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Low rate of YMDD motif mutations in polymerase gene of hepatitis B virus in chronically infected patients not treated with lamivudine

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Background. Lamivudine is used for the treatment of chronic hepatitis B (CH-B), and exhibits excellent antiviral activity. However, longterm administration increases the likelihood of the emergence of resistant viruses, with an accompanying relapse of hepatitis. However, recent studies have reported lamivudine-resistant viruses in patients with CH-B before such treatment. The aim of this study was to investigate whether YMDD mutants occur in nature. ***Methods.*** The existence of lamivudine-resistant viruses was examined in 20 asymptomatic carriers of hepatitis B virus (ASC), 10 patients who lost hepatitis B surface antigen (HBsAg) during follow-up and in 20 lamivudine-treated patients with and without breakthrough hepatitis. Both polymerase chain reaction (PCR) restriction fragment length polymorphism and SMITEST hepatitis B virus (HBV)-YMDD mutation detection methods were used to detect resistant viruses. ***Results.*** No YMDD mutants were detected in the sera of the 20 ASC at the initial and final medical examinations, nor were YMDD mutants detected in sera collected at the initial medical examination, about 6 months before, or immediately after the loss of HBsAg in the 10 patients. In the 20 patients treated with lamivudine, YMDD mutants were not detected in any of them before treatment, whereas mutants were detected in the sera of 10 patients during treatment. ***Conclusions.*** Our results suggest that lamivudine-resistant YMDD mutant viruses were present in a few patients with HBV infection who before they have been treated with lamivudine.

Key words: hepatitis B virus, lamivudine, YMDD mutant

Introduction

Lamivudine is used for the treatment of chronic hepatitis B, for which it exhibits excellent antiviral activity, and is clinically useful.[1-4] One of the problems with lamivudine therapy, however, is that longterm administration increases the likelihood of the emergence of resistant viruses.[5-10] It has been reported that lamivudine-resistant viruses emerge after more than 6 months' treatment, with accompanying relapse of hepatitis. However, it was reported recently that lamivudine-resistant viruses were present in patients with chronic hepatitis B before such patients were treated with lamivudine.[11] The method for detecting YMDD motif mutation combines polymerase chain reaction (PCR)-enzyme-linked immunosorbent assay (ELISA) and the minisequence method (PCR-enzyme-linked mini-sequence assay [ELMA] method).[12] Of interest subjects found to be infected with mutant viruses before lamivudine therapy were asymptomatic hepatitis B virus carriers (ASC) who were positive for anti-hepatitis B e (HBe) antibody.[11]

The aim of the present study was to determine whether YMDD mutants exist spontaneously in nature. For this purpose, we investigated the existence of lamivudine-resistant viruses in ASC with normal transaminase levels for 10 years or more, irrespective of treatment with lamivudine, and in patients with hepatitis B who had lost hepatitis B surface antigen (HBsAg) and were treated with lamivudine. Studies were conducted using two methods for the detection of YMDD motif mutation; the above PCR-ELMA method and a PCR restriction fragment length polymorphism (PCR-RFLP) method, which was previously reported by Chayama et al.[5]

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Subjects

The study subjects were 50 patients with hepatitis B who were being followed-up at Toranomon Hospital. Three groups were investigated: (1) group A, 20 ASC who showed normal transaminase levels for 10 or more years (aged 25 to 77 years; median, 47.5 years), comprising 8 men and 12 women. Hepatitis B e antigen (HBeAg) was positive in 1 patient and negative in 19 patients, as determined by radioimmunoassay (RIA) or enzyme immunoassay (EIA). None of these patients had been treated with antiviral agents. (2) Group B, 10 patients with hepatitis B who had lost HBsAg during the follow-up (aged 31 to 51 years; median, 43 years), comprising 8 men and 2 women. At the initial medical examination at Toranomon Hospital, HBeAg was positive in 1 patient and negative in 9 patients. The HBeAg-positive patient became antigen-negative during the follow-up. (3) Group C, 20 patients who were treated with lamivudine at Toranomon Hospital, 10 of whom had breakthrough hepatitis and the other 10 who did not. They were aged 26 to 56 years (median, 43 years) and comprised 18 men and 2 women. The patients were treated with lamivudine for 25 to 290 weeks (median, 108.5 weeks), and 7 of the 10 patients who had breakthrough hepatitis were concomitantly treated with interferon (IFN). In patients in group A, serum samples were collected at the initial medical examination and at the final examination during follow-up. In patients in group B, the samples were collected at the initial medical examination, and about 6 months before, and immediately after the loss of HBsAg. In patients in group C who had breakthrough, the samples were collected before lamivudine treatment and at breakthrough (before IFN treatment in IFN-treated patients) and in patients in group C who did not have breakthrough, the samples were collected before lamivudine treatment and at the final examination at the end of lamivudine treatment.

Methods

Detection of YMDD mutant viruses

HBV-DNA was extracted from 100 μl of serum by using SMITEST EX-R&D (Genome Science, Tokyo, Japan). YMDD mutant viruses were detected by a combination of PCR-ELISA and a minisequence method (PCR-ELMA method; SMITEST HBV-YMDD mutation detection kit; Genome Science)[11,12] and by a PCR-RFLP method.[5]

HBV DNA levels were measured by transcription-mediated amplification and hybridization protection assay (TMA-HPA) (Chugai Diagnostics Science, Tokyo, Japan)[13] in patients treated with lamivudine, at baseline and 3 months after commencement of the therapy. The lower and higher limits of detection of this assay are 3.7 and 8.7 log genome equivalents per milliliter (LGE/ml), respectively.

Results

Serum samples obtained from ASC at the initial medical examination were HBV-DNA negative in 2 of the 20 patients and positive (YMDD) in the remaining 18 patients by PCR-RFLP, whereas the serum samples were HBV-DNA-positive (YMDD) in all patients by PCR-ELMA. Serum samples obtained from ASC at the final examination were HBV-DNA-negative in 7 patients and positive (YMDD) in the remaining 13 patients by PCR-RFLP, whereas they were HBV-DNA-negative in 6 patients and positive (YMDD) in 14 patients by PCR-ELMA. No YMDD mutant virus was detected by either method (Table 1).

Sera obtained at the initial medical examination from the patients who had lost HBsAg were HBV DNA-positive (YMDD) in all patients by PCR-RFLP, but were HBV DNA-positive (YMDD) in five of the ten patients and negative in the remaining five patients by PCR-ELMA. Serum samples obtained approximately 6 months before the loss of HBsAg were HBV DNA-negative in four patients and positive (YMDD) in six patients by both PCR-RFLP and PCR-ELMA. The serum samples obtained immediately after the loss of HBsAg were HBV-DNA-negative in four patients and positive (YMDD) in six patients by PCR-RFLP, but they were negative in nine patients and positive (YMDD) in 1 patient by PCR-ELMA. No YMDD mutant virus was detected by either method (Table 2).

Sera obtained from lamivudine-treated patients before such treatment were HBV DNA-positive in all patients by the PCR-RFLP method as well as by PCR-ELMA. Sera obtained during lamivudine treatment were HBV DNA-negative in ten patients by PCR-RFLP, whereas YIDD and/or YVDD was detected in the sera of the remaining ten patients by this method. By PCR-ELMA, the sera were HBV DNA-negative in three patients and positive (YMDD) in seven patients, whereas YIDD and/or YVDD was detected in the sera of ten patients by this method. The YMDD mutant viruses were detected in the same ten patients by both methods, and all of these patients had breakthrough (Table 3). On the other hand, HBV DNA levels were decreased after 3 months of lamivudine treatment in all patients (Fig. 1).

Table 1. Detection of mutant viruses in asymptomatic carriers

No.	Age (years)	Sex	ALT (IU/l)	HBeAg	YMDD motif at initial medical examination			YMDD motif at final examination		
					PCR-RFLP[a]	PCR-ELMA[b]	Date of measurement	PCR-RFLP[a]	PCR-ELMA[b]	Date of measurement
1	77	F	14	−	Negative	YMDD	07/10/1987	YMDD	YMDD	15/11/2000
2	62	F	20	−	YMDD	YMDD	22/06/1983	Negative	YMDD	20/03/1998
3	56	F	12	−	YMDD	YMDD	19/06/1986	YMDD	YMDD	16/02/1999
4	55	F	18	−	YMDD	YMDD	22/06/1983	Negative	Negative	29/05/1998
5	55	M	28	−	YMDD	YMDD	08/03/1984	YMDD	Negative	15/10/1998
6	54	F	24	−	YMDD	YMDD	07/09/1989	Negative	YMDD	24/01/2001
7	53	M	18	−	YMDD	YMDD	23/05/1988	Negative	YMDD	06/12/2000
8	52	F	26	−	YMDD	YMDD	02/07/1986	YMDD	YMDD	21/05/1996
9	51	M	16	−	YMDD	YMDD	30/05/1988	YMDD	YMDD	11/06/1996
10	51	M	16	−	YMDD	YMDD	14/05/1987	Negative	Negative	03/06/1996
11	44	F	18	−	YMDD	YMDD	25/09/1985	YMDD	Negative	01/11/2000
12	37	F	16	−	YMDD	YMDD	08/06/1983	Negative	Negative	13/07/1999
13	37	M	20	−	YMDD	YMDD	25/09/1984	YMDD	Negative	22/12/2000
14	36	M	38	−	YMDD	YMDD	26/06/1980	YMDD	YMDD	29/07/1996
15	30	F	18	+	YMDD	YMDD	13/03/1985	YMDD	YMDD	19/07/2000
16	29	M	26	−	YMDD	YMDD	06/06/1979	YMDD	YMDD	03/07/1996
17	29	F	14	−	YMDD	YMDD	19/12/1984	YMDD	YMDD	21/02/2001
18	29	F	66	−	YMDD	YMDD	12/03/1981	YMDD	YMDD	24/01/1996
19	26	M	16	−	YMDD	YMDD	21/09/1983	YMDD	YMDD	28/02/1996
20	25	F	18	−	Negative	YMDD	06/06/1985	Negative	YMDD	18/10/2000
			Sum[c]		0/20 (0%)	0/20 (0%)		0/20 (0%)	0/20 (0%)	

PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; ELMA, enzyme-linked mini-sequence assay

[a] By the method of Chayama et al.[5]

[b] By SMITEST HBV-YMDD mutation detection kit

[c] Numbers of YMDD mutations/all samples

Table 2. Detection of mutant viruses in patients who showed clearance of HBsAg during treatment

No.	Age (years)	Sex	ALT (IU/l)	HBeAg	YMDD motif at initial medical examination			YMDD motif 6 months before loss of HBsAg			YMDD motif immediately after loss of HBsAg		
					PCR-RFLP[a]	PCR-ELMA[b]	Date of measurement	PCR-RFLP[a]	PCR-ELMA[b]	Date of measurement	PCR-RFLP[a]	PCR-ELMA[b]	Date of measurement
1	31	M	286	+	YMDD	Negative	26/08/1982	Negative	Negative	10/04/1996	YMDD	Negative	21/10/1998
2	43	M	28	−	YMDD	YMDD	15/12/1976	YMDD	YMDD	15/03/1990	YMDD	Negative	28/02/1991
3	43	M	22	−	YMDD	Negative	29/09/1982	Negative	YMDD	20/12/1995	Negative	Negative	28/08/1996
4	38	M	28	−	YMDD	YMDD	25/05/1981	YMDD	YMDD	24/01/1994	Negative	Negative	19/10/1994
5	38	M	66	−	YMDD	Negative	05/02/1987	YMDD	YMDD	30/10/1996	YMDD	Negative	03/03/1999
6	41	M	44	−	YMDD	YMDD	04/11/1988	YMDD	YMDD	29/11/1995	Negative	Negative	24/06/1998
7	51	F	60	−	YMDD	Negative	29/09/1982	Negative	Negative	12/12/1990	YMDD	YMDD	07/08/1991
8	46	F	14	−	YMDD	YMDD	14/10/1991	YMDD	YMDD	28/02/1996	YMDD	Negative	24/06/1998
9	45	M	22	−	YMDD	YMDD	21/01/1987	Negative	Negative	18/01/1994	Negative	Negative	23/08/1994
10	50	M	30	−	YMDD	Negative	14/04/1992	YMDD	Negative	10/11/1998	YMDD	Negative	31/08/1999
				Sum[c]	0/10 (0%)	0/10 (0%)		0/10 (0%)	0/10 (0%)		0/10 (0%)	0/10 (0%)	

[a] By the method of Chayama et al.[5]
[b] By SMITEST HBV-YMDD mutation detection kit
[c] Numbers of YMDD mutations/all samples

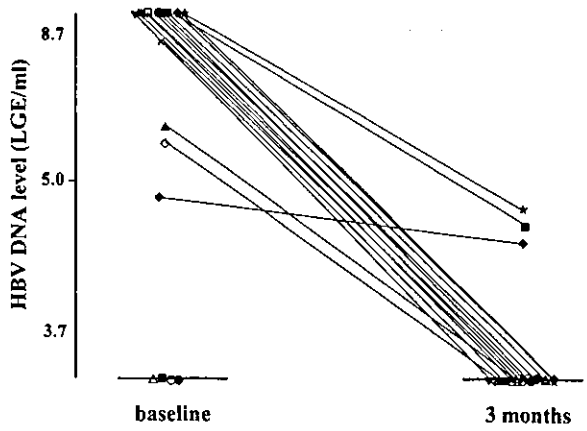

Fig. 1. Hepatitis B virus (HBV) DNA levels in 20 patients at baseline and after 3 months of lamivudine therapy. HBV DNA level was measured by a transcription-mediated amplification and hybridization protection assay (TMA-PHA) method. The lower and higher limits of detection of this assay were 3.7 and 8.7 log genome equivalents per milliliter (*LGE/ml*), respectively (*dotted lines*). *Each symbol* represents an individual patient

Discussion

Lamivudine is widely used for the treatment of hepatitis B mainly in Western and Asian countries. Among the problems associated with lamivudine are the relapse of hepatitis when such treatment is discontinued, which results in the release of HBV replication suppressed by lamivudine, and the emergence of resistant viruses during the treatment.[5–10] The latter factor may cause a relapse of hepatitis, necessitating the concomitant use of other antiviral agents.[14–16] Prediction of the emergence of lamivudine-resistant viruses before treatment with lamivudine would provide clinically useful information. Lamivudine is an antiviral agent that inhibits HBV replication through the suppression of RNA-dependent DNA polymerase. What are the mechanism(s) underlying the appearance of YMDD mutant viruses? One possible mechanism is that YMDD motif mutations represent the induction of a new resistant viral strain(s) to cope with this stress. Another possible mechanism is the selection of minor preexisting resistant strains. Recently, Kobayashi et al.[11] reported that resistant viruses were present in the blood of patients who had never been treated with lamivudine. We attempted to confirm their observation by looking for YMDD mutations in patients with various stages of hepatitis B. We used two methods for the detection of the mutations; one method that was employed by Kobayashi et al.[12] and the other method that we have been using in our hospital.[5] In our present study, no YMDD mutant viruses were detected in any of the patients not treated with lamivudine or in

Table 3. Detection of mutant viruses in patients treated with lamivudine

No.	Age (years)	Sex	ALT (IU/l)	HBeAg	YMDD motif before lamivudine treatment			YMDD motif during lamivudine treatment		
					PCR-RELP[a]	PCR-ELMA[b]	Date of measurement	PCR-RELP[a]	PCR-ELMA[b]	Date of measurement
1	43	M	74	+	YMDD	YMDD	16/11/1995	Negative	Negative	22/06/2001
2	43	F	469	+	YMDD	YMDD	07/12/1995	Negative	Negative	18/06/2001
3	28	M	22	+	YMDD	YMDD	22/09/1998	Negative	YMDD	06/06/2001
4	45	M	926	+	YMDD	YMDD	12/02/1999	Negative	YMDD	06/06/2001
5	45	M	71	+	YMDD	YMDD	06/11/1998	Negative	Negative	11/05/2001
6	45	M	53	−	YMDD	YMDD	07/11/1995	Negative	YMDD	30/05/2001
7	47	M	355	−	YMDD	YMDD	22/01/1996	Negative	YMDD	16/03/2001
8	35	M	79	−	YMDD	YMDD	18/06/1997	Negative	YMDD	20/06/2001
9	38	M	68	−	YMDD	YMDD	19/01/1994	Negative	YMDD	04/04/2001
10	31	F	31	−	YMDD	YMDD	15/03/1999	Negative	YMDD	21/06/2001
11	26	M	204	+	YMDD	YMDD	28/09/1995	YVDD	YVDD	24/10/1996
12	56	M	130	+	YMDD	YMDD	14/11/1995	YIDD + YVDD	YIDD	08/12/1997
13	38	M	182	+	YMDD	YMDD	18/12/1995	YIDD	YIDD	06/01/1997
14	55	M	104	+	YMDD	YMDD	26/01/1996	YIDD + YVDD	YIDD + YVDD	04/04/1997
15	45	M	206	+	YMDD	YMDD	27/10/1999	YIDD + YVDD	YVDD	12/10/2000
16	47	M	109	+	YMDD	YMDD	09/11/1999	YIDD	YIDD	02/10/2000
17	47	M	209	+	YMDD	YMDD	29/11/1999	YIDD + YVDD	YIDD + YVDD	20/02/2001
18	29	M	416	+	YMDD	YMDD	13/01/2000	YIDD	YIDD	09/08/2000
19	42	M	64	+	YMDD	YMDD	06/03/2000	YIDD + YVDD	YIDD	01/11/2000
20	43	M	37	−	YMDD	YMDD	24/11/1995	YVDD	YVDD	16/01/2000
				Sum[c]	0/20 (0%)	0/20 (0%)		10/20 (50%)	10/20 (50%)	

[a] By the method of Chayama et al.[5]
[b] By SMITEST HBV-YMDD mutation detection kit
[c] Numbers of YMDD mutations/all samples

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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Editorial on page 1125

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.

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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 alanme ammotransferase (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/mm^3, platelet count >80000/mm^3, and serum bilirubin <2.0 mg/ml before the initial period of IFN therapy. These 165 patients were given IFN-α2b (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
n	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 ($\times 10^4$/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
[a] Data are median (range)

Table 2. Patient characteristics at and after reduction of ribavirin

Characteristic	Continuation group	Discontinuation group	*P* value
n	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/≥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

[a] Data are median (range)
[b] Serum ribavirin level 4 weeks after reduction of ribavirin
[c] Serum 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 discon tinuation 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.

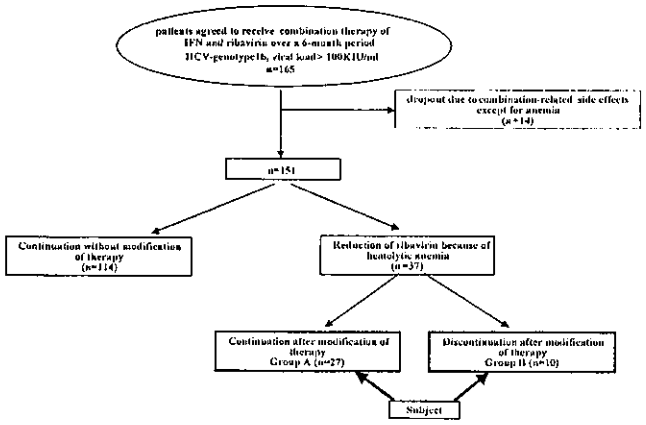

Fig. 1. Flowchart for this trial

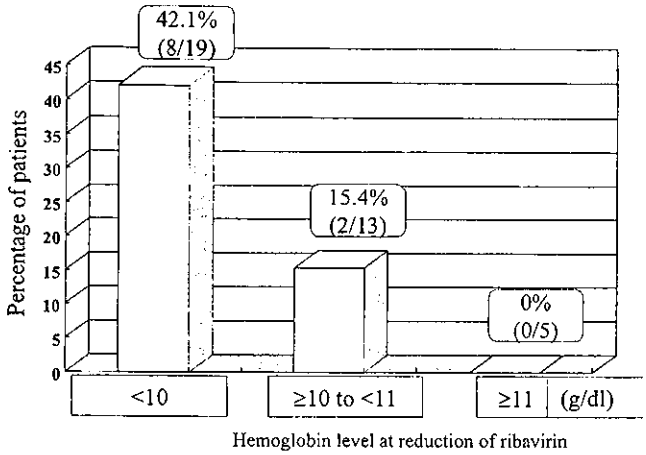

Fig. 2. Relationship between discontinuation of combination therapy and hemoglobin level at time of ribavirin reduction

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)

[a] Data 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 patents 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 Japan[17] as well as in many European[13] 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 300 h; 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–

3000ng/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 ≥3000ng/ml at 8 weeks after the initiation of combination therapy and to be maintained 2000–3000ng/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 >10g/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.

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/μl and/or platelet count $<1 \times 10^5$/μl; (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

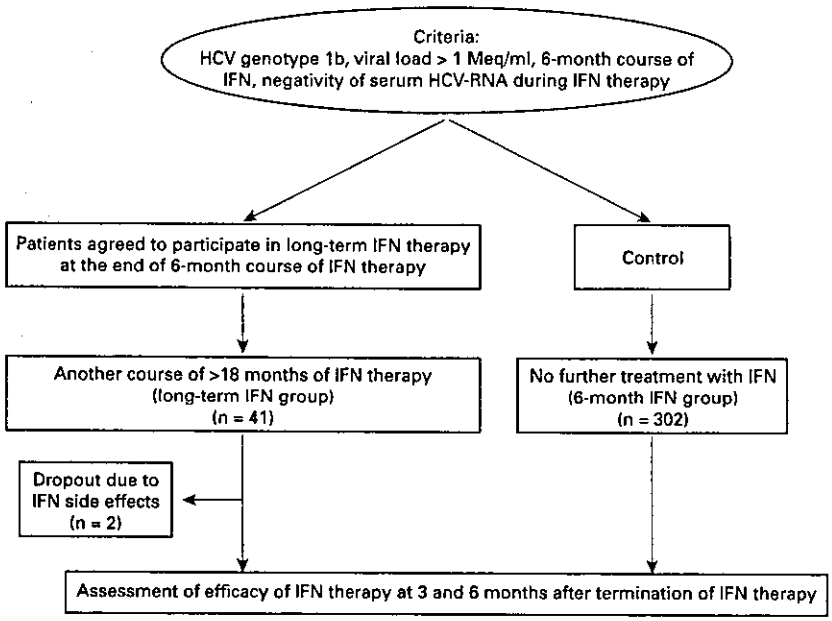

Fig. 1. Study protocol and treatment groups.

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 attaining 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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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.

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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