring HBV DNA. HBsAg, HBeAg, and anti-HBe were determined by radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA). HBV DNA was measured by branched-chain DNA probe assay (bDNA; Chiron Laboratory Service, Van Nuys, CA, USA); the lower limit of detection of this assay is 0.7×10^6 viral genomic equivalents/ml (0.7 Meq/ml).

HBV genotype

The six major genotypes of HBV (A, B, C, D, E, and F) were determined by enzyme-linked immunosorbent assay (ELISA; HBV Genotype EIA, Institute of Immu-

nology, Tokyo, Japan) according to the method described by Usuda et al.¹⁴ This method involves the use of monoclonal antibodies directed against five epitopes, which are exposed on the product of the preS2 region of the HBV genome. Because the expression of the five preS2 epitopes is influenced by the HBV genotype, their combination enables determination of the genotypes serologically. Thus, the genotype was determined as A to F. The validity of this ELISA for serological determination of the five HBV genotypes has been verified previously.¹⁴

Subgroups Ba and Bj of genotype B were determined by a polymerase chain reaction (PCR) method. For this purpose, DNA was extracted from 100 µl of serum. The first PCR for detection of the precore and core region (nucleotide [nt] 1690 to 2600) of HBV DNA was performed using primers BJF3 (5'-CCGACCTTGAGGCATACTTC-3'; sense) and BJR4 (5'-GGGTCCCACA AATTGCTTAC-3'; antisense) under conditions of initial denaturation for 4 min, 35 cycles of amplification at 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and 72°C for 7 min. The second PCR reaction was performed under the same reaction conditions, using primers BJF1 (5'-GCTGTGCCTTGGGTGGCTTTG-3'; sense) and BJR2 (5'-GCGACGCGGTGATTGAGACCT-3'; antisense). The amplified PCR products were purified and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Japan, Tokyo, Japan). Phylogenetic analysis was performed by the following method. The total numbers of synonymous and non-synonymous substitutions among nucleotide sequences were estimated by the method of Gojobori et al.¹⁵ Using this number, a phylogenetic tree was constructed by the neighbor-joining method.¹⁶ Genotype B subgroups (Ba and Bj) were determined by these results.

Statistical analysis

Differences between groups were examined for statistical significance using the χ^2 test or Fisher's exact test and the Mann-Whitney *U*-test where appropriate. The above calculations were performed using StatView software (version 4.5J; Abacus Concepts, Berkeley, CA, USA). Independent predictive factors associated with response to IFN treatment were determined using multivariate multiple logistic regression. The following 12 potential predictors of the response to IFN treatment were assessed in this study: age, sex, family history of an HBV carrier, pretreatment with IFN, IFN total dose, method of IFN administration, HBV genotype, severity of liver disease (mild fibrosis [F1] or not [F2–4]), aspartate aminotransferase (AST), ALT, HBeAg, and HBV DNA level. All factors found to be at least marginally

associated with response to IFN therapy ($P < 0.15$) were entered into the multivariate multiple logistic regression analysis. Multivariate multiple logistic regression was performed using the Windows SPSS software package (SPSS, Chicago, IL, USA). The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Study population

One (2%), 8 (12%), and 51 (77%) patients were infected with HBV genotypes A, B, and C, respectively. The genotype in the remaining 6 patients could not be determined. The baseline characteristics of the patients are shown in Table 1. Although the numbers of patients with genotypes A and B were small, the distribution of HBV genotype was similar to that in patients with chronic hepatitis B who received care in our hospital, with a follow-up period of more than 2 years.¹³ The 1 patient with genotype A, 1 of the 8 with genotype B, 37 of the 51 with genotype C, and all 6 with unknown genotype had HBeAg at the commencement of treatment. Seven of the 8 patients with genotype B were Bj and the other was the Ba type. At the commencement of IFN therapy, the ALT levels of 2 patients (both HBeAg-positive) were normal, and the HBV DNA levels of 9 patients (3 who were HBeAg-positive and 6 who were-negative) were less than 0.7 Meq/ml. However, these parameters in the above patients had been elevated within the 3 months before the treatment started.

Response to interferon therapy

Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Table 2 shows the demographic and clinical characteristics of responders and non-responders in these patients with HBeAg. Patients of younger age or higher ALT level had significantly higher rates of antiviral response to IFN than the others. Other characteristics were not related to the response to therapy. The one patient with HBeAg of genotype B (Bj) responded to IFN therapy.

On the other hand, of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in the clinical characteristics of responders and non-responders in this group (Table 3). Among the patients negative for HBeAg, the genotype (B or C) did not correlate with response to IFN therapy. The single patient with genotype Ba was a non-responder. Of the 6 patients with genotype Bj, 4 were responders while the remaining 2 were non-responders.

Table 2. Analysis of predictors of response to interferon therapy in patients positive for HBeAg

	Responders (n = 9)	Non-responder (n = 36)	P value
Sex (female/male)	4/5	11/25	0.45
Age (years) ^a	30 (21–35)	39 (23–59)	0.0048
Family history of liver disease	5 (56%)	22 (61%)	1.0
Previous interferon treatment	1 (11%)	8 (22%)	0.66
Total dose of interferon (MU) ^a	408 (120–1892)	408 (120–774)	0.80
Method of interferon administration (ED + I/I) ^b	5/4	21/15	1.0
Staging of liver history (F1/2/3/4/ND)	3/1/1/1/3	21/9/4/0/2	0.11
ALT (IU/l) ^a	263 (126–500)	149 (47–701)	0.049
Serum HBV DNA (bDNA; Meq/ml) ^a	7.9 (0.5–4000)	303 (0.5–4000)	0.18
HBV genotype (A/B/C/unknown)	0/1/7/1	1/0/30/5	0.12

^aData values are medians (ranges)^bED + I, initially every day following intermittent therapy; I, only intermittent therapy**Table 3.** Analysis of predictors of response to interferon therapy in patients negative for HBeAg

	Responders (n = 13)	Non-responders (n = 8)	P value
Sex (female/male)	3/10	1/7	1.0
Age (years) ^a	42 (30–60)	37 (28–61)	0.54
Family history of liver disease	6 (46%)	4 (50%)	1.0
Previous interferon treatment	3 (23%)	1 (13%)	1.0
Total dose of interferon (MU) ^a	219 (129–624)	152 (120–533)	0.45
Method of interferon administration (ED + I/I) ^b	3/10	1/7	1.0
Staging of liver histology (F1/2/3/4/ND)	6/4/1/1/1	7/1/0/0/0	0.36
ALT (IU/l) ^a	185 (58–712)	153 (54–802)	0.66
Serum HBV DNA (bDNA; Meq/ml) ^a	7.7 (0.5–770)	1 (0.5–47)	0.32
HBV genotype (A/B/C/unknown)	0/4/9/0	0/3/5/0	1.0

^aData values are medians (ranges)^bED + I, initially every day following intermittent therapy; I, only intermittent therapy

Two patients with normal ALT levels at commencement were non-responders. Of six HBeAg-negative patients with undetectable levels of HBV DNA at commencement, three were responders. On the other hand, of three HBeAg-positive patients with undetectable levels of HBV DNA at commencement, one patient was a responder.

Long-term outcome after IFN therapy

In this study, the median follow-up period after IFN therapy was 2 years (range, 0.5–6 years). We analyzed long-term outcome in 31 patients positive for HBeAg and 18 patients negative for HBeAg, in whom the follow-up period was 1 year or more. In the 31 HBeAg-positive patients, the number of responders had decreased from 8 (26%) after 6 months to 5 (16%) at 1–5 years. On the other hand, in the 18 HBeAg-negative patients, the number of responders decreased from 11 (61%) after 6 months to 6 (33%) at 1–6 years.

Evaluation of efficacy of IFN in relation to clinical factors

Data for all patients were subjected to univariate analysis to determine the clinical factors that contributed to

the efficacy of IFN treatment. In this analysis, the following two factors significantly influenced the response to IFN: HBeAg negativity (OR, 6.5; 95% CI, 2.1–20.4; $P = 0.0013$) and low HBV DNA level (<100 vs ≥ 100 Meq/ml; OR, 3.4; 95% CI, 1.0–10.8; $P = 0.043$). Moreover, ALT level (≥ 200 vs <200 IU/l) and age (≤ 35 vs >35 years of age) showed borderline significance with a higher chance of response among all patients ($P = 0.056$ and $P = 0.082$, respectively). Next, we investigated the significance of response to IFN therapy by multivariate logistic regression analysis. Both HBeAg and age independently and significantly influenced the outcome of IFN therapy (Table 4).

Discussion

Although IFN is reported to have beneficial effects in the treatment of chronic hepatitis B, the response rate is not high. A metaanalysis published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN- α for 4–6 months; loss of HBeAg occurred in 33% of the treated patients.³ In our study, the response rate to IFN among the HBeAg-positive patients was lower than that in the above studies. The reasons for the difference between studies may

Table 4. Factors associated with response to interferon therapy

Variable	Multivariate odds ratio	95% Confidence interval ^a	P value
HBeAg (negative vs positive)	11.1	2.7–46.1	0.0009
Age (≤ 35 vs > 35 years)	5.2	1.3–21.0	0.0209

^a Values are the odds of having a response to interferon

be differences in ethnic groups and/or in HBV genotype. Kao et al.¹¹ reported that HBV genotype C, compared to genotype B, was associated with a lower response rate to IFN- α therapy among chronic hepatitis B patients with HBeAg. The response rate among our patients with genotype C was low similar to the results of Kao et al.¹¹ (response rate; 15%). In our study, in the HBeAg-positive patients, young patients, especially those with a high ALT level at baseline, were significantly more likely to respond to IFN. These prognostic factors were similar to those reported in previous studies,^{4–6} although the sample size in our study was small. On the other hand, our previous report¹⁷ showed that 16 of 52 (31%) patients who received IFN- α , given twice per week for 52 weeks, were responders. Therefore, a long-term therapeutic regimen may be necessary to secure a better response.

On the other hand, in the present study the response rate in patients negative for HBeAg was higher than that in those with HBeAg. Previous reports showed that response rates to a 6- to 12-month course of IFN- α in patients with HBeAg-negative CHB ranged from 10% to 47% (average, 24%).^{18–21} Moreover, another previous study of ours²² showed that 9 of 12 (75%) patients who received IFN- β , given twice per week for 24 weeks, responded to the therapy. Considered together, these findings show that the efficacy of IFN in patients negative for HBeAg is high. However, the factors that could predict a sustained response are less well defined in HBeAg-negative patients than in HBeAg-positive patients.² The dose of IFN also had little effect, but the duration of therapy (12 vs 5–6 months) was associated with a doubling of sustained response rates.²³

Our study included two patients with normal ALT levels and 9 patients with undetectable levels of HBV DNA at the commencement of IFN therapy. In these patients, these parameters had decreased by chance at the commencement of IFN therapy, although they had been increased in the 3 months before the treatment. However, the two patients with normal ALT levels at commencement were non-responders. While the response to IFN of patients with normal ALT levels may be poor, that of patients with undetectable levels of HBV DNA at commencement seems similar to other patients. Furthermore, among patients with undetectable levels of HBV DNA at commencement there was

no difference in the response rate of those with and without HBeAg.

In our study, no difference in the response to IFN monotherapy was noted between genotypes B and C in patients without HBeAg. Previous reports showed that HBV genotype B was associated with a higher rate of antiviral response to IFN- α treatment in Chinese patients with HBeAg-positive chronic hepatitis B than genotype C.^{11,24} It is not clear at present whether this phenomenon applies in patients who are negative for HBeAg.

Recently, Sugauchi et al.¹² proposed that genotype B could be provisionally classified into Ba and Bj subgroups. In Japan, Bj is the major group (93% with genotype B) and most patients with Bj are HBeAg-negative (92%).²⁵ In our study, six of the seven patients with genotype Bj were HBeAg-negative. Therefore, it is difficult to investigate whether HBV genotype B is associated with a higher rate of antiviral response to IFN treatment than genotype C in Japanese patients with HBeAg-positive chronic hepatitis B.

In our study, HBeAg negativity and younger age were identified as independent and significant determinants of the outcome of IFN therapy. However, an important issue in the treatment of HBeAg-negative chronic hepatitis B is the sustainability of the response to treatment. Our results, which showed a decrease in the response to treatment at long-term follow-up, were similar to those reported by Papatheodoridis et al.²⁶ In their long-term follow-up of treated patients, it was reported that the sustained response rates decreased from 41% after 6 months to 22% at 2–5 years and thereafter.²⁶ Lampertico et al.²⁷ recently reported that 24-month IFN treatment resulted in sustained disease suppression in a significant proportion of patients with HBeAg-negative chronic hepatitis B.²⁷ Long-term IFN therapy may improve the response to IFN therapy in HBeAg-negative patients. On the other hand, lamivudine, another approved treatment for chronic hepatitis B, requires long-term continuous therapy and could potentially be associated with the development of viral resistance.²⁸ Considering these aspects of treatment modalities, patients of younger age may require long-term IFN therapy.

In conclusion, we investigated the efficacy of 6-month IFN therapy for Japanese patients. The response rate to

IFN among HBeAg-positive patients was low. In this group, patients of young age with high ALT levels were significantly more likely to respond to IFN monotherapy than other patients. On the other hand, the response rate to IFN among HBeAg-negative patients was high. Multivariate analysis identified HBeAg negativity and young age as independent determinants of the outcome of 6-month IFN therapy. Further studies, such as longer-term therapy (over 1 year) may be necessary in order to confirm these findings and establish the true response to IFN therapy.

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