Original Article

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Nucleic Acid Substitutions and Response to Interferon in HBeAg-positive Chronic Hepatitis B Patients with Genotype C HBV Infection

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Abstract

To elucidate the effect of nucleic/amino acid substitutions on the response to interferon treatment, we retrospectively analyzed 27 HBeAg-positive patients infected with genotype C hepatitis B virus (HBV), who were treated with interferon (IFN). Nucleic/amino acid sequences of the core upstream regulatory sequences (CURS), basic core promoter (BCP), and precore-core regions (nucleotide (nt.) 1643-2452) HBV DNA were determined. Sustained virological response (SVR) was defined as normalization of serum alanine aminotransferase (ALT) level, HBeAg loss, and decrease in HBV DNA level to less than 5 log copies/ml at 24 weeks after the end of treatment. The other patients were defined as having no response (NR). Of the 27 patients, 6 achieved SVR and 21 showed NR. There were no significant differences in clinical characteristics between both groups. The total number of substituted nucleic acids in the CURS and BCP is higher in the patients who achieved SVR (SVR: 4.33, NR: 3.05, p=0.018). Amino acid mutations in the precore-core region were not related to the response to IFN treatment. Our study suggests that nucleic acid sequences in the CURS and BCP may be related to the response to IFN.

Key words

hepatitis B virus, core, precore, genotype, interferon

Introduction

Chronic hepatitis B (CHB) is an intractable disease that sometimes leads to progressive liver disease with advanced fibrosis and hepatocellular carcinoma. Interferon (IFN) and nucleoside analogues (NAs) are the current approved treatments for CHB in most countries. IFN is superior to NAs in that IFN can remit hepatitis even after the withdrawal of treatment[1]. Furthermore, IFN, which mainly act as a immunomodulator, does not induce drug-resistant mutants as seen in the case of NAs[1-3]. Therefore, the role of IFN treatment is still considerable even after the use of NAs.

Although long-term virological and biochemical responses may be expected in IFN treatment, the proportion of patients who exhibit long-term response is not large[3-5]. Furthermore, IFN treatment is accompanied by several adverse effects in most patients[1]. Therefore, prediction of outcome

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before the start of IFN treatment is important.

Previous studies have shown some factors that may affect the treatment efficacy of IFN in CHB treatment. The major pretreatment factors that correlated with long-term response were high ALT levels, low HBV DNA, female sex, greater degrees of activity, and fibrosis on liver biopsy[3)6)].

HBe antigen (HBeAg) has a major role in determining the response to IFN. HBeAg modulates the immune response to HBV[7)] and affects the therapeutic efficacy of IFN. Patients who are positive for serum HBe antigen (HBeAg) respond better to IFN treatment than those who are negative for HBeAg[3-5)].

HBV genotype is another factor that affects treatment efficacy. Studies from Europe show that a higher rate of HBeAg seroconversion following IFN treatment was found in those infected with genotype A than in those with genotype D[8-10)].

The rate of HBeAg loss was also significantly higher in patients with genotype B than in those with genotype C in Taiwanese studies[11) 12)].

In addition to HBeAg and HBV genotype, variations in nucleotide sequences have been shown to affect the response to IFN. Some reports have shown that patients with nucleic acid mutations at nt. 1762/1764 in the basic core promoter (BCP) region respond better to IFN treatment[9)]. Another report has shown that patients with nucleic acid substitution at nt. 1896 in the precore (preC) region, which terminate HBeAg translation, have a poorer response to IFN than those without the substitution[13)]. However, most previous studies have focused on nt. 1762/1764/1896. The effect of other regions on the efficacy of interferon treatment has not been determined.

Therefore, we examined the nucleic/amino acid sequences of core upstream regulatory sequences (CURS), nt. 1643–1742, BCP, preC, and core regions of HBV DNA in the serum of HBeAg positive chronic hepatitis B patients who were infected with genotype C and were treated with IFN-α. The relationship between sequence polymorphisms and the response to treatment was also studied.

Materials and Methods

Patients

Twenty-seven consecutive patients with chronic hepatitis B with HBeAg who received IFN-α treatment from 1996 to 2007 for chronic active hepatitis with abnormal ALT were retrospectively analyzed. Six megaunits of interferon alpha was administered daily in the first 2 weeks, followed by 3 times per week for the next 22 weeks.

Patients whose serum alanine aminotransferase (ALT) level was normalized and whose serum hepatitis B virus (HBV) DNA level decreased to less than 5 log copies/ml 24 weeks after the end of treatment were categorized as achieving sustained virological response (SVR). This is because DNA above this level usually accompanies ALT elevation and HBV DNA over 5-log copies/ml was indicated for antiviral therapy in HBeAg-positive patients by The clinical study group for the treatment of viral liver disease in Japan. The other patients were categorized as showing no response (NR).

Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines stipulated in the 1975 Declaration of Helsinki and was approved by the Ethics Committees of St. Marianna University School of Medicine (No. 1163) and Seizankai Kiyokawa Hospital.

HBV DNA and genotype

HBV DNA was measured using transcription mediated amplification TMA-HPA (Chugai Diagnostics Science, Tokyo, Japan) assay or PCR (Amplicor HBV monitor. Roche Diagnostics, Tokyo, Japan).

The HBV genotypes were determined using a commercial enzyme-linked immunosorbent assay kit (SMITEST HBV genotype detection kit, Genome Science Laboratories, Fukushima, Japan).

Nucleic/amino acid sequences of HBV DNA

DNA was extracted from the serum using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The serum samples, collected just before treatment and 24 weeks after treatment, were preserved at −80°C until use. DNA was dissolved in 200μl of distilled water.

For amplification and sequencing, the CURS, BCP, and preC-core regions were divided into three fragments, namely, those spanning nt. 1590–1974, nt. 1632–2389, and nt. 2111–2680, and amplified by PCR using KOD-Plus-Ver. 2 (Toyobo Co., Ltd., Osaka, Japan). The primers used in this study, which were selected using "primer 3" on Website (http://frodo.wi.mit.edu/primer3/), are shown in **Table 1**. The cycle profile for the first round of PCR was 94°C for 2 min, followed by 98°C for 10 s for

Talbe 1. Primers in This Study

Primer	Sequence
HBVCURSIS	5'-TTCACCTCTGCACGTCGCAT-3' (nt.1590-1609)
HBVCURSIAS	5'-GGAAAGAAGTCAGAAGGCAAA-3' (nt.1974-1954)
HBVR4OS	5'-CAAGTCTTGCCCAAGGTCTTA-3' (nt.1632-1652)
HBVR4OAS	5'-AGGCGAGGGAGTTCTTCTTCT-3' (nt.2389-2369)
HBVR5OS	5'-TGGGTGGGAAGTAATTTGGA-3' (nt.2111-2130)
HBVR5OAS	5'-AAGGGCAAATATTTGGTAAGG-3' (nt.2680-2660)

Talbe 2. Pretreatment Background of the Patients

	SVR	NR
Mean Age (median)	38.5 (34)	37.4 (39)
Sex (Male:Female)	5: 1	19: 2
Mean ALT (IU/L)	206.2±206.4	254.2±277.2
HBV DNA (logcopy/ml)	7.65±1.00	7.34±1.15

The pretreatment background is not statistically different between SVR and NR patients.

denaturation, 52°C for 30 s for annealing, and 68°C for 30 s for polymerization for 40 cycles. Standard precautions to avoid contamination were taken during PCR, with a negative control included in each run. The PCR products were analyzed by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized by ultraviolet transillumination.

Sequencing of PCR products

The amplification products were purified using a QIAquick@Spin purification system (QIAGEN, Hilden, Germany) and sequenced in forward and reverse directions using the PCR primers.

Statistical analysis

The data were analyzed by chi-square test, Student's t-test, and Mann-Whitney U-test. *P* values less than 0.05 were regarded as statistically significant.

Results

Clinical features of patients

Of the 27 enrolled patients, 6 patients achieved SVR and the other 21 patients showed NR. The clinical background of the patients is shown in **Table 2**. Age, sex, levels of ALT, and HBV DNA were not different between SVR patients and NR patients.

Nucleic acid sequences of nt. 1762/1764 in the BCP and nt. 1896 in the preC regions and response to IFN treatment

Nucleic acid sequences of nt. 1762/1764 in the BCP regions, which were reported to be frequently substituted in responders to the IFN treatment, were studied. The BCP sequences were determined in 6 SVR and 18 NR patients. The preC sequence was determined in 6 SVR and 20 NR patients.

As shown in **Table 3**, the nucleic acids in the BCP region (both nt. 1762 and 1764) were substituted in 5 of 6 SVR patients and 11 of 18 NR patients

Talbe 3. Nucleic Acid Substitutions in the BCP (nt. 1762/1764) and PreC Regions (nt. 1896)

	BCP1762	BCP1764	PC1896	Number of patients
SVR	W	W	W	1
	M	M	W	4
	M	M	M	1
NR	W	W	W	7
	M	M	W	9
	M	M	M	2
	NT	NT	NT/W	3

W: wild, M: mutant, NT: not tested

(p=0.32). The nucleic acid sequence at nt. 1896 in the preC region was substituted in 1 of 6 SVR patients and 2 of 20 NR patients (p=0.88). The nucleic acid sequences in the three positions (nt. 1762, 1764 and 1896) did not change after treatment except for 3 NR patients (NR3, NR10 and NR20).

Nucleic acid sequences in the CURS/BCP region and response to IFN treatment

The mean number of substituted nucleic acid sequences in the BCP region including nt. 1762/1764 was not different between SVR patients and NR patients (2.67 vs. 1.79, p=0.06). The mean number of substituted nucleic acids in the region ranging from CURS to BCP was larger in SVR patients than in NR patients (4.33±2.26 vs. 3.05±1.29, p= 0.018).

Table 4 shows the frequency of mutation in each position of nucleic acid. The nucleic acid at position 1688 in the CURS region was substituted from G to A in 5 of 19 NR patients and in none of the SVR patients (p=0.41). The nucleic acid at position 1719 in the CURS region was substituted from T to G in 7 of 19 NR patients and in 1 of the 6 SVR patients (p=0.67). The sequences at these positions after treatment did not change in most cases.

Amino acid sequences in the preC-core region and response to IFN treatment

The mean number of amino acid substitutions in the preC-core region before treatment is shown in **Table 5**. No significant difference was found between SVR and NR patients (3.00 vs. 2.71). The total number of mutated nucleic acids in CURS and BCP regions, which differed between SVR and NR patients, was not different. Two SVR patients and 5 NR patients showed no amino acid substitution in the region. There were no significant changes in the amino acids that are related to the response to IFN treatment (data not shown).

Discussion

IFN treatment for chronic hepatitis B was first reported by Greenberg *et al.* in 1976[14]. Eradication of HBV by IFN is somehow difficult because the effect of IFN was mainly on messenger RNA as the precursor of viral protein. HBV DNA including covalently closed circular DNA (cccDNA) is cleared or decreased by immunological response indirectly mediated by IFN. Therefore, elucidating the factors, including viral ones, related to the favorable response to IFN treatment is important, which prompted us to conduct this study.

Nucleic acid substitution at nt. 1896 from G to A creates a stop codon, which abolishes the translation of HBeAg at the transcriptional level[15-17]. Therefore, few patients in this study who were positive for HBeAg do not have G1896A. The mutation at nt. 1896, which may show that only minor clone produce 1896, may decrease the level of HBeAg and change both viral load and immune response viral protein. However, the nucleic acid mutation, found in one SVR and two NR patients, is not useful for predicting response to IFN treatment.

Nucleic acid substitutions at nt. 1762 from A to T and at nt. 1764 from G to A are known to upregulate transcription of pregenomic RNA and downregulate transcription of preC mRNA, which lead to lowered HBeAg production[18]. In this study,

Talbe 4. Changes in the Nucleic Acid Sequence in the CURS and BCP Regions after IFN Treatment

before

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
SVR1	W	W	W	W	3
SVR2	W	W	W	M	5
SVR3	W	W	W	W	6
SVR4	M	W	M	W	6
SVR5	M	W	W	W	3
SVR6	W	W	W	M	3
mean					4.33*

after

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
SVR1	W	W	W	W	3
SVR2	W	W	W	M	4
SVR3	W	W	W	W	---
SVR4	M	W	M	W	---
SVR5	M	W	W	W	---
SVR6	W	W	W	M	---
mean					3.50

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
NR1	W	W	W	W	3
NR2	NT	NT	NT	NT	---
NR3	W	W	M	W	5
NR4	NT	NT	NT	NT	0
NR5	W	W	M	W	4
NR6	W	M	W	W	3
NR7	W	W	M	W	3
NR8	W	M	W	W	3
NR9	W	M	W	W	1
NR10	W	W	W	W	4
NR11	W	W	M	W	3
NR12	W	W	M	W	3
NR13	W	W	W	W	3
NR14	W	W	W	W	3
NR15	W	W	W	W	4
NR16	W	W	W	W	2
NR17	W	M	W	M	4
NR18	W	W	W	W	3
NR19	W	M	W	W	2
NR20	W	W	M	W	2
NR21	W	W	M	W	3
mean					3.05*

	CURS 1652	CURS 1688	CURS 1719	BCP 1753	Substituti on in total
NR1	W→M	W	W	W	6
NR2	NT	NT	NT	NT	---
NR3	W	W→M	M→W	W	3
NR4	NT	NT	NT	NT	0
NR5	W	W	M	W	5
NR6	W	M	W	W	3
NR7	W	W	M	W	3
NR8	W	M	W	W→M	4
NR9	W	M	W	W	1
NR10	W	W	W	W	3
NR11	W	W	M	W→M	5
NR12	W	W	M	W	3
NR13	W	W	W	W	2
NR14	W	W	W	W	---
NR15	W	W	W	W	3
NR16	W	W	W	W	4
NR17	W	M	W	M	4
NR18	W	W	W	W	2
NR19	W	M	W	W	4
NR20	W	W	M	W	1
NR21	W	W	M	W	---
mean					3.11

W: wild, M: mutant, NT: not tested
*: p=0.018

Talbe 5. Amino Acid Changes in the PreC and Core Regions after IFN Treatment

	Number of substituted amino acid in the precore and core regions	
	Pre	Post
SVR1	8	2
SVR2	4	5
SVR3	2	0
SVR4	0	Not done
SVR5	4	3
SVR6	0	Not done
Mean(SVR)	3	2.5
NR1	9	3
NR2	0	3
NR3	1	2
NR4	4	5
NR5	3	2
NR6	4	4
NR7	2	3
NR8	1	2
NR9	0	1
NR10	6	5
NR11	4	0
NR12	4	4
NR13	2	2
NR14	1	1
NR15	5	6
NR16	3	2
NR17	2	4
NR18	6	5
NR19	0	3
NR20	0	4
NR21	0	Not done
Mean(NR)	2.71	3.05

83% of SVR and 61% of NR patients have nucleic acid substitution at these positions. The results were inconsistent with a previous report showing that patients whose serum HBV DNA has nucleic substitutions at nt. 1753, 1766, and 1764 respond better to IFN treatment than those without the substitutions[8]. The reason for this inconsistency may be attributed to the HBV genotype. In this study, we investigated patients who are infected with genotype C HBV, many of whom were resistant to IFN treatment. Therefore, studies on a large number of patients may be necessary to show the effect of nucleic acid substitutions at nt. 1753/1766/1764 on IFN treatment.

Nucleic acid substitutions in the BCP region at nt. 1762/1764 are often seen in patients with genotype C HBV. These mutations predict delayed HBe clearance and development of advanced liver disease[19]. In our study, 16 patients had the substitutions. Among the 16 patients, 5 (31%) achieved SVR. In contrast, in 8 patients without the substitutions, only 1 (13%) patient achieved SVR (p=0.31). Therefore, we might expect that nucleic acid substitutions in the BCP region may be favorable for IFN treatment.

The mean total number of substituted nucleic acid sequences in the CURS and BCP regions is larger in SVR patients than in NR patients (4.33 vs. 3.05, p=0.018). The mechanism is unclear but it may be hypothesized that nucleic acid substitutions in the regions reduce HBe production and render tolerance to HBV, which may trigger clearance of HBV and remission of hepatitis[8].

Nucleic acid substitutions from G to A at nt.

1688 and T to G at nt. 1719 in the CURS region tend to be found more often in NR than in SVR patients. Twelve out of 20 NR patients have nucleic acid substitutions at nt. 1688 and/or nt. 1719. In contrast, only 1 out of 6 SVR patients has the substitutions ($p=0.16$). As nt. 1719 is included in the HNF-3 binding region, the nucleic acid substitution at this position may lead to upregulation of the transcription of HBV and may reduce the response to IFN[8].

Nucleic and amino acid substitutions in the preC-core region were not related to response to IFN treatment in this study. It was reported that amino acid substitutions in the hypervariable region (AA 84–101) occur around the onset of hepatitis in HBeAg positive patients and may be related to breaking tolerance[20]. Our results, written above, did not show the relation between amino acid sequences in the core region and clinical outcome. The role of amino acid substitution in the core region in the response to IFN treatment seems low, which should be further studied.

The results of nucleotide/amino acid sequence was obtained using a set of primers. HBV clones that do not hybridize the primers may be overlooked. Using another set of primers and subcloning of PCR samples may show more accurate results.

We have shown that the nucleotide sequences of the regulatory regions upstream of the core region may be related to favorable response to IFN in HBeAg-positive patients. However, the results did not show that the sequences of these regions were related to the natural seroconversion to anti-HBe. However, the sequences might be related to the durability of anti-HBe, which should be further investigated.

In conclusion, nearly 20% of the patients who were infected with genotype C HBV and positive for HBeAg showed sustained virological response to IFN treatment. Nucleic acid substitutions in the regulatory regions upstream of the core region might be related to the response.

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Original Article

Occurrence of clinical depression during combination therapy with pegylated interferon alpha or natural human interferon beta plus ribavirin

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Aim: The onset of depression symptoms during pegylated interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to treatment discontinuation in some cases. In the present study, we conducted a questionnaire survey during treatment to determine whether natural human interferon β plus ribavirin (IFNβ/RBV) therapy is associated with a lower incidence of depression symptom onset compared with PEG-IFN/RBV therapy.

Methods: Seventy-seven patients with chronic hepatitis C received PEG-IFN/RBV (PR) or IFNβ/RBV (FR) therapy. A questionnaire survey was administered at the start of treatment, and at 4 and 12 weeks, using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).

Results: BDI-II scores in the PR group increased at 4 and 12 weeks, but remained unchanged in the FR group. At 12 weeks, the mean BDI-II score and incidence of abnormalities with a BDI-II score of ≥14 were significantly lower in the FR group than in the PR group. BDI-II scores during IFNβ/RBV therapy in 11 patients currently using antidepressants remained unchanged up to 12 weeks. None of these 11 patients required addition or dose increases of antidepressants, and there was no evidence of worsened depression symptoms. Nine PR patients had BDI-II scores of ≥14 and PSQI scores of ≥11 at 12 weeks.

Conclusions: IFNβ/RBV therapy was associated with a lower incidence of depression symptom onset during treatment. In patients already diagnosed with depression, there was no evidence that IFNβ/RBV therapy caused any worsening of symptoms, indicating that IFNβ/RBV therapy is safe for patients with depression.

Key words: Beck Depression Inventory II, chronic hepatitis C, depression, natural interferon β, pegylated interferon α, Pittsburgh Sleep Quality Index.

INTRODUCTION

INTRODUCTION OF PEGYLATED interferon α plus ribavirin (PEG-IFN/RBV) combination therapy has led to an improved sustained virological response (SVR) in patients with chronic hepatitis C who are receiving interferon therapy.[1–6] An additional new treatment regimen has been introduced by adding Telaprevir to this PEG-IFN/RBV therapy.[7,8] However, adverse effects of PEG-IFN/RBV include the onset of symptoms of depression.[9–11] Thus, there are some difficulties in treating patients with depression or sleep disorders with PEG-IFN/RBV therapy.

In Japan, natural human interferon β (IFNβ), which has a low association with the onset of symptoms of depression, has been used in interferon therapy for chronic hepatitis C.[12,13] IFNβ plus ribavirin (IFNβ/RBV) combination therapy is now used.[14] However, there are no existing reports on the relationship between PEG-IFN/RBV or IFNβ/RBV therapy and the onset of depression symptoms. Therefore, in the present study, in order to determine if IFNβ/RBV therapy is associated with a lower incidence of the onset of symptoms of depression compared to PEG-IFN/RBV therapy, and to evaluate the safety of the IFNβ/RBV therapy in patients with depression, we conducted a questionnaire survey during PEG-IFN/RBV or IFNβ/RBV therapy to investigate the frequency, timing, and intensity of depression symptoms.

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METHODS

Study population

A TOTAL OF 77 Shinkokura Hospital patients with chronic hepatitis C who received IFN therapy for at least 12 weeks between January 2010 and April 2011 were included in the study. The study protocol was in compliance with both the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were assigned to one of the following three groups: (1) the PEG-IFN/RBV (PR) group, consisting of 41 patients who received PR therapy for a period of 24 to 48 weeks; (2) the IFNβ/RBV (FR) group, consisting of 25 patients who received the FR therapy for a period of 24 to 48 weeks; and (3) the FR-d group, consisting of 11 patients with depression who were on antidepressants and who received the FR therapy for a period of 24 to 48 weeks. Patients in the FR-d group received regular psychiatric consultation and experienced dose reduction, dose increase, or addition of antidepressants during treatment. Patients with depression, those with a previous history of depression, those who were on antidepressants, or those who were on sleep-inducing drugs were excluded from the PR and FR groups. Patients reporting some type of sleep disorder during treatment were given sleep-inducing drugs at the discretion of their primary physician. Treatment regimens of PR or FR therapy were determined by the physician. None of the patients required dose reduction of IFN due to neutropenia or thrombocytopenia prior to 12 weeks. This study is a prospective, non-randomized open trial.

Criteria for exclusion from the study were as follows: (i) clinical or biochemical evidence of hepatic decomposition and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) IFNβ/RBV: white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3, PEG-IFN/RBV: white blood cell count of less than 4000/mm^3 and platelet count of less than 80 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment.

Interferon treatment

Patients in the PR group received the following treatment regimen. In brief, PEG-IFNα-2b (PEG-Intron; MSD Co., Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 lg/kg (range: 1.3–2.0 lg/kg) once a week. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Patients in the FR and FR-d groups received the following treatment regimen. Briefly, IFNβ (Feron; Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 20–44 weeks. Ribavirin (Rebetol; MSD Co., Tokyo, Japan) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). Hepatitis C virus (HCV) RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/mL. Patients were considered to have an SVR if HCV RNA remained undetectable at 24 weeks after the completion of treatment. Urinalysis and measurement of serum albumin levels were performed once every 4 weeks, from the start of treatment to Week 24.

Questionnaire

A questionnaire survey was conducted immediately before the start of treatment and at 4 weeks and 12 weeks using the Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI).[15,16] The questionnaire survey was administered by one expert investigator, who remained blinded to the treatment regimens prescribed to patients, the timing of treatment, and other information. Patients with a BDI-II score of 14 or more were considered to have the onset of depression symptoms. Patients with a PSQI score of 11 or more were identified as having sleep disorder. All patients were given a questionnaire at 12 weeks, while a questionnaire was administered to 58 subjects at the baseline and at 4 weeks, including 28, 19, and 11 patients in the PR, FR, and FR-d groups, respectively.

Statistical analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups, as well as the BDI-II score and the PSQI score at 12 weeks. Univariate and multivariate logistic regression analyses were used to determine the factors that significantly contributed to the onset of symptoms of depression. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P*-values less than 0.05, as determined by the two-tailed test, were considered significant. Variables were entered into

multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with patients having the onset of depression included the following variables: age, sex, HCV genotype, type of IFN, hemoglobin, platelet count, alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γ-GTP), total cholesterol, fasting blood sugar, and HCV RNA level.

RESULTS

Baseline background and IFN treatment

TABLE 1 SHOWS THE background of patients in the PR and FR groups. The mean age was significantly higher in the FR group (64.1 years) than in the PR group (52.5 years; $P < 0.001$). The PR group had more men than the FR group, although statistical significance was not reached. Baseline laboratory data showed a significantly lower platelet count in the FR group ($P < 0.05$). Significantly lower γ-GTP values were observed in the FR group ($P < 0.05$). The other laboratory parameters were comparable between the two groups. More patients with genotype 1 were in the PR group than the FR group, although no statistical significance was found. A total of 59 of 66 patients were evaluable for SVR. The proportion of patients with genotype 1 achieving an SVR was 33% (3/9) in the FR group and 48% (12/25) in the PR group. The PR group had a higher SVR rate, although statistical significance was not reached. The SVR rate among patients with genotype 2 was similar in the FR (85%, 11/13) and PR (83%, 10/12) groups. Over 24 weeks of treatment, 8% of patients (3/36) experienced at least one proteinuria event. None of the patients had a serum albumin level of ≤3.3 g/dL.

Change in the BDI-II score and the PSQI score during IFN treatment

Changes in the BDI-II score over time are shown in Figure 1. BDI-II scores in the PR group were increased relative to baseline at 4 and 12 weeks. Corresponding scores in the FR group remained unchanged. At 12 weeks, BDI-II scores were significantly lower in the FR group (5.8) than in the PR group (12.6; $P < 0.05$). The FR-d group had already high BDI-II scores of 23.0 at baseline, but BDI-II scores remained unchanged during treatment. No patients required dose increase or addition of antidepressants during treatment. There was no evidence of worsened depression symptoms during FR therapy.

In the PR group, the incidence of the onset of symptoms of depression, defined as a BDI-II score of 14 or more, increased from 0% at baseline to 21% at 4 weeks

Table 1 Clinical background before combination therapy of interferon β plus ribavirin (IFNβ/RBV) or pegylated interferon α plus ribavirin (PEG-IFN/RBV) in chronic hepatitis C patients

		IFNβ /RBV $n = 25$		PEG-IFN/RBV $n = 41$		IFNβ /RBV with depression $n = 11$	
Study variables		Mean	(SD)	Mean	(SD)	Mean	(SD)
Age	years	64.1	(12.7)**	52.5	(10.2)**	49.2	(9.7)
Gender							
Male		13	(52%)	30	(73%)	5	(45%)
Female		12	(48%)	11	(27%)	6	(55%)
Baseline hemoglobin	g/dL	14.0	(1.4)	14.7	(1.4)	14.0	(2.0)
Baseline platelet	10^9/L	165	(57)*	192	(59)*	202	(78)
Baseline ALT	IU/L	81.2	(81.1)	73.4	(64.0)	65	(43.1)
Baseline γ-GTP	IU/L	47.9	(36.5)*	92.0	(58.5)*	92.1	(96.3)
Baseline total cholesterol	mg/dL	177.1	(23.3)	177.5	(43)	201.5	(38.3)
Baseline fasting blood sugar	mg/dL	118.7	(58.4)	117.5	(33)	10.5.0	(30.8)
Baseline HCV	log IU/mL	5.8	(1.1)	6.1	(0.9)	5.9	(1.1)
HCV genotype							
1		12	(48%)	28	(68%)	5	(45%)
2		13	(52%)	13	(32%)	6	(55%)

*$P < 0.05$ (IFNβ/RBV vs. PEG-IFN/RBV).
**$P < 0.001$ (IFNβ/RBV vs. PEG-IFN/RBV).
ALT, alanine aminotransferase; HCV, hepatitis C virus; γ-GTP, albumin, gamma-glutamyl transpeptidase.

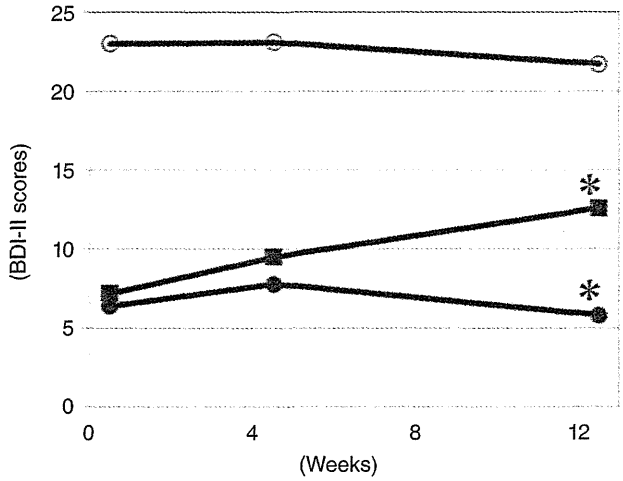

Figure 1 Changes in Beck Depression Inventory II (BDI-II) score for pegylated interferon α plus ribavirin (PEG-IFN/RBV) or interferon β plus ribavirin (IFNβ/RBV) therapy (●: IFNβ/RBV [FR] group, ○: FR-d group [FR patients with depression], ■: PEG-IFN/RBV [PR] group. *$P < 0.05$, FR vs. PR at week 12).

($n = 6$) and 34% at 12 weeks ($n = 14$). In the FR group, the incidence of the onset of symptoms of depression was 10% at 4 weeks ($n = 2$) and 8% at 12 weeks ($n = 2$), compared with 0% at baseline, indicating that the incidence did not change between 4 and 12 weeks. The incidence of the onset of depressive symptoms at 4 weeks was lower, but not significantly, in the FR group than in the PR group. Figure 2 shows the BDI-II score with a treatment regimen of IFN therapy at 12 weeks. The incidence of the onset of depressive symptoms (BDI-II score of 14 or more) was significantly lower in the FR group (8%, $n = 2$) than in the PR group (34%, $n = 14$; $P < 0.05$). The incidence of the onset of moderate depression symptoms (a BDI-II score of 20 or more) was higher in the PR group (20%, $n = 8$) than in the FR group (4%, $n = 1$). Mean PSQI scores at baseline, 4 weeks, and 12 weeks were 5.44, 6.62, and 7.37 in the PR group and 5.69, 6.01, and 6.88 in the FR group, respectively, indicating higher scores in the PR group than in the FR group from Week 4 onward. The incidence of sleep disorder, defined as a PSQI score of 11 or more, was higher in the PR group at both 4 and 12 weeks (18% and 27%, respectively) than in the FR group (0% and 8%, respectively).

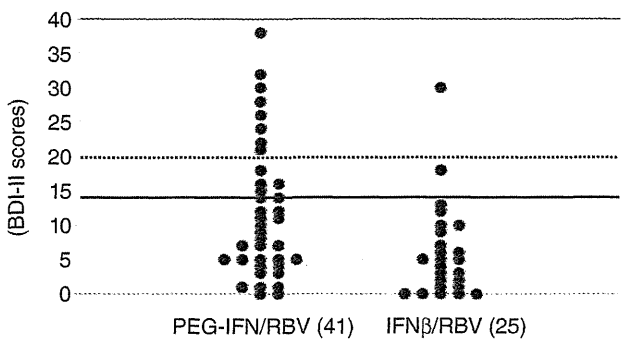

Figure 2 Distribution of Beck Depression Inventory II (BDI-II) scores for treatment regimens of interferon (IFN) therapy at 12 weeks (solid line: BDI-II score of 14, dotted line: BDI-II score of 20).

BDI-II score and PSQI score at 12 weeks

Figure 3 shows the correlation between the BDI-II score and the PSQI score at 12 weeks. Some correlation was found between these scores with an overall coefficient of correlation (r) of 0.6755 ($P < 0.0001$). A strong correlation was noted between the BDI-II score and the PSQI score in the PR group, with an r-value of 0.7586 ($P < 0.0001$). In contrast, no correlation was observed in the FR group, with an r-value of 0.3589 ($P = 0.0786$). The incidence of sleep disorder (a PSQI score of 11 or more) at 12 weeks was lower in the FR group (8%, $n = 2$) than in the PR group (27%, $n = 11$). Only nine patients in the PR group had a BDI-II score of 14 or more and a PSQI score of 11 or more, whereas there were no such patients in the FR group, with the difference reaching statistical significance ($P < 0.05$). Three of the nine patients with a BDI-II score of 14 or more

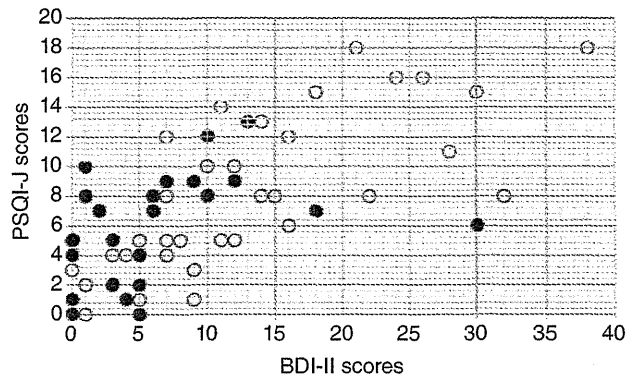

Figure 3 Graph showing correlation between Beck Depression Inventory II (BDI-II) and the Pittsburgh Sleep Quality Index (PSQI) scores at 12 weeks (correlation coefficient, Total: r = 0.6755, $P < 0.0001$; pegylated interferon α plus ribavirin [PEG-IFN/RBV]: r = 0.7586, $P < 0.0001$; interferon β plus ribavirin [IFNβ/RBV]: r = 0.3589, $P = 0.0786$).

and a PSQI score of 11 or more at 12 weeks discontinued treatment prior to 24 weeks due to depression symptoms.

Predictive factors contributing to the onset of depression symptoms during IFN therapy

Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depression symptoms during IFN therapy are shown in Table 2. The univariate regression analysis showed that the type of IFN (PEG-IFNα) was the only factor that contributed to the onset of depressive symptoms ($P < 0.027$). The multivariate logistic regression analysis confirmed that the type of IFN (PEG-IFNα/RBV) was the only contributing significant independent predictive factor.

DISCUSSION

PR THERAPY FOR chronic hepatitis C involves long-term treatment, ranging from 24 to 48 weeks. The duration of treatment in patients with HCV genotype 1 and a high viral load may range from 48 and 72 weeks.[17] Currently available PR therapy yields only a low SVR rate in patients who discontinue treatment early. Thus, it is important to complete treatment as prescribed. The onset of depression symptoms associated with PEG-IFNα treatment is one of the reasons for early discontinuation of treatment due to adverse effects. In Japan, IFNβ, which is associated with a low incidence of the onset of depression symptoms, has been used in patients with depression.[12–14] In addition, due to the milder side effects of IFNβ, we have used it in IFN therapy for hemodialyzed patients with chronic hepatitis C.[18] The SVR rate among patients with HCV genotype 1 who were treated with IFNβ/RBV was lower (approximately 40%) than that among those treated with PEG-IFN/RBV[11], while patients with HCV genotype 2 who were treated with IFNβ/RBV had an SVR rate of approximately 87%, which was similar to that observed in those treated with PEG-IFN/RBV [19].

There have been no reported studies on the relationship between FR therapy and the onset of depression symptoms. In the present study, we demonstrated that FR therapy produced a significantly lower frequency of depression symptoms than PR therapy. We also found no evidence of worsened depression symptoms during the FR therapy in patients with depression.

In the present study, a questionnaire was conducted using BDI-II and PSQI scores to assess depression symptoms and sleep disorder. The BDI-II is way to measure the severity of depression symptoms and consists of 21 questions. Symptoms with a total score of ≥14, ≥20, and ≥29 are considered mild, moderate, and severe, respectively.[15] The PSQI is a questionnaire that is used to measure the quality of sleep. Original versions of both questionnaires have been translated into Japanese, and the translated versions were used in our study.

In the present study, we found that the percentage of patients with a BDI-II score of 14 or more in the PR group was approximately 20% as early as 4 weeks after

Table 2 Results from univariate and multivariate logistic regression analyses of the factors contributing to the onset of depressive symptoms

			Simple regression		Multiple logistic regression	
Factor	Range		Odds ratio	*P*-value	Odds ratio	*P*-value
Age	≧60 / <60	(years)	0.308	0.066	–	–
Sex	Male / Female		0.808	0.728	–	–
Genotype	1 / 2		0.900	0.859	–	–
Type of IFN	PEG-IFN/IFNβ		0.168	0.027	0.168	0.027
Hemoglobin	<14 / ≧14	(g/dL)	1.310	0.647	–	–
Platelet	<15 / ≧15	(10^4/μL)	3.294	0.143	–	–
ALT	≧50 / <50	(IU/L)	1.269	0.682	–	–
γ-GTP	≧45 / <45	(IU/L)	0.990	0.986	–	–
Total cholesterol	≧220 / <220	(mg/dL)	1.667	0.652	–	–
FBS	<110 / ≧110	(mg/dL)	0.682	0.531	–	–
Viral load	≧6.0 / <6.0	(LogIU/mL)	0.829	0.750	–	–

ALT, alanine aminotransferase; FBs, fasting blood sugar; IFN, interferon; γ-GTP, gamma-glutamyl transpeptidase; PEG-IFN/RBV, pegylated interferon α plus ribavirin.

the start of treatment and increased to 34% within the first 12 weeks. However, in the FR group, 10% or less of patients only experienced the onset of mild depressive symptoms and the percentage was comparable at 4 and 12 weeks, after which no patients discontinued treatment due to depression symptoms. At 12 weeks particularly, both the mean BDI-II score and the incidence of abnormalities (a BDI-II score of 14 or more) were significantly lower in the FR group than in the PR group, indicating that FR therapy was less likely to induce the onset of depression symptoms than PR therapy. It appears that assessing the onset of depressive symptoms is useful at 12 weeks of IFN treatment. However, assessment at 4 weeks of treatment also appears to be necessary, when possible, because the onset of depression symptoms may be observed as early as 4 weeks.

The onset of depression symptoms during PR therapy has been associated with sleep disorder. In the present study, there was a strong association between the BDI-II scores and PSQI scores. Careful management is required in patients reporting sleep disorder, which is one of the early symptoms of depression.

Some of the patients receiving PR therapy, who had a BDI-II score of 14 or more and a PSQI score of 11 or more at 12 weeks, discontinued treatment due to the subsequent onset of depressive symptoms; more careful management is required in these patients.

Patients with depression were also included in the present study (FR-d group). There was no increase over time in the BDI-II score of patients with depression and none of the patients with depression required additional or an increased dose of antidepressants; there was no evidence that the depression symptoms worsened. This suggests that FR therapy is safe in both patients with depression and patients at risk for symptoms of depression.

The BDI-II and the PSQI, which were used in the present study, are simple questionnaires, which take several minutes to complete and appear to be useful instruments in assessing the onset of depressive symptoms during IFN therapy. IFNβ/RBV therapy should be used in patients with depression or sleep disorder. Patients showing the onset of depression or sleep disorder during PEG-IFN/RBV therapy should be switched to IFNβ/RBV therapy to continue IFN therapy, having given due consideration to the discontinuation of therapy.

IFNB/RBV THERAPY WAS associated with a low incidence of the onset of depression symptoms during treatment, and was also safe in patients with depression, who showed no evidence of worsening of symptoms during treatment. Depression symptoms during PEG-IFN/RBV therapy were strongly associated with sleep disorders and commonly occurred within the first 12 weeks of treatment. Patients with the onset of both symptoms of depression and sleep disorders should be closely monitored, as they are more likely to discontinue treatment after these conditions develop.

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Original Article

Pegylated interferon α-2b plus ribavirin for Japanese chronic hepatitis C patients with normal alanine aminotransferase

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Aim: To investigate the efficacy and safety of a pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination treatment for patients with chronic hepatitis C virus (HCV) infection who have persistently normal alanine aminotransferase (NALT).

Methods: This multicenter study included 989 patients with HCV genotype 1 (114 with NALT and 875 with elevated ALT) who received weight-based doses of PEG-IFN α-2b plus RBV for 48 weeks. We compared the sustained viral response (SVR) rates of patients with NALT and elevated ALT who received at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target RBV (minimum acceptable dosage).

Results: No significant difference was found in the overall SVR rate between the NALT (42.1%) and elevated ALT groups (37.3%). No significant difference in the SVR rates was found between NALT (63.3%) and elevated ALT group (61.6%) patients who received minimum acceptable dosage. Multivariate analysis showed that age (<65 years old) and total cholesterol (≧220 mg/dL) were significantly independent positive factors associated with an SVR in the NALT group. Twenty-four weeks after treatment, an ALT increase above the normal range was observed for 34.0% (18 of 53) of the non-responsive group of NALT patients.

Conclusions: The efficacy and safety of PEG-IFN α-2b plus RBV combination therapy for patients with chronic HCV infection are similar for patients with NALT and those with elevated ALT levels. These results indicate that patients with NALT should be considered for treatment with PEG-IFN α-2b plus RBV.

Key words: hepatitis C virus, normal alanine aminotransferase, pegylated interferon, ribavirin

INTRODUCTION

IT IS WELL documented that hepatocellular carcinoma (HCC) caused by HCV infection develops at a high rate in patients with advanced chronic hepatitis (CH) and liver cirrhosis.[1] Interferon (IFN) therapy for chronic hepatitis C is useful for eliminating hepatitis C virus (HCV)[2,3] and for reducing the progression of hepatic fibrosis,[4] and consequently the development of HCC.[5] Alanine aminotransferase (ALT) values are persistently normal for 20–40% of HCV patients,[6–9] with these patients generally having milder disease and a relatively favorable prognosis, and thus they have in the past been excluded from antiviral treatment.[10,11] However, the current American Association for the Study of Liver Disease (AASLD) practice guidelines recommend that

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the decision to treat HCV-infected patients with persistently normal ALT (NALT) should be individualized based on the severity of liver disease by liver biopsy, the potential for serious side effects, the likelihood of response, and the presence of comorbid conditions.[12] Because, several studies conducted over the past several years have shown that the liver histology of patients with NALT levels shows advanced fibrosis, and in some reports, 5–30% of these patients were found to have marked fibrosis or even cirrhosis (1.3%).[13–15] Further, previous studies reported that the efficacy and safety of pegylated interferon (PEG-IFN) α-2a and ribavirin (RBV) combination treatment for NALT patients with chronic hepatitis C were comparable or even higher than was found for patients with elevated ALT levels.[16–18]

Most patients in previous studies were from western countries and were aged in their 40s on average. The influence of aging of the patient population has not been adequately studied. In Japan, patients with chronic hepatitis C currently under treatment with IFN are 10 to 15 years older than corresponding patients in the United States and other western countries, where patients treated with antiviral therapy tend to average 45 years of age.[19,20] Moreover, a racial analysis reported that being Asian (non-south) is a strong independent predictor of sustained virological response to antiviral therapy.[21] However, there is no Asian data concerning the response and safety of this combination therapy from large scale trials of NALT patients with chronic HCV infection. The present prospective study was done to analyze the efficacy and safety of a combination treatment of PEG-IFN α-2b plus RBV for Japanese NALT patients with HCV genotype 1.

METHODS

Patients

A MULTICENTER STUDY of the efficacy and safety of antiviral treatments for Japanese chronic liver disease patients, the Kyushu University Liver Disease Study (KULDS), was launched in 2003.[22,23] For the present study, combination PEG-IFN α-2b and RBV treatment was done from December 2004 to September 2008, and chronic hepatitis C patients were enrolled with exclusion criteria that included: (i) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by bleeding-risky esophageal varices, history of gastrointestinal bleeding, ascites, encephalopathy, or hepatocellular carcinoma; (ii) hemoglobin level <11.5 g/dL, white blood cell count $<3 \times 10^9$/L, and platelet count $<50 \times 10^9$/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or HIV positive); (iv) excessive active alcohol consumption >60 g/day or drug abuse; (v) severe psychiatric disease; or (vi) antiviral or corticosteroid treatment within 12 months prior to enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital and 40 affiliated hospitals in the northern Kyushu area of Japan. We have treated 2270 Japanese patients aged 18 years or older with PEG-IFN α-2b plus RBV. Of the 2270 patients, 989 were HCV genotype 2, and the remaining 292 patients were currently undergoing combination treatment or we were not yet able to judge the effect of combination treatment. The 989 HCV genotype 1 patients were enrolled for analysis in the present study. All who were positive for both antibody to HCV and HCV RNA for over 6 months were enrolled in the KULDS study. Within 3 months before the start of the treatment and every 3 months during the treatment period, each patient was tested for α-fetoprotein (AFP) and had abdominal ultrasonographic examination. If an abnormal AFP level of 40 ng/mL and/or an appearance of focal lesions on ultrasonographic examination was found at any testing, further testing for HCC was done, which included dynamic computed tomography, angiography, and/or tumor biopsy. In this study, NALT was defined as ALT persistently below 30 IU/L in at least three measurements within the past 6 months, and we defined an ALT-flare up as an ALT level ≧30 IU/L at the 24-week follow-up after the end of treatment. Of the enrolled patients, 114 were assigned to a NALT group (group A) and the remaining 875 to an elevated ALT group (group B) (Table 1). The number of the women and platelet count were significantly higher in group A than in group B. Furthermore, in group A, body mass index, γ-glutamyltranspeptidase and hemoglobin were significantly lower than for group B ($P < 0.001$), and the total cholesterol level was significantly lower in group B than group A ($P < 0.001$).

Informed consent was obtained from all patients before enrollment. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of Guidelines for Good Clinical Practice.

Liver histology

Liver biopsy was done for 63 (55.3%) of the group A and 518 (59.2%) of the group B patients: The other patients refused biopsy. Fibrosis was staged on a 0–4

Table 1 Characteristics of 989 chronic hepatitis C virus (HCV) infected patients treated with a combination of pegylated interferon IFN) α-2b plus RBV

	Group A (ALT < 30 IU/L) (n = 114)	Group B (ALT ≧ 30 IU/L) (n = 875)	P-value
Men/Women	37/77	502/373	<0.001
Age (years)	57.4 ± 11.9	58.0 ± 10.1	0.607
Body mass index (kg/m^2)	22.5 ± 2.9	23.6 ± 3.2	<0.001
Prior non-pegylated IFN monotherapy n (%)	26 (22.8)	235 (26.9)	0.350
Prior combined non-pegylated IFN plus RBV treatment n (%)	6 (5.3)	77 (8.8)	0.200
Alanine aminotransferase (IU/L)	22.9 ± 4.4	82.9 ± 56.3	<0.001
γ-glutamyltranspeptidase (IU/L)	31.6 ± 24.8	64.3 ± 57.1	<0.001
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.4	0.015
White blood cell (×10^9/L)	5.1 ± 1.6	5.0 ± 1.4	0.629
Hemoglobin (g/dL)	13.4 ± 1.3	13.9 ± 1.4	<0.001
Platelet count (×10^9/L)	188 ± 5.5	157 ± 5.2	<0.001
Creatinine (mg /dL)	0.7 ± 0.2	0.8 ± 0.9	0.284
Creatinine clearance (mL/min)	93.9 ± 32.6	97.6 ± 28.6	0.168
Total cholesterol (mg/dL)	182.6 ± 31.7	167.6 ± 30.5	<0.001
Tryglyceride (mg/dL)	102.6 ± 429	105.8 ± 52.7	0.638
HDL-C (mg/dL)	54.4 ± 15.7	50.1 ± 14.4	0.058
LDL-C (mg/dL)	100.2 ± 26.5	95.6 ± 25.9	0.233
Fasting plasma glucose (mg/dL)	95.8 ± 15.2	99.8 ± 21.9	0.075
HbA1c (%)	5.2 ± 0.5	5.4 ± 0.8	0.100
HOMA-IR	2.4 ± 1.8	2.7 ± 1.8	0.158
Serum HCV RNA level (logIU/mL)	6.5 ± 0.6	6.5 ± 0.6	0.332
Histological fibrosis F0/F1/F2/F3/F4	10/31/14/5/3	31/166/165/97/59	0.008

Data are shown as the mean ±standard deviation Group A; ALT<30 IU/L, Group B; ALT ≧ 30 IU/L.
ALT, alanine aminotransferase; HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance (plasma fasting glucose (mg/dL) × IRI(ng/mL) ÷ 405); LDL-C, Low density lipoprotein-cholesterol; RBV, ribavirin.

scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Liver fibrosis was more advanced in group B than group A (P = 0.008).

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dosage of subcutaneous PEG-IFN α-2b (PegIntron, Schering-Plough, Osaka, Japan), in combination with RBV (Rebetol, Schering-Plough), which was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing 80 kg or more). The length of treatment was 48 weeks, and the above duration and dosage are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to less than 10.0 g/dL. In such cases, a reduction in the dosage of RBV was required. Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dose of PEG-IFN α-2b was required. Both PEG-IFN α-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dL, 1×10^9/L, and 25×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment.

Determination of baseline HCV RNA level and HCV genotype

The pretreatment, baseline, serum HCV RNA level was measured by COBAS TaqMan HCV assay (TaqMan)

(Roche Diagnostics, Tokyo, Japan). TaqMan has a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL (1.2 to 7.8 logIU/mL referred to $\log_{10}$ units/mL).[24,25] Therefore, TaqMan assay is able to do both qualitative and quantitative analysis for HCV RNA. The HCV genotype was determined by type-specific primers of the core region of the HCV genome. The protocol for genotyping was carried out as previously described.[3]

Efficacy of treatment

Sustained virological response (SVR) was defined as serum HCV RNA undetectable at 24 weeks follow-up after the end of treatment. SVR was defined as non-detectable HCV-RNA as measured by TaqMan assay, with the results labeled as positive or negative. The analysis of SVR rate was done on an intention-to-treat basis.

Minimum acceptable dosage

We previously reported that the minimum acceptable dosage necessary for Japanese genotype 1 patients to obtain an SVR is at least 80% or more of the target dosage of PEG-IFN α-2b and a minimum acceptable dosage of 60% or more of the target RBV.[23,26] Therefore, we compared the SVR rates of patients with NALT and elevated ALT who received at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target RBV (minimum acceptable dosage).

Statistical analysis

Continuous data are expressed as mean values, the values ± standard deviation (SD), or the values ± standard error (SE) of the mean. The statistics were done using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The χ^2 test, Student's *t*-test and Fisher's exact test were used to determine the differences in baseline clinical characteristics, safety, efficacy of the combination therapy, adherence to the total dose, and the association between the adherence and SVR. Univariate analysis was carried out on 13 background factors that had previously been evaluated in the literature for their possible association with SVR. Logistic regression models were used to evaluate possible predictors of SVR, and results were reported as odds ratios (OR) and their 95% confidence intervals (CI). A *P*-value of less than 0.05 was considered significant.

RESULTS

SVR rate by intention-to-treat analysis

ANALYSIS OF VIRAL response and ALT change was done at 24 weeks after the end of treatment. Of the 989 patients, 374 (37.8%) achieved SVR in the intention-to-treat analysis. The SVR rate was not significantly different between group A (48 of 114, 42.1%) and group B (326 of 875, 37.3%) ($P = 0.749$). The SVR rate was significantly higher for the women of group A (37 of 77, 48.1%) than for those of group B (120 of 373, 32.2%) ($P = 0.009$), but no significant difference was found for the men (group A: 11 of 37, 29.7% vs. group B: 206 of 502, 41.0%).

The SVR rates of patients with at least the minimum acceptable dosage during treatment were 43.0%, 49 of 114 patients in group A and 33.4%, 292 of 875 in group B. When the men received at least the minimum acceptable dosage, the SVR rate was not significantly different between groups A and B (group A: 9 of 16, 56.3% vs. group B: 122 of 185, 65.9%), and no significant difference was found between groups A and B for the women (group A: 22 of 33, 66.7% vs. group B: 58 of 107, 54.2%). The rate of SVR for patients under 65 years was significantly higher than for patients 65 years or older in groups A and B (41 of 80, 51.3% vs. 7 of 34, 20.6%:$P = 0.003$, 274 of 627, 43.7% vs. 52 of 248,21.0%: $P < 0.001$). Among the group B patients who received at least the minimum acceptable dosage of treatment, the SVR rate was significantly higher for patients under 65 years than for patients 65 years or older (158 of 239, 66.1% vs. 22 of 53, 41.5%: $P = 0.002$). However, there was no significant difference of SVR rate between patients under 65 years and patients 65 years or older in group A (25 of 39, 64.1% vs. 6 of 10, 60.0%, $P = 0.810$).

In our analysis of whether or not the SVR rate differed according to the age and sex of patients who received at least the minimum acceptable dosage, the rate of SVR of group A patients was not significantly different by sex or age (men: 8 of 15, 53.3% vs. 1 of 1, 100%, women: 17 of 24, 70.8% vs. 5 of 9, 55.6%). On the other hand, among the men of group B, the SVR rate was significantly higher for patients under 65 years than for patients 65 years or over (108 of 154, 70.1% vs. 8 of 22, 36.4%, $P = 0.003$). There was no significant difference of the rate between patients under 65 years and patients 65 years or older among the women of group B (50 of 85, 58.8% vs. 14 of 31, 45.2%).

We compared the SVR rates by platelet count status, over 150×10^9/L or not, and by whether or not the

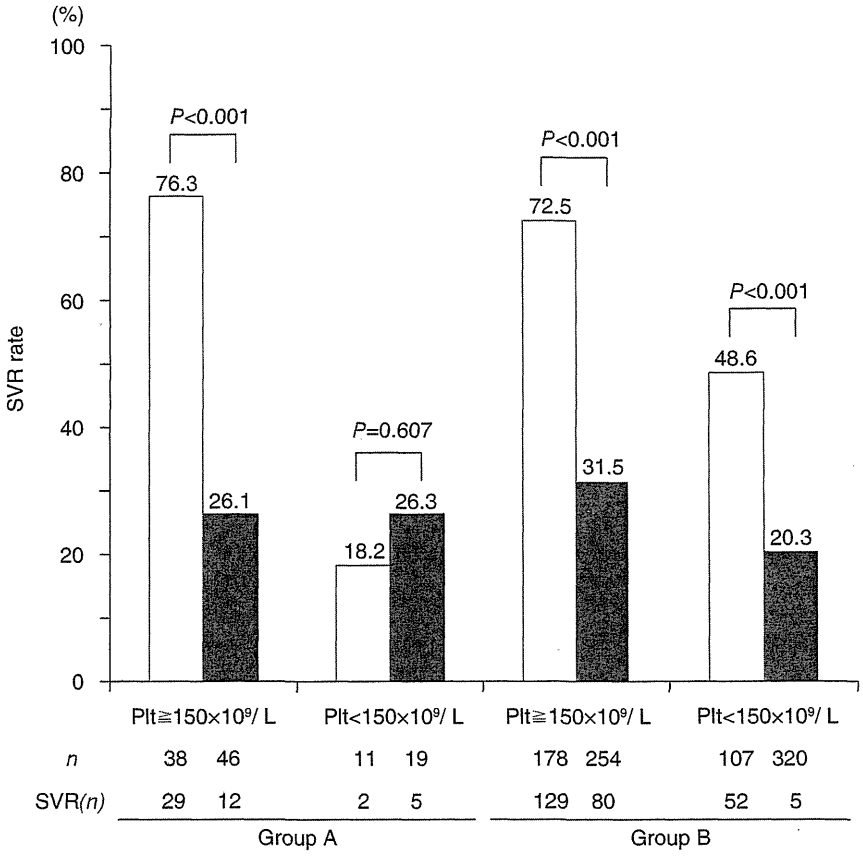

Figure 1 Comparison of the sustained virological response (SVR) rate and platelet count of patients who received the minimum acceptable dosage of pegylated interferon α-2b and ribavirin. In group A (alanine aminotransferase [ALT] <30 IU/L) patients whose platelet count was over 150×10^9/L, the SVR rate was significantly higher for those who received the minimum acceptable dosage than for those who did not (29 of 38, 76.3% vs. 12 of 46, 26.1%, $P < 0.001$). In group B (ALT ≧ 30 IU/L), the SVR rate was significantly higher for those who received the minimum acceptable dosage, with no relation to platelet count. The white column means an SVR rate of patients who received the minimum acceptable dosage. The black column means an SVR rate of patients who did not receive the minimum acceptable dosage.

patient received at least the minimum acceptable treatment dosage. In group A patients whose platelet count was over 150×10^9/L, the SVR rate was significantly higher for those who received at least the minimum acceptable dosage than for those who did not (29 of 38, 76.3% vs. 12 of 46, 26.1%, $P < 0.001$). In group B, the SVR rate was significantly higher for those who received the minimum acceptable dosage with no relation to platelet count (over 150×10^9/L: 129 of 178, 72.5% vs. 80 of 254, 31.5%, $P < 0.001$, under 150×10^9/L: 52 of 107, 48.6% vs. 65 of 320, 20.3%, $P < 0.001$) (Fig. 1). Further, in group A patients whose platelet count was over 150×10^9/L and who received at least the minimum acceptable dosage, the SVR rate was not significantly different by sex or age (under 65 men: 8/11, 72.7%, under 65 women: 15/20,75.0%, over 65 men: 1/1, 100%, over 65 women, 5/6, 83.3%). Furthermore, we compared the SVR rates of patients whose liver fibrosis was F2-4, and found no significant difference between groups A and B.

In a comparison of the SVR rate of patients with or without one or more previous courses of IFN plus RBV, there was no significant difference between groups A and B.

Background factors associated with SVR

To determine the relative weight of the background factors influencing SVR, both univariate and multivariate analyses were performed. Univariate analysis showed that age (<65 years old), homeostasis model assessment-insulin resistance (HOMA-IR) (<2) and total cholesterol (≧220 mg/dL) were significantly associated with SVR in the NALT group, butγGTP, HCVRNA level and LDL-C were not (Table 2). In the multivariate analysis, age (odds ratio [OR] 0.236, $P = 0.017$) and total cholesterol (OR 4.098, $P = 0.039$) were independent factors associated with an SVR in the NALT group (Table 3).

Change of ALT levels after the combination therapy of PEG-IFN α-2b plus RBV

After 6 months of the combination therapy, the mean ALT level of the group A patients who achieved an SVR