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Research Paper

Peginterferon Alfa-2a plus Ribavirin in Japanese Patients Infected with Hepatitis C Virus Genotype 2 Who Failed Previous Interferon Therapy

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Abstract

Some patients infected with hepatitis C virus (HCV) genotype 2 could be cured with treatment shorter than 24 weeks using peginterferon plus ribavirin, but there are still treatment-refractory patients. Direct-acting antivirals (DAAs) are not currently available for HCV genotype 2 patients, different from genotype 1 patients, in clinical practice. We investigated 29 HCV genotype 2-infected Japanese patients who had been previously treated and failed to clear HCV. We retreated them with peginterferon alfa-2a plus ribavirin and measured HCV RNA level to assess the efficacy and safety of this treatment in patients who had failed previous therapy. We found that retreatment of HCV genotype 2-infected Japanese patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks led to 60 to 66.6% sustained virological response (SVR) in patients previously treated with (peg-)interferon monotherapy and to 69.9% SVR in relapsers previously treated with peginterferon plus ribavirin. Attention should be paid to certain patients with unique features. Selection of patients according to their previous treatment could lead to optimal therapy in HCV genotype 2 treatment-experienced patients.

Key words: Retreatment, HCV G2, Japanese

INTRODUCTION

Hepatitis C virus (HCV) infection causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) [1]. HCV is also a major causative agent of HCC in Japan [2]. HCV is a positive-sense single stranded RNA virus with ~9.6 kb length, belonging to the genus Hepacivirus, a member of *the family Flaviviridae*. It is known that there exist at least 6 main genotypes of HCV [3]. These approximately equidistant genetic groups each contain a variable number of more closely related, genetically and epidemiologically distinct "subtypes". Genotypes differ from each other by 31 to 33% at the nucleotide level,

compared with 20 to 25% between subtypes [3]. In Japan, HCV genotype 1b, 2a and 2b, respectively, are observed in ~70, 20 and 10% of HCV-infected patients [4].

Treatment with peginterferon plus ribavirin for 24 weeks leads to 70-80% sustained virological response (SVR) in treatment-naïve patients infected with HCV genotype 2 [5-7]. Combination of peginterferon with ribavirin for 24 weeks is the current standard of care (SOC) for treatment-naïve patients infected with HCV genotype 2 or 3. Some selected HCV genotype 2-infected patients achieved SVR with treatment periods shorter than 24 weeks [5-9]. However, in treatment-naïve patients infected with HCV genotype 1, treatment with peginterferon plus ribavirin for 48 weeks leads to only ~50% SVR [7]. Thus, HCV genotype is one of the important factors influencing the outcome of interferon treatments [7,10].

Retreatment of chronic hepatitis C patients failing to achieve SVR with combination peginterferon plus ribavirin could only obtain 10 to 15% SVR in non-responders and 40 to 50% SVR in relapsers [11]. In North America and European countries, retreatment for HCV genotype 2 or 3 patients failing to achieve SVR with combination peginterferon plus ribavirin could lead to 37 to 46% SVR in non-responders and 52 to 63% in relapsers [12, 13], even though retreatment with SOC was performed for 48 weeks. In our previous study [14], we observed that retreatment for HCV genotype 2 Japanese patients who failed to achieve SVR with combination peginterferon alfa-2b plus ribavirin for 16, 24 or 48 weeks resulted in 71.4% SVR, but the proportions of non-responders and relapsers were unclear and HCV RNA was measured with COBAS AMPLICOR HCV Monitor Test v. 2.0 (range: 0.5 - 850 kIU/mL) (Roche Diagnostics, Tokyo, Japan).

In the present study, we investigated 29 HCV genotype 2-infected Japanese patients who had been previously treated and failed to clear HCV. We retreated them with peginterferon alfa-2a plus ribavirin and measured HCV RNA with the more sensitive COBAS TaqMan HCV test (Roche) to assess the efficacy and safety of peginterferon alfa-2a with ribavirin in patients who had failed previous therapy in clinical practice. We focused on 3 females retreated with peginterferon alfa plus ribavirin and resulting in non-SVR, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected. This would indicate that treatment with SOC should be stopped in HCV genotype 2 female patients with these features.

MATERIALS AND METHODS

Patients

Patients were recruited from Chiba University Hospital and 29 hospitals in Chiba, Ibaraki, and Saitama Prefectures between March 2008 and September 2011. Patients were eligible if they met the following inclusion criteria: (i) infected with HCV genotype 2 alone, (ii) age ≥ 20 years, (iii) diagnosed as chronic hepatitis C, (iv) negative for HBs antigen, (V) negative for human immunodeficiency virus, (vi) no autoimmune liver diseases, (vii) no severe renal disease, (viii) no severe heart disease, (ix) no mental disorders, (x) no current intravenous drug abuse, and (xi) no pregnancy. Thirty-four of the patients had previously been included in an investigation of the incidence of HCC during and immediately after peginterferon alfa-2a and ribavirin treatment in patients with chronic hepatitis C in Japan [2].

Study design

We recruited previously treated patients infected with HCV genotype 2. In Japan, combination therapy for treatment-naïve patients infected with HCV genotype 2 was not supported by the Japanese health insurance system at that time [15]. Concerning previously treated patients, they had to have failed previous treatment with either conventional interferon monotherapy, peginterferon monotherapy, conventional interferon/ribavirin combination therapy, or peginterferon/ribavirin combination therapy, different from the previous study by Sherman et al. [12]. Twenty-nine consecutive patients were enrolled in this study. Informed consent was obtained from all patients prior to enrolment. The Ethics Committee of Chiba University School of Medicine approved the study protocol. In this study, 180 μg of peginterferon alfa-2a per week plus 600-800 mg ribavirin per day were usually given in the treatment of patients for as long as 24, 48, or 72 weeks, according to the patient's will, as combination therapy for retreated patients infected with HCV genotype 2 was supported for only 24 weeks by the Japanese health insurance system at that time [15]. Clinical and laboratory assessments were performed at least every 4 weeks during treatment and a 12-week follow-up period. Adverse events were noted by oral inquiry (patient interview), physical examinations and laboratory tests.

Determination of HCV RNA titers and HCV genotype

Serum HCV RNA titer was measured using COBAS TaqMan HCV test (Roche), with levels ranging from 1.2 to 7.8 log IU/mL [16]. HCV genotype was

determined by the antibody serotyping method of Tukiyama-Kohara et al. [17,18]. According to this assay, HCV serotypes 1 and 2 correspond to HCV genotypes 1a/1b and 2a/2b [3]. HCV RNA titer and HCV genotype were determined before treatment, and HCV RNA was measured every 4 weeks before, during, and for at least 24 weeks after the end of treatment.

Serum liver function tests and hematology tests

Serum aminotransferase concentrations, other liver function tests and hematology tests were performed according to standard methods every 1 to 3 months before, during, and for at least 24 weeks after the end of treatment.

Assessment of efficacy

SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Relapse was defined as undetectable HCV RNA at the end of therapy, followed by the reappearance of HCV RNA [11]. Non-response was defined as detectable HCV RNA at the end of therapy. Patients with undetectable HCV RNA within the initial 4 weeks of treatment were considered to have had rapid virological response (RVR). Patients who had undetectable HCV RNA within the initial 12 weeks of treatment were considered to have had complete early virological response (cEVR) (described as EVR in this article) [16].

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences were evaluated by Student's *t*-test, chi-square test, or Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The characteristics of the 29 patients in the present study are shown in Table 1. They had a history of peginterferon/conventional interferon with or without ribavirin, and 4 were unknown regarding previous treatment response (Table 1). In these 29 patients, 3 received conventional interferon monotherapy, 10 peginterferon alfa-2a monotherapy, 12 peginterferon alpha-2b plus ribavirin, 3 peginterferon alfa-2a plus ribavirin, and 1 had details unknown. HCV RNA levels (≥5log IU/mL, <5log IU/mL, and unknown) were 24, 4 and 1, respectively. Concerning virological response, 18 (62.0%) had SVR, 9 (31.0%) relapsed, and 2 (6.8%) discontinued treatment due to side effects.

Table 1. Baseline and demographic characteristics of patients in the present study

Number of patients	29
Age (years)	60.1 ± 8.6
Gender (male/female)	15/14
Body mass index (kg/m^2)	26.2 ± 3.6
HCV RNA (log IU/mL)	5.5 ± 2.0
ALT (IU/L)	57.8 ± 50.7
γ-GTP (IU/L)	46.0 ± 40.7
AFP (ng/mL)	5.7 ± 3.4
Leukocyte count (/mm^3)	4940 ± 1670
Hemoglobin (g/dL)	14.0 ± 1.6
Platelet count (x10^4/mm^3)	16.2 ± 5.2
Treatment response	
Duration of treatment (~24/48/72 wks)	9/18/2
RVR rates, %	34.4 (10/29)
HCV RNA negativity at 8 wks	81.4 (22/27)
EVR rates, %	88.8 (24/27)
SVR rates, %	62.0 (18/29)

Data are expressed as mean ± SD. ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transferase; AFP, alpha-fetoprotein; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response.

Comparison of SVR patients with non-SVR patients among previously treated patients

Next, we compared 18 SVR patients with 11 non-SVR patients among the previously treated patients (Table 2). The platelet count of SVR patients tended to be higher than that of non-SVR patients ($P = 0.061$). We did not see any differences in the baselines of other factors and treatment responses (Table 2). In the 18 SVR patients previously treated, 3 received conventional interferon monotherapy, 5 peginterferon alfa-2a monotherapy, 7 peginterferon alpha-2b plus ribavirin, 2 peginterferon alfa-2a plus ribavirin and 1 with details unknown. In the 11 non-SVR patients previously treated, 5 received peginterferon alfa-2a monotherapy, 5 peginterferon alpha-2b plus ribavirin, and 1 peginterferon alfa-2a plus ribavirin. Concerning previous treatment response of the 29 previously treated patients, 18 were relapsers, 7 non-responders, and 4 had details unknown. In the 18 SVR patients, 12 were relapsers, 4 non-responders, and 2 had details unknown. In 11 non-SVR patients, 6 were relapsers, 3 non-responders, and 2 had details unknown.

Table 2. Baseline and demographic characteristics of SVR- and non-SVR-retreated patients

	SVR	Non-SVR	*P*-value*
Number of patients	18	11	N.S.
Age (years)	60.0 ± 10.0	60.3 ± 6.3	N.S.
Gender (male/female)	8/10	7/3	N.S.
Body mass index (kg/m^2)	26.0 ± 3.4	26.5 ± 4.0	N.S.
HCV RNA (log IU/mL)	5.5 ± 1.9	5.5 ± 2.1	N.S.
ALT (IU/L)	57.8 ± 50.7	55.6 ± 52.8	N.S.
γ-GTP (IU/L)	46.0 ± 40.7	30.4 ± 17.6	N.S.
AFP (ng/mL)	5.7 ± 3.4	6.2 ± 5.7	N.S.
Leukocyte count (/mm^3)	4940 ± 1670	4670 ± 940	N.S.
Hemoglobin (g/dL)	14.0 ± 1.6	13.6 ± 2.0	N.S.
Platelet count (x10^4/mm^3)	16.2 ± 5.2	12.6 ± 4.1	0.061
Treatment response			
Duration of treatment (~24/48/72 wks)	6/11/1	3/7/1	N.S.
RVR rates, %	44.4 (8/18)	18.1 (2/11)	N.S.
HCV RNA negativity at 8 wks	88.8 (16/18)	66.6 (6/9)	N.S.
EVR rates, %	88.8 (16/18)	88.8 (8/9)	N.S.
Adherence (≥80/≥80/≥80), yes	44.4 (8/18)	54.5 (6/11)	N.S.

Data are expressed as mean ± SD. *P-value, between groups with and without SVR by Student's t-test or chi-square test; N.S., not statistically significant; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transferase; AFP, alpha-fetoprotein; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response; adherence was classified according to the previous report [19].

Previous treatment response and SVR rates in HCV genotype 2 retreated patients

The relationship between previous treatment response and SVR rates of HCV genotype 2 retreated patients is shown in Table 3. In 1 patient previously treated with peginterferon plus ribavirin and non-response, treatment was discontinued due to side effects by ~8 weeks and SVR was not obtained. Of 13 patients previously treated with peginterferon plus ribavirin who had relapsed, 2 discontinued treatment due to side effects by ~8 weeks.

Female cases retreated, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected

Furthermore, we tried to determine the clinical features of non-SVR HCV genotype 2 patients retreated with peginterferon alfa-2a plus ribavirin. We noticed 3 females retreated with peginterferon alfa-2a plus ribavirin and resulting in non-SVR, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected (Figure 1). HCV RNA finally relapsed in all 3 cases. Treatment with SOC might need to be stopped in HCV genotype 2 female patients with these features.

Table 3. Previous treatment response and SVR rates in 25 retreated patients

Number of patients	Previous treatment (Treatment response)	Formula of re-treatment	SVR rates (%)
6	Peginterferon alfa-2a (NR)	Peginterferon alfa-2a plus ribavirin (~24wks)	66.6
1	Peginterferon plus ribavirin (NR)	Peginterferon alfa-2a plus ribavirin (~24wks)	0
5	(Peg-)interferon (relapse)	Peginterferon alfa-2a plus ribavirin (~48wks)	60
13	Peginterferon plus ribavirin (relapse)	Peginterferon alfa-2a plus ribavirin (24~48wks)	69.9

NR, non-response

DISCUSSION

In the present study, we focused on the virological response in HCV genotype 2-infected Japanese patients retreated with peginterferon alfa-2a plus ribavirin. We did not observe any differences in baseline background between SVR patients retreated and non-SVR patients retreated, although we must admit that the number of patients was small. However, during this study, we did find 3 females who did not obtain SVR by the retreatment and had unique features. That is, in their sera, a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected (Figure 1). These 3 cases did not discontinue peginterferon alfa-2a or ribavirin. In Figure 1, cases 1 and 2 had reduced peginterferon alfa-2a but not reduced ribavirin. On the other hand, case 3 had reduced ribavirin due to anemia, but did not have a reduction of peginterferon alfa-2a. In cases 2 and 3, adherence (≥80/≥80/≥80) [19] based on the calculation at 48 weeks was not lower. These 3 cases were relapsers and seemed different from non-responders having anti-interferon-alfa neutralizing antibody [20]. We do not know the exact reasons at this time.

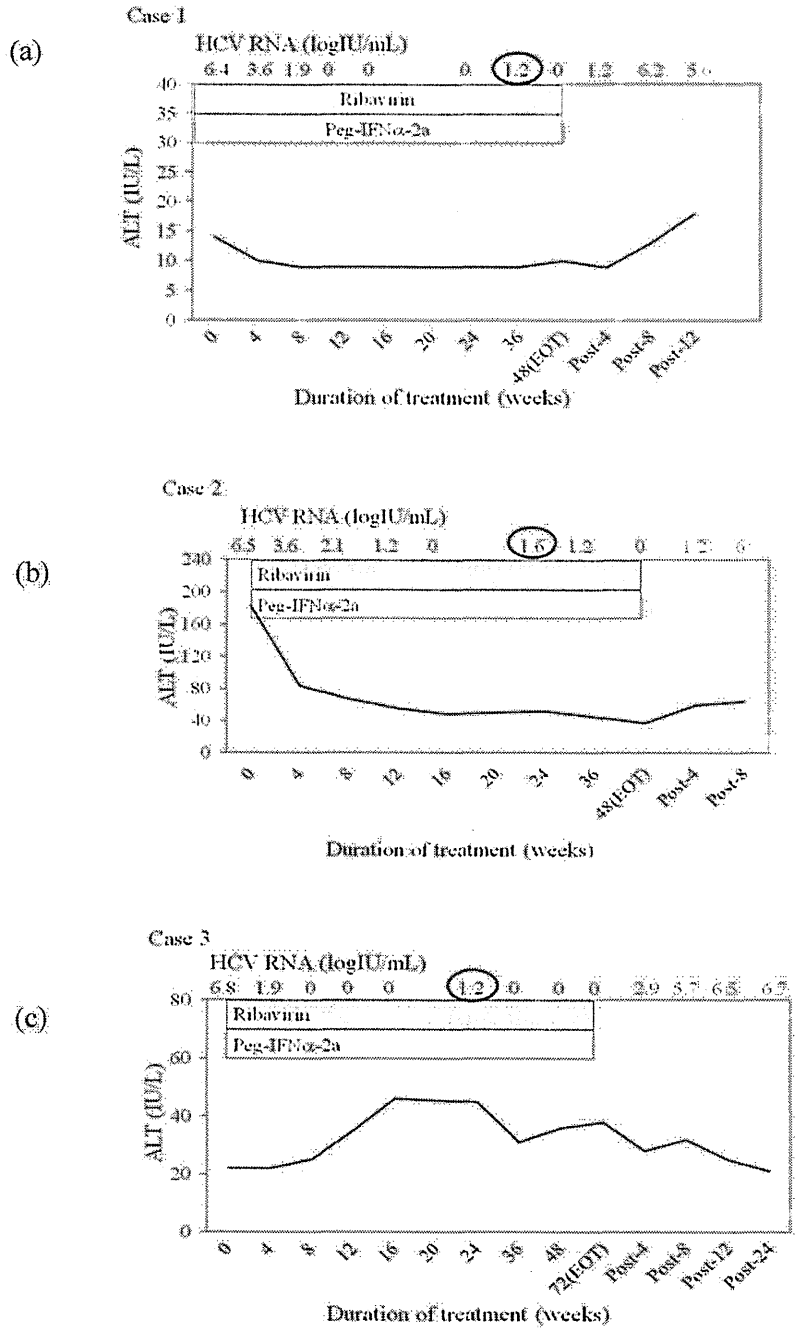

Figure 1. Three females retreated with peginterferon alfa plus ribavirin and resulting in non-sustained virological response, in whose sera a single very low-titer fluctuation of HCV RNA from negative to positive was detected after HCV RNA had been undetected. (a) Case 1, 68 years, female, IL28Brs8099917 TT. She was previously treated with peginterferon alfa-2a for 48 weeks, with details unknown. (b) Case 2, 58 years, female, IL28Brs8099977, not determined. She was previously treated with peginterferon alfa-2a for 48 weeks, with relapse. (c) Case 3, 58 years, female, IL28Brs8099917 TG. She was previously treated with peginterferon alfa-2b plus ribavirin, with details unknown. HCV RNA was determined by COBAS TaqMan HCV test (Roche), with levels ranging from 1.2 to 7.8 log IU/mL [16].

In the present study, 44% of patients had rapid virological response (RVR) and 89% of the patients had EVR (cEVR) in the retreated genotype 2 chronic hepatitis C patients with an SVR (Table 2). These results were concordant with previous studies. However, 89% of the non-SVR patients also had EVR (Table 2). Among the 8 non-SVR patients, 3 had lower adherence (≥80/≥80/≥80) (data not shown). In the present study, the adherence rates were quite low (44% in patients with SVR, and 54% in patients without SVR). In certain cases, lower adherence may be one of the reasons for non-SVR.

For HCV genotype 1 patients, direct acting antivirals (DAAs) such as telaprevir and boceprevir have been available in clinical practice [7, 21-23]. The addition of telaprevir or boceprevir to peginterferon plus ribavirin resulted in significantly higher rates of SVR in previously treated patients with chronic HCV genotype 1 infection [7, 21-23]. It will require more time until the more frequent use of DAAs for the treatment of HCV genotype 2 patients will become possible [24, 25]. Until then, we have to retreat HCV genotype 2-infected patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks.

Recently, it was reported by several groups that genetic variations in IL28B-SNP predict HCV genotype 1 treatment-induced viral clearance [7, 26-29]. Yu et al. [30] reported that rs8099917 TT genotype is significantly independently predictive of RVR, which is the single best predictor of SVR, in Asian HCV genotype 2 patients. Further study will be needed.

In conclusion, we showed that retreatment of HCV genotype 2-infected Japanese patients with peginterferon alfa-2a plus ribavirin for 24-48 weeks resulted in 60 to 66.6% SVR in patients previously treated with (peg-)interferon monotherapy and in 69.9% SVR in relapsers previously treated with peginterferon plus ribavirin, which supports the previous reports [12, 13]. Attention should be paid to certain patients with unique features. Selection of patients according to previous treatment could lead to optimal therapy in HCV genotype 2 treatment-experienced patients.

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CONFLICT OF INTEREST

Dr. Tatsuo Kanda reports receiving lecture fees from Chugai Pharmaceutical, MSD, and Ajinomoto, and Prof. Osamu Yokosuka receiving grant support from Chugai Pharmaceutical, Bayer, MSD, Daiichi-Sankyo, and Mitsubishi Tanabe Pharma.

ABBREVIATIONS

cEVR: Complete early virological response; DAA: Direct-acting antiviral; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; IL28B: Interleukin-28B; RVR: Rapid virological response; SNP: Single nucleotide polymorphism; SD: Standard deviation; SOC: Standard of care; SVR: Sustained virological response.

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HEPATOLOGY

Lead-in treatment with interferon-β/ribavirin may modify the early hepatitis C virus dynamics in pegylated interferon alpha-2b/ribavirin combination for chronic hepatitis C patients with the *IL28B* minor genotype

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Key words
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Conflict of interest: The authors declare that they have no conflict of interest.

Abstract

Background and Aim: The most important factor influencing the effect of pegylated interferon (PEG-IFN)/ribavirin therapy (PEG) for chronic hepatitis C genotype 1b with high viral load is the interleukin 28B (*IL28B*) genotype. We investigated the usefulness of lead-in twice-daily interferon (IFN)-β/ribavirin therapy (IFN-β), and the early hepatitis C virus RNA (HCV-RNA) dynamics was compared between PEG and IFN-β groups according to the *IL28B* genotype.

Methods: Forty-six patients were randomly allocated to PEG and IFN-β groups, and HCV-RNA dynamics in an early phase of treatment were analyzed.

Results: The patients with minor *IL28B* genotype was 6/23 and 8/23 in IFN-β and PEG groups, respectively. In the patients with *IL28B* major genotype, viral load reduction was marginally greater in IFN-β group than in PEG group. In contrast, in the patients with the *IL28B* minor genotype, viral load reduction was significantly and numerically greater in IFN-β group than in PEG group at 1 week (2.07 vs 0.76 log IU/mL, $P = 0.038$), 2 weeks (2.73 vs 1.01, $P = 0.009$), 4 weeks (2.72 vs 1.55, $P = 0.059$), and 12 weeks (4.56 vs 3.24, $P = 0.104$). The sustained virological response rates in the *IL28B* major genotype were similar between IFN-β group (47.1%, 8/17) and PEG group (53.3%, 8/15). In contrast, the sustained virological response rates in the *IL28B* minor genotype were numerically higher in IFN-β group (50.0%, 3/6) than in PEG group (12.5%, 1/8), although not statistically significant.

Conclusion: It was suggested that lead-in twice-daily IFN-β/ribavirin treatment followed by PEG-IFN/ribavirin combination therapy may modify the HCV-RNA dynamics compared with that by PEG-IFN/ribavirin therapy, and it is particularly useful for the *IL28B* minor genotype.

Introduction

Pegylated interferon (PEG-IFN)/ribavirin combination therapy is a standard-of-care treatment for chronic hepatitis C patients infected with hepatitis C virus (HCV) genotype 1b and high viral load, and the treatment results have been improved substantially.[1–4] However, outcomes are still unsatisfactory in that only about 50% of patients achieve a sustained virological response (SVR),[5] and many intractable cases are also present. Further improvement of the therapeutic outcome is needed, and the development of various novel therapeutic drugs is being investigated.[6]

The interferon sensitivity-determining region[7] in the NS5A region of HCV genome and amino acid substitutions at 70 and 91 in the HCV core region[8] have been reported as viral factors influencing the therapeutic effect of PEG-IFN/ribavirin combination therapy for chronic hepatitis C. On the other hand, host factors are strongly related to treatment outcome. It has been reported that single nucleotide polymorphisms (SNPs) located in the locus adjacent to the interleukin 28B (*IL28B*) gene of chromosome no. 19, rs8099917 and rs12979860, are one of the strongest factors associated with the treatment outcome in PEG-IFN/ribavirin therapy for chronic hepatitis C.[9–11] Especially, the rate of SVR has been

reported to be low in combination therapy for *IL28B* SNPs minor genotype patients with HCV-RNA core amino acid mutant type,[12] indicating that improvement of the therapeutic effect in patients with such unfavorable factors should be needed.

NS3/4A protease inhibitors, such as telaprevir[13–16] and boceprevir,[17–20] have been recently developed as new direct-acting antivirals (DAAs) against chronic hepatitis C. It has been reported that combination of DAAs with PEG-IFN/ribavirin increased the SVR rate to about 70% in patients with genotype 1.[21–23] However, PEG-IFN/ribavirin/telaprevir triple-combination therapy achieved SVR in only 30–50% of patients with the *IL28B* minor genotype.[24]

Currently, IFN plays the central role in treatment of chronic hepatitis C, and there are IFN-α and IFN-β. IFN-α is mainly used worldwide, while IFN-β is also used in Japan, and its high anti-HCV effect is known.[25,26] As a characteristic of IFN-β, the blood IFN level rapidly rises compared with that after administration of other preparations because it is intravenously administered.[27] In addition, a low incidence of adverse effects, such as depressive symptoms caused by IFN-α, is another major characteristic.[28] On the other hand, the half-life of IFN-β is short, and the blood level becomes undetectable within 12 h after administration, for which daily or every-other-day administration is necessary. Later, a twice-daily dosing regimen was proposed to maximize the effect of IFN-β, in which the virus load reduction rate in an early phase of treatment was high. Earlier conversion to virus-negative and a favorable SVR rate by lead-in treatment with IFN-β before PEG-IFN/ribavirin therapy have also been reported.[29–31] Furthermore, combination therapy of IFN-β and ribavirin has become possible and employed for treatment of chronic hepatitis C.

SNPs near the *IL28B* is the strongest independent factor that influences the effect of PEG-IFN/ribavirin combination therapy. However, it is unclear whether it serves as a significant therapeutic effect-predicting factor of lead-in twice-daily IFN-β/ribavirin combination. There have been a few reports on the effect of IFN-β/ribavirin therapy on chronic hepatitis C according to *IL28B* genotype.[32] Furthermore, no study reported the efficacy or safety of lead-in treatment with twice-daily IFN-β and ribavirin. In this study, we performed lead-in twice-daily IFN-β/ribavirin treatment followed by PEG-IFN/ribavirin therapy and investigated HCV-RNA dynamics in an early phase of treatment, and focused especially on the outcome in patients with the *IL28B* minor genotype, which is an intractable factor.

Methods

Patients and methods. This was an open-label, prospective, randomized, controlled trial. Of 50 consecutive chronic hepatitis C patients with genotype 1b and high viral loads who visited Nippon Medical School Chiba Hokusoh Hospital between January 2010 and December 2010, 46 patients were judged to require treatment, met the inclusion criteria, and provided written informed consent. They were randomly and equally allocated to either treatment group, whose regimens were described later. The remaining four patients were excluded; two had cytopenia, and two did not provide written informed consent. Patients were eligible for the enrollment, if they fulfilled the following criteria: HCV-RNA persistently detectable in serum, a high viral load (defined as >5.0 log IU/mL), white blood cell count of more than 3000/mm^3, neutrophil count of more than 1500/mm^3, platelet count of more than 80 000/mm^3, and hemoglobin concentration of more than 10 g/dL at the entry. Patients with the following conditions were considered as ineligible for this study: complications by other liver diseases including autoimmune hepatitis and alcoholic hepatitis, decompensated liver cirrhosis, liver failure, severe renal disorder, abnormal thyroid function, poorly controlled diabetes or hypertension, medication with Chinese herbal medicine, past medical history of interstitial pneumonia, pregnancy or possibility of pregnancy, lactation, severe depression, past medical history of allergy to biological preparations, and interferon or ribavirin. The study protocol followed the ethical guidelines established in accordance with the 2004 Declaration of Helsinki and were approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital.

Treatment and definition of virological response. Patients assigned to receive PEG-IFN/ribavirin combination therapy were designated as the PEG group. Other patients assigned to receive lead-in twice-daily IFN-β/ribavirin combination followed by PEG-IFN/ribavirin therapy were designated as the IFN-β group (Fig. 1). The patients received a subcutaneous injection of PEG-IFNα-2b (Pegintron; MSD, Tokyo, Japan) of 1.5 μg/kg/week and oral administration of ribavirin (Rebetol; MSD). The oral dose of ribavirin was determined on the basis of body weight (600 mg/day for <60 kg, 800 mg/day for 60–80 kg, 1000 mg/day for >80 kg) according to the manufacturer's instructions. In the IFN-β group, the daily dose of IFN-β (Feron; Toray Medical, Tokyo, Japan) was 6 MIU, and it was divided into twice a day: 3 MIU was administered by intravenous drip infusion every 12 h for the first 2 weeks as lead-in treatment. When marked proteinuria or thrombocytopenia was noted during the twice-daily administration period, the dosing regimen of IFN-β was changed to 6 MIU once a day. Following the lead-in treatment, PEG-IFNα-2b was administered for the next 46 weeks. In the PEG group, PEG-IFN-α2b

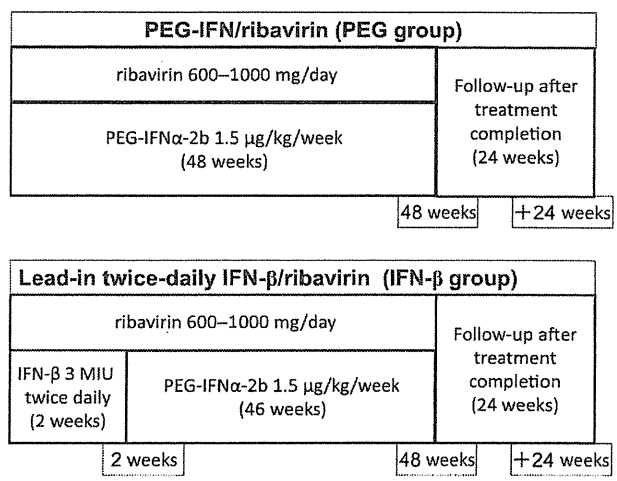

Figure 1 Treatment protocol. PEG group, pegylated interferon (PEG-IFN)/ribavirin treatment group; IFN-β group, lead-in twice-daily interferon (IFN)-β/ribavirin followed by PEG-IFN/ribavirin combination treatment group.

was administered for 48 weeks without the lead-in IFN-β. In both groups, ribavirin was concomitantly administered for 48 weeks. The doses of agents were appropriately reduced when an adverse event, such as cytopenia, occurred during the treatment course according to the package insert.

When HCV-RNA was undetectable 4 and 12 weeks after the treatment initiation, patients were considered to achieve rapid virological response (RVR) and complete early virological response (cEVR), respectively. Patients who were negative for the virus at the time of treatment completion were defined as having end-of-treatment response (ETR). The patients were followed for 24 weeks after the treatment completion. SVR was defined as negative for the virus at 24 weeks after the treatment completion. The patients who exhibited an ETR but were positive for the virus at 24 weeks after the completion were considered to have a relapse.

Laboratory tests. Peripheral blood examination, liver function tests, and urinalysis were performed weekly until 8 weeks after the treatment initiation, and then monthly until 24 weeks after the treatment completion. After the treatment initiation, HCV-RNA was measured at 24 and 48 h, 1 and 2 weeks, 4 weeks, and once a month thereafter throughout the study period in both groups. The HCV-RNA levels were measured by using the real-time polymerase chain reaction (PCR) method (COBAS AmpliPrep, Roche Diagnostics, Tokyo, Japan). Gene mutations in the core and NS5A regions of the HCV genome were determined by using the direct sequencing method. Genomic DNA was extracted from whole blood using a DNA ISOLATION kit on a MagNA Pure LC instrument (Roche Diagnostics). SNP at rs8099917 located in the locus adjacent to the *IL28B* gene on chromosome no. 19 were determined by real-time detection PCR using TaqMan SNP Genotyping Assays on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into two categories: T/T (major genotype) and non-T/T (minor genotype: T/G or G/G).

Statistical analysis. The primary end-points were HCV-RNA dynamics within the first 12 weeks of treatment and the rates of RVR and cEVR. Especially, they were analyzed according to the *IL28B* genotype. Safety was evaluated as a secondary end-point. Differences between the groups were tested by the Mann–Whitney *U*-test or Fisher's exact test. Statistical analyses were performed using IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan). $P < 0.05$ was considered statistically significant.

Results

Forty-six patients were randomly and equally allocated to the PEG and IFN-β groups (23 patients in each). The patient characteristics of the groups are shown in Table 1. There were no significant differences in the baseline characteristics between the IFN-β and PEG groups. Six of 23 IFN-β and 8 of 23 PEG group patients had the *IL28B* minor genotype. All of the *IL28B* minor genotype patients in the IFN-β group had core amino acid 70 mutant type (Table 2).

In the early HCV-RNA dynamics during the first 12 weeks of treatment, viral load reduction from the baseline was significantly and marginally greater in the IFN-β than in the PEG group

Table 1 Baseline clinical and demographic characteristics of 46 patients with chronic hepatitis C

	PEG-IFN/ribavirin (PEG group) ($n = 23$)	Lead-in twice-daily IFN-β/ribavirin (IFN-β group) ($n = 23$)	*P* value
Age (years), median(range)	63 (47–83)	66 (55–75)	0.552†
Gender (male/female)	17/6	10/13	0.071†
Body mass index (kg/m/m)	23.3 (17–26.1)	23.1 (20.3–30.2)	0.338†
History of interferon therapy (naïve/retreatment)	21/2	16/7	0.135‡
White blood cell count (/μL)	5600 (3280–11 100)	5200 (3010–13 640)	0.302†
Hemoglobin (g/dL)	14.2 (12.8–16.8)	14.0 (10.9–16.0)	0.194†
Platelet count (/mm^3) × 10^3	159 (95–301)	144 (100–320)	0.328†
Alanine aminotransferase (IU/L)	56 (16–280)	61 (26–139)	0.660†
Gamma-glutamyl transpeptidase (U/L)	58 (17–293)	35 (14–231)	0.114†
Albumin (g/dL)	4.1 (2.6–4.6)	4.1 (2.5–4.4)	0.387†
Low density lipoprotein cholesterol (mg/dL)	97.5 (66–150)	87.5 (52–149)	0.275†
α-fetoprotein (ng/mL)	5.5 (1.6–80.4)	7.5 (1.7–80.4)	0.382†
Procollagen type 3 propeptide (U/mL)	1.2 (0.5–82)	1.2 (0.7–2.1)	0.733†
Hyaluronic acid (ng/mL)	138 (21–500)	153 (21–1566)	0.825†
Type 4 collagen (ng/mL)	4.9 (1.3–11)	4.9 (1.8–12.5)	0.341†
HCV-RNA (log IU/mL)	6.6 (5.1–7.3)	6.5 (5.0–7.5)	0.758†
ISDR (0–1/2<)	11/12	12/11	1.0‡
Core aa70 (wild/mutant)	16/7	9/14	0.075‡
Core aa91 (wild/mutant)	13/10	7/16	0.136‡
IL28B (TT/TG or GG)	15/8	17/6	1.0‡

†Mann–Whitney *U*-test.

‡Fisher's exact test.

Categorical variables are given as number. Continuous variables are given as median (range).

HCV, hepatitis C virus; IFN-β, interferon-β; *IL28B*, interleukin 28B; ISDR, interferon sensitivity determining region; PEG-IFN, pegylated interferon

(Fig. 2): 24 h (1.59 vs 1.64 log IU/mL, respectively; $P = 0.796$), 48 h (2.39 vs 2.20 log IU/mL, respectively; $P = 0.556$), 1 week (2.94 vs 1.47 log IU/mL, respectively; $P = 0.0004$), 2 weeks (3.85 vs 2.07 log IU/mL, respectively; $P = 0.0001$), 4 weeks (4.15 vs 3.18 log IU/mL, respectively; $P = 0.053$), 8 weeks (5.07 vs 4.92 log IU/mL, respectively; $P = 0.375$), and 12 weeks (5.68 vs 5.26 log IU/mL, respectively; $P = 0.871$). The RVR rate was numerically higher in the IFN-β group (27%, 6/22) than in the PEG group (9%, 2/23), although not statistically significant ($P = 0.135$, Fig. 3). However, the cEVR rates were similar between the groups (58% and 59%, respectively; $P = 1.000$).

Table 2 Clinical characteristics of patients with the *IL28B* (rs8099917) minor genotype ($n = 14$)

Treatment group	Age	Gender	HCV-RNA	Core aa70	ISDR	*IL28B*
IFN-β	61	M	5.3	Mutant	1	GG
IFN-β	63	F	5.1	Mutant	0	TG
IFN-β	73	F	6.4	Mutant	1	TG
IFN-β	68	F	6.5	Mutant	1	TG
IFN-β	58	F	6.6	Mutant	0	TG
IFN-β	57	F	6.1	Mutant	1	TG
PEG	60	M	6.6	Wild	2	TG
PEG	77	M	7.0	Wild	1	TG
PEG	46	F	7.2	Wild	0	TG
PEG	54	M	6.8	Wild	2	TG
PEG	46	M	5.9	Wild	0	TG
PEG	57	F	7.1	Mutant	1	TG
PEG	68	M	7.2	Wild	1	TG
PEG	54	M	6.1	Mutant	2	TG

HCV, hepatitis C virus; IFN-β, interferon-β/ribavirin treatment group; *IL28B*, interleukin 28B; ISDR, interferon sensitivity determining region; PEG, pegylated interferon /ribavirin treatment group.

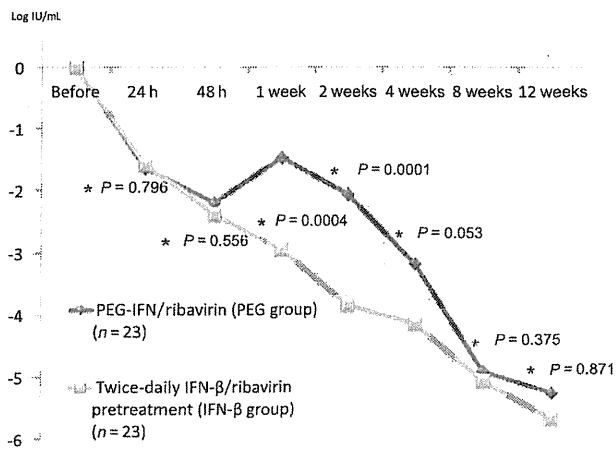

Figure 2 Comparison of viral load kinetics during the first 12 weeks between two treatment groups. Line connecting points denote mean values at each time points. Viral load reduction was significantly greater in the IFN-β group than in the PEG group at 1 week (2.94 log IU/mL vs 1.47 log IU/mL, $P = 0.0004$), 2 weeks (3.85 log IU/mL vs 2.07 log IU/mL, $P = 0.0001$) of treatment. IFN, interferon; PEG-IFN, pegylated IFN.

Next, the early HCV-RNA dynamics was compared between the two groups according to the *IL28B* genotype (Fig 4). As for patients with the *IL28B* major genotype, viral load reduction was significantly greater in the IFN-β group than in the PEG group at 1 and 2 weeks: 24 h (1.69 vs 2.02 log IU/mL, respectively; $P = 0.098$), 48 h (2.6 vs 2.58 log IU/mL, respectively; $P = 0.883$), 1 week (3.25 vs 1.88 log IU/mL, respectively; $P = 0.007$), 2 weeks (4.24 vs 2.67 log IU/mL, respectively; $P = 0.003$), 4 weeks (4.69 vs 4.05 log IU/mL, respectively; $P = 0.172$), 8 weeks (5.71 vs 5.58 log IU/mL, respectively; $P = 0.604$), and 12 weeks (6.05 vs 6.20 log IU/mL, respectively; $P = 0.479$). On the other hands, as for patients with the *IL28B* minor genotype, by contrast, viral load reduction was significantly and numerically greater in the IFN-β group than in the PEG group at 1, 2, 4, 8, and 12 weeks: 24 h (1.28 vs 1.03 log IU/mL, respectively; $P = 0.476$), 48 h (1.78 vs 1.58 log IU/mL, respectively; $P = 0.561$), 1 week (2.07 vs 0.76 log IU/mL, respectively; $P = 0.038$), 2 weeks (2.73 vs 1.01 log IU/mL, respectively; $P = 0.009$), 4 weeks (2.72 vs 1.55 log IU/mL, respectively; $P = 0.059$), 8 weeks (3.48 vs 2.41 log IU/mL, respectively; $P = 0.198$), and 12 weeks (4.56 vs 3.24 log IU/mL, respectively; $P = 0.104$). The RVR rates in the *IL28B* minor genotype were 16.7% in the IFN-β group and 0% in the PEG group ($P = 0.500$). The cEVR rates in the IL28B minor genotype were 33.3% and 0% ($P = 0.227$), respectively, showing more favorable outcomes in the IFN-β group than in the PEG group.

Finally, the ETR and SVR rates in the *IL28B* minor genotype were numerically higher in the IFN-β group (66.7%, 4 of 6; and 50.0%, 3 of 6) compared with that 37.5% (3 of 8) and 12.5% (1 of 8) in the PEG group, respectively, although not statistically significant. On the other hand, the sustained response (SVR) rates in the *IL28B* major genotype were similar, 47.1% (8 of 17) in the IFN-β group and 53.3% (8 of 15) in the PEG group.

Regarding adverse effects, no severe complication occurred in either group. The lead-in regimen of IFN-β was changed to 6 MIU once a day from day 11 to 14 because of thrombocytopenia ($<50 \times 10^3/mm^2$) in two patients and serum albumin level reduction (<3.0 g/dL) because of proteinuria in 1 of the 23 IFN-β

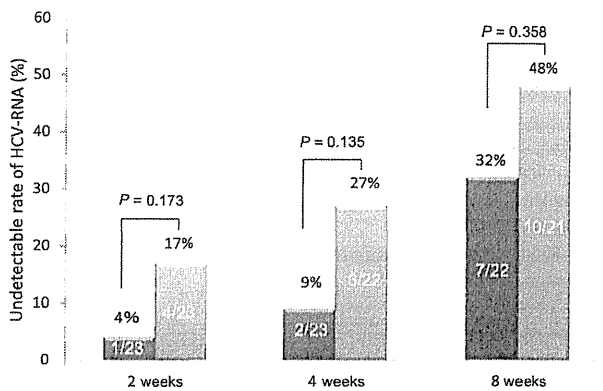

Figure 3 Comparison of undetectable hepatitis C virus (HCV) RNA rates between two treatment groups. Virological response rates are shown as a percentage and the number of patients at 2, 4, and 8 weeks of treatment. (■) pegylated interferon/ribavirin therapy group; (■) interferon-β/ribavirin therapy group.

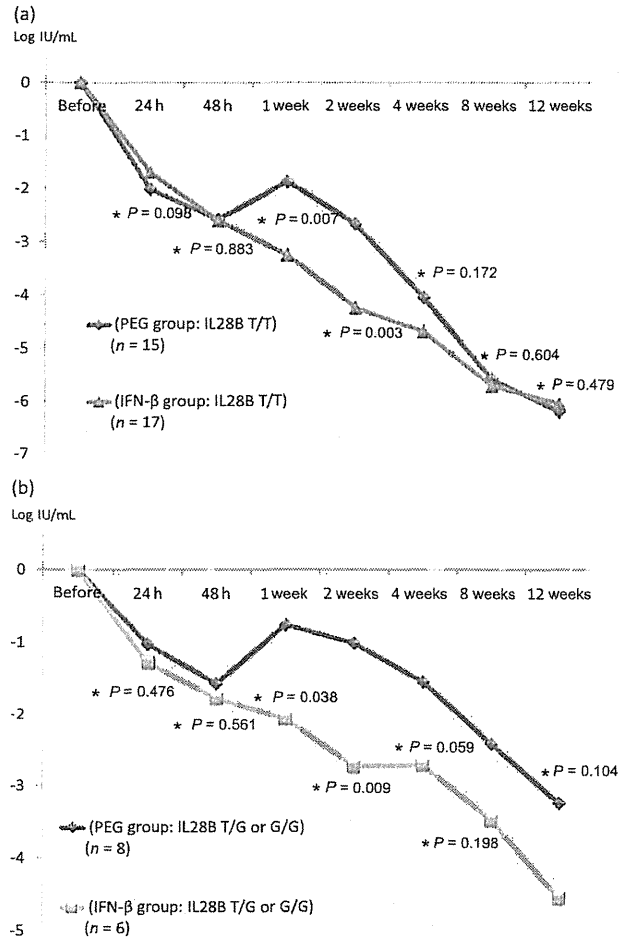

Figure 4 (a) Comparison of viral load kinetics during the first 12 weeks between two treatment groups in patients with interleukin 28B (*IL28B*) (rs8099917) major genotype T/T. Line connecting points denote mean values at each time points. When patients with the *IL28B* major genotype alone were analyzed, viral load reduction was significantly greater in the interferon (IFN)-β/ribavirin treatment group (IFN-β group) than in the pegylated interferon (PEG-IFN)/ribavirin treatment group (PEG group) at 1 week (3.25 vs 1.88 log IU/mL, $P = 0.007$), 2 weeks (4.24 vs 2.67 log IU/mL, $P = 0.003$) of treatment. (b) Comparison of viral load kinetics during the first 12 weeks between two treatment groups in patients with *IL28B* (rs8099917) minor genotype T/G or G/G. Line connecting points denote mean values at each time points. When patients with the *IL28B* minor genotype alone were analyzed, viral load reduction was significantly and numerically greater in the IFN-β group than in the PEG group at 1 week (2.07 vs 0.76 log IU/mL, $P = 0.038$), 2 weeks (2.73 vs 1.01 log IU/mL, $P = 0.009$), 4 weeks (2.72 vs 1.55 log IU/mL, $P = 0.059$), 8 weeks (3.48 vs 2.41 log IU/mL, $P = 0.198$), and 12 weeks (4.56 vs 3.24 log IU/mL, $P = 0.104$) of treatment.

group patients. The conditions were reversible and rapidly improved after the modification of treatment. Ophthalmological examination of fundus revealed no abnormal findings during the treatment.

Discussion

This report was an open-label, prospective, randomized, controlled trial. Forty-six patients were allocated to the PEG and IFN-β groups. In the present study, HCV-RNA dynamics in an early phase of treatment were analyzed according to the *IL28B* genotype influencing the effect of PEG-IFN/ribavirin therapy for chronic hepatitis C genotype 1b with high viral load.

The timing of disappearance of serum HCV-RNA is important for virus elimination by IFN therapy.[33] Because virus elimination in the early phase of treatment is closely associated with the SVR rate, we focused on this point. The viral dynamics after treatment is divided into the first phase showing a rapid decrease within 24 h and subsequent slow decrease as the second phase. The first phase reflects the direct antiviral effect of IFN on HCV-RNA, and the second phase is related to HCV-RNA reduction through elimination of HCV-RNA-infected hepatocytes. It has been reported that the SVR rate rose with an increase in the virus elimination rate within 2 weeks after treatment initiation, which is the second phase.[34]

It has been indicated that the hepatic cells exposed to IFN lead to modulate the IFN receptor downregulation.[35] On the other hand, the blood half-life of IFN-β is short, and IFN receptor expression rapidly re-increases. Every-12-h administration may increase the area under the blood concentration time curve and facilitate efficient intracellular signal transmission. Actually, it has been reported that lead-in twice-daily IFN-β administration achieved a high virological response rate and a high antiviral effect. Asahina *et al.* showed that the virus load reduction rate was markedly high in the second phase of twice-daily IFN-β administration[25] and reported that expressions of protein kinase assumed to play an important role in virus elimination and an IFN-inducing protein, MxA, were maintained at a high level.[26]

Favorable anti-HCV effects of lead-in twice-daily IFN-β treatment alone followed by PEG-IFN/ribavirin therapy have been reported,[30,31] but there have been no report on the efficacy or safety of lead-in twice-daily IFN-β and ribavirin combination treatment. In the HCV-RNA dynamics in the early phase of treatment, virus load reduction was greater in the lead-in IFN-β/ribavirin treatment group compared with that in the PEG group, and the RVR rate was 27%, showing a favorable outcome, although the difference was not significant.

Prediction of the therapeutic effect of PEG-IFN/ribavirin based on the host factor, *IL28B* SNP, was investigated in many reports, but the therapeutic effect of IFN-β was investigated in only a few reports on IFN-β/ribavirin combination therapy,[32] and there has been no report on lead-in IFN-β/ribavirin combination therapy according to the *IL28B* genotype. When analysis was limited to the *IL28B* genotype, the HCV-RNA dynamics in the minor genotype were favorably similar to that in the *IL28B* major genotype in the IFN-β group, and a significant difference was noted on comparison with that in the minor genotype in the PEG group. In addition, the RVR and cEVR rates in the minor genotype were higher in the IFN-β group.

However, some limitations still remained. First, the number of the *IL28B* minor genotype patients (six in IFN-βgroup, eight in PEG group) was insufficient for analysis. Second, there were no significant differences in the SVR rates in the *IL28B* major genotype. On the other hand, in the early HCV-RNA dynamics during

the first 12 weeks of treatment, viral load reduction from the baseline was significantly greater in the IFN-β than in the PEG group. In addition, the SVR rate was numerically higher in the IFN-β group than in the PEG group, although not statistically significant. The small number of cases may have influenced the results, and it may be possible to elevate the SVR rate by modifying the study protocol, such as prolongation of the IFN-β administration period from 2 to 4 weeks.

The effectiveness of lead-in twice-daily IFN-β/ribavirin therapy in patients with the IL28B minor genotype remains to be determined. This study showed that lead-in twice-daily IFN-β/ribavirin treatment more markedly decreased the early-phase viral load through its potent antiviral activity compared with the conventional combination therapy and suggested that the lead-in treatment could improve the SVR rate especially in patients with the minor genotype. As depicted in Figure 4, it is possible that greater viral reduction during the first 12 weeks of treatment may lead to higher SVR rate in patients with the minor genotype, with being different from those with the major genotype (although not statistically significant). However, this small RCT failed to reach a definitive conclusion that the lead-in treatment is truly advantageous to patients with the minor genotype. Conversely, a previous study reported that patients with the minor genotype did not respond to IFN-β/ribavirin therapy.[32] Taken together, the potent antiviral activity of the two-divided IFN-β administration may be demanded for patients with the unfavorable genetic genotype. Although the exact reason is unclear, one possible explanation is that the different antiviral mechanisms between IFN-α and -β may cause different treatment outcome according to diverse genetic characteristics. A large-scale study is required to resolve the issue.

Triple-combination therapy including protease inhibitors may become the main stream of chronic hepatitis treatment, but virus elimination may remain difficult in the patients with the *IL28B* minor genotype. We expect that lead-in twice-daily IFN-β/ribavirin treatment before triple-combination therapy including protease inhibitor improves the therapeutic effect in patients with the *IL28B* minor genotype.

In conclusion, it was suggested that lead-in twice-daily IFN-β/ribavirin treatment followed by PEG-IFN/ribavirin combination therapy may modify the early HCV dynamics, and it may be particularly useful for the *IL28B* minor genotype.

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Combination of fluvastatin with pegylated interferon/ribavirin therapy reduces viral relapse in chronic hepatitis C infected with HCV genotype 1b

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Abstract

Background and Aim: Although the anti-hepatitis C virus (HCV) effect of statins *in vitro* and clinical efficacy of fluvastatin combined with Pegylated interferon (PEG-IFN)/ribavirin therapy for chronic hepatitis C (CHC) have been reported, the details of clinical presentation are largely unknown. We focused on viral relapse that influences treatment outcome, and performed a post-hoc analysis by using data from a randomized controlled trial.

Methods: Thirty-four patients in the fluvastatin group and 33 patients in the non-fluvastatin group who achieved virological response (complete early virological response [cEVR] or late virological response [LVR]) with PEG-IFN/ribavirin therapy were subjected to this analysis. Factors contributing to viral relapse were identified by using multiple logistic regression analysis.

Results: Relapse rate in patients with cEVR was significantly lower in the fluvastatin group (2 of 23, 8.7%) than in the non-fluvastatin group (9 of 26, 34.6%; $P = 0.042$). The use of fluvastatin decreased relapse rate in patients with LVR (27.3% *vs* 57.1%), though not significantly. Overall, relapse rate was significantly lower in the fluvastatin group (14.7%; 5 of 34) than in the non-fluvastatin group (39.4%; 13 of 33; $P = 0.027$). Multivariate analysis identified absence of fluvastatin ($P = 0.027$, odds ratio [OR] = 3.98, 95% confidence interval [CI] = 1.05–15.11) and low total ribavirin dose ($P = 0.002$, OR = 2.41, 95% CI = 1.38–4.19) as independent factors contributing to relapse.

Conclusion: The concomitant addition of fluvastatin significantly suppressed viral relapse, resulting in the improvement of sustained virological response rate, in PEG-IFN/ribavirin therapy for CHC patients with HCV genotype 1b and high viral load.

Introduction

Pegylated interferon (PEG-IFN)/ribavirin combination therapy is a standard-of-care treatment for chronic hepatitis C patients infected with hepatitis C virus (HCV) genotype 1b and high viral load.[1,2] The treatment outcome has been substantially improved, but it is still unsatisfactory. Approximately 70% of those patients show virological response to PEG-IFN/ribavirin at the end of treatment, whereas only 45–50% of them achieve sustained virological response (SVR). The remaining responders develop virological relapse after the completion of treatment.[3–6] Therefore, the occurrence of virological relapse has an impact on the final treatment outcome. One way to further improve the treatment outcome is that the occurrence rate of virological relapse is decreased with combination of various agents, which are still being developed and under investigation. Alternatively, identification of factors contributing to virological relapse may provide a critical clue to understand the mechanism of relapse and to develop a novel therapeutic strategy.

In vitro and *in vivo* studies have demonstrated that fluvastatin could inhibit HCV replication effectively.[7,8] Concomitant use of statins has been reported to be an independent positive predictor of achieving SVR in chronic hepatitis C patients with or without diabetes.[9] A preliminary pilot trial has suggested that fluvastatin combined with PEG-IFN/ribavirin might improve the treatment outcome in patients with HCV genotype 1b and high viral load.[10] A prospective randomized controlled study has proven that fluvastatin-combined PEG-IFN/ribavirin therapy was safe and significantly improved the SVR rate.[11] However, it remains to be

clarified whether fluvastatin could influence the virological response and suppress virological relapse in the combination therapy of PEG-IFN/ribavirin/fluvastatin. The present investigation focused on the impact of fluvastatin on virological relapse by post-hoc analysis of data obtained from a randomized controlled study of PEG-IFN/ribavirin combination therapy with *versus* without fluvastatin.

Methods

Subjects and data. The present analyses were based on data obtained from a randomized controlled trial.[11] To focus on the impact of fluvastatin on virological relapse, data of patients with non-virological response were excluded from the present analyses. Of 119 consecutive chronic hepatitis C patients with genotype 1b and high viral load who visited Nippon Medical School Chiba Hokusoh Hospital between July 2008 and December 2009, 101 patients met the inclusion criteria and were randomly allocated to two arms: the PEG-IFN/ribavirin-treated group, provisionally designated as the non-fluvastatin group, and the PEG-IFN/ribavirin/fluvastatin-treated group, provisionally designated as the fluvastatin group. All patients provided written informed consent. The study protocol was prepared following ethics guidelines established in conformity with the 2008 Declaration of Helsinki after approval by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital. Leading inclusion criteria were as follows: genotype 1b as determined by a conventional polymerase chain reaction (PCR) technique, viral load of > 5.0 log IU/mL as measured by a quantitative real-time PCR method, hemoglobin concentration of > 10 g/dL, white blood cell count of > 2500/mm^3, or platelet count of > 70 000/mm^3 at the entry. Leading exclusion criteria were as follows: existence of decompensated liver cirrhosis, other liver diseases, severe renal disorder, abnormal thyroid function, poorly controlled diabetes or hypertension, past medical history of interstitial pneumonia, pregnancy, severe depression, allergy to interferon, ribavirin or fluvastatin, or medication with fibrates and other statins.

Treatment protocol. Patients received a subcutaneous injection of PEG-IFNα-2b (PEGINTRON, MSD, Tokyo, Japan) in a dose of 1.5 μg/kg per week and oral administration of ribavirin (REBETOL, MSD). The oral dose of ribavirin was determined based on the body weight (600 mg/day for < 60 kg, 800 mg/day for 60–80 kg, 1000 mg/day for > 80 kg), as specified by the package insert in Japan. The doses were appropriately reduced when a critical adverse event occurred during the treatment course. Fluvastatin was orally administered in a dose of 20 mg/day. The duration of treatment period was 48 weeks when serum HCV RNA was undetectable at week 12 of treatment, and was prolonged to 72 weeks when serum HCV RNA became undetectable at week 13 and later. Patients were followed for 24 weeks after the treatment completion.

Complete early virological response (cEVR) was defined as serum HCV RNA undetectable at week 12 of treatment. Late virological response (LVR) was defined as HCV RNA that became undetectable between weeks 13 and 36. Undetectable HCV RNA at the time of treatment completion was defined as end-of-treatment response (ETR). SVR was defined as HCV RNA undetectable at 24 weeks after treatment completion. Virological relapse was defined as achievement of ETR and reappearance of HCV RNA at 24 weeks after the treatment completion.

Laboratory. Peripheral blood examinations were performed weekly until week eight of treatment, and thereafter once a month until 24 weeks after the treatment completion. Biochemical tests were performed in the fasting-state. The HCV-RNA level was measured by using a quantitative real-time PCR method (COBAS AmpliPrep, Roche Diagnostics, Tokyo, Japan). Gene mutations in the core and NS5A regions of the HCV genome were determined by using a direct sequencing method. Genomic DNA was extracted from whole blood using a DNA Isolation kit on a MagNA Pure LC instrument (Roche Diagnostics, Basel, Switzerland). Single nucleotide polymorphisms (SNPs) at rs8099917 located in the locus adjacent to the *interleukin 28B* (*IL28B*) gene on chromosome No.19 were determined by real-time detection PCR using TaqMan SNPs Genotyping Assays on a 7500Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into two categories: T/T (major genotype) and non-T/T (minor genotype: T/G or G/G).

Statistical analysis. Fisher's exact test and Mann–Whitney *U*-test was performed for comparison of baseline characteristics and treatment outcome between the fluvastatin group and non-fluvastatin group. Multiple logistic regression analysis was performed to identify factors that contributed to virological relapse. Statistical analysis was performed using IBM SPSS version 17.0 (IBM Japan, Tokyo, Japan).

Results

Forty-six patients in the fluvastatin group and 48 patients in the non-fluvastatin group completed the treatment as scheduled. Twenty-three (50.0%) patients in the fluvastatin group and 26 (54.2%) patients in the non-fluvastatin group achieved cEVR ($P = 0.837$), and 11 (23.9%) and seven (14.6%) achieved LVR ($P = 0.431$). Thus, 34 patients in the fluvastatin group and 33 patients in the non-fluvastatin group who achieved virological response (either cEVR or LVR) were subjected to the subsequent analyses (Table 1). There were no significant differences in the baseline characteristics between the two groups.

Among patients who showed cEVR, SVR was achieved in 21 of 23 (91.3%) fluvastatin group patients and 17 of 26 (65.4%) non-fluvastatin group patients. Thus, only two (8.7%) patients in the fluvastatin group and nine (34.6%) patients in the non-fluvastatin group showed virological relapse ($P = 0.042$, Fig. 1).

Among patients who showed LVR, the SVR rate was 72.7% (8 of 11 patients) in the fluvastatin group and 42.9% (3 of 7 patients) in the non-fluvastatin group. Thus, the relapse rates were 27.3% (3 of 11 patients) and 57.1% (4 of 7 patients), respectively. The fluvastatin group was numerically lower in the relapse rate than the non-fluvastatin group, although not statistically significant ($P = 0.332$).

No patients showed virological breakthrough during the treatment period. Accordingly, the number of patients who achieved ETR was 34 in the fluvastatin group and 33 in the non-fluvastatin group. The relapse rate was significantly lower in the fluvastatin

Table 1 Baseline characteristics of patients who achieved complete early virological response (cEVR) and late virological response (LVR) in each group

Factor		PEG-IFN/ribavirin/fluvastatin ($n = 34$)	PEG-IFN/ribavirin ($n = 33$)	*P*-value
Age (years)	Median (range)	58 (41–79)	58 (32–80)	0.6469
Gender (no.)	Male	20	20	1.0000
	Female	14	13	
BMI (kg/m^2)	Median (range)	23.3 (15.6–31.8)	22.5 (17.9–27.7)	0.4724
Previous treatment with IFN	No	23	26	0.4101
	Yes	11	7	
AST (IU/L)	Median (range)	53 (18–151)	52 (18–152)	0.9284
ALT (IU/L)	Median (range)	66 (19–379)	76 (17–202)	0.7443
White blood cells (/mm^3)	Median (range)	5820 (3300–9620)	5380 (2900–7720)	0.1796
Hemoglobin (g/dL)	Median (range)	14.5 (11.1–17.0)	14.2 (11.0–16.4)	0.1320
Platelets (× 10^3/μL)	Median (range)	177 (90–244)	170 (72–236)	0.8705
LDL-C (mg/dL)	Median (range)	108 (66–195)	109 (63–177)	0.3757
Gamma-glutamyltransferase (IU/L)	Median (range)	40 (19–141)	44 (14–208)	0.9898
Glucose (mg/dL)	Median (range)	100 (77–198)	102 (77–198)	0.3902
Triglyceride (mg/dL)	Median (range)	106 (42–343)	102 (57–261)	0.8946
Total bilirubin (mg/dL)	Median (range)	0.7 (0.2–1.1)	0.6 (0.2–1.3)	0.6256
Serum Albumin level (g/dL)	Median (range)	4.3 (3.5–4.8)	4.3 (3.6–5.0)	0.8347
HCV-RNA (Log IU/mL)	Median (range)	6.5 (5.1–7.5)	6.3 (5.3–7.6)	0.3322
ISDR mutation	0 or 1	19	15	0.8031
	$ 2	15	14	
Core aa 70 substitution	Wild type	25	26	0.1222
	Mutant type	9	3	
Core aa 91 substitution	Wild type	27	23	1.0000
	Mutant type	7	6	
IL28B SNPs (rs8099917)	T/T	29	25	0.3389
	T/G or G/G	4	7	
PEG-IFN (μg/kg per week)	Median (range)	1.58 (1.27–1.67)	1.45 (0.74–1.85)	0.0779
Ribavirin (mg/kg per day)	Median (range)	11.0 (7.5–13.3)	10.9 (8.0–12.9)	0.1653

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; LDL-C, low density lipoprotein-cholesterol; PEG-IFN, Pegylated interferon; SNPs, Single nucleotide polymorphisms.

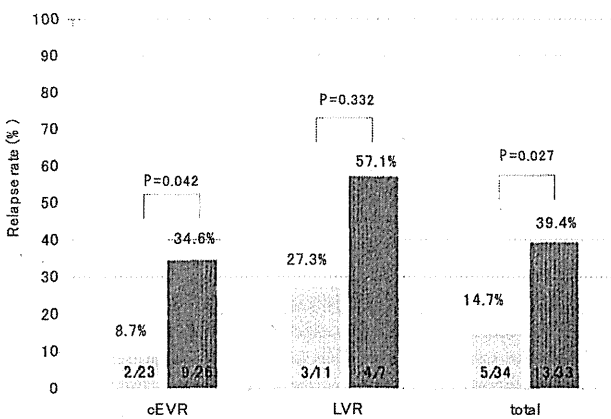

Figure 1 Comparison of the relapse rate between the fluvastatin group and non-fluvastatin group. (□) Fluvastatin group, (■) Non-fluvastatin group.

group (14.7%; 5 of 34 patients) than in the non-fluvastatin group (39.4%; 13 of 33 patients) ($P = 0.027$).

Multiple logistic regression analysis identified two independent factors contributing to virological relapse: female gender ($P = 0.030$, odds ratio [OR] = 4.33, 95% confidence interval [CI] = 1.14–12.44) and absence of fluvastatin ($P = 0.021$, OR = 4.33, 95% CI = 1.25–15.01) (Table 2). Actually, the relapse rates were 40.7% (11 of 27 female patients) *versus* 17.5% (7 of 40 male patients), and 39.4% (13 of 33 non-fluvastatin group patients) *versus* 14.7% (5 of 34 fluvastatin group patients).

Next, on-treatment factors, including total ribavirin dose, total PEG-IFN dose and presence or absence of cEVR, were added into univariate and multivariate analyses to investigate whether they could contribute to virological relapse. Although total ribavirin dose was not different between the fluvastatin and non-fluvastatin groups ($P = 0.165$), it was significantly lower in patients with relapse than in those with SVR ($P = 0.002$). Multiple logistic regression analysis identified absence of fluvastatin ($P = 0.027$, OR = 3.98, 95% CI = 1.05–15.11) and low total ribavirin dose ($P = 0.002$, OR = 2.41, 95% CI = 1.38–4.19) as independent factors contributing to relapse (Table 3).

Discussion

Several studies have reported the advantage of concomitant addition of statins to PEG-IFN/ribavirin combination therapy for chronic hepatitis C.[9,10,12] However, the exact mechanism by which fluvastatin improves SVR rate has not yet been determined. This

Table 2 Univariate and multiple logistic regression analyses of baseline factors associated with virological relapse in patients who achieved complete early virological response (cEVR) and late virological response (LVR)

		Univariate			Multivariate*		
Factor	Category	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Age (years)	By 1 year up	1.0337	0.9784–1.0922	0.2375			
Gender	Female/male	3.2411	1.0574–9.9339	0.0396	3.7643	1.1387–12.4440	0.0298
BMI (kg/m^2)	By 0.1 kg/m^2 up	0.9242	0.7691–1.1105	0.4001	—	—	—
Previous treatment with IFN	+/–	0.7143	0.2001–2.5495	0.6042	—	—	—
AST (IU/L)	By 1 IU/L up	0.9981	0.9807–1.0157	0.8275	—	—	—
ALT (IU/L)	By 1 IU/L up	0.9989	0.9894–1.085	0.8214	—	—	—
Serum Albumin level (g/dL)	By 0.1 mg/dL down	2.8216	0.4131–19.2678	0.2900	—	—	—
Total bilirubin (mg/dL)	By 0.1 mg/dL up	4.1684	0.3974–43.6681	0.2339	—	—	—
Gamma-glutamyltransferase (IU/L)	By 1 IU/L up	1.0001	0.9890–1.0121	0.9304	—	—	—
White blood cells (/mm3)	By 100/mm^3 down	1.0158	0.9803–1.0527	0.3867	—	—	—
Hemoglobin (g/dL)	By 1 g/dL down	1.7446	1.0838–2.8090	0.0220	—	—	—
Platelets (× 10^3/μL)	By 1 × 10^3/μL down	1.0601	0.9495–1.1836	0.2993	—	—	—
LDL-C (mg/dL)	By 1 mg/dL down	1.0128	0.9913–1.0346	0.2462	—	—	—
Glucose (mg/dL)	By 1 mg/dL up	0.9988	0.9728–1.0276	0.9912	—	—	—
Triglyceride (mg/dL)	By 1 mg/dL up	0.9988	0.9866–1.0111	0.8424	—	—	—
Serum HCV-RNA level (Log IU/mL)	By 0.1 LIU/mL up	1.0041	0.4446–2.2677	0.9921	—	—	—
ISDR mutation	$ 2/0,1	2.3571	0.7693–7.2220	0.1334	—	—	—
Core aa70 substitution	Mutant/wild	2.0879	0.5639–7.7310	0.2704	—	—	—
Core aa91 substitution	Mutant/wild	0.3023	0.3023–4.3202	0.8440	—	—	—
IL28B SNPs (rs8099917)	TG/GG or TT	2.3071	0.6876–10.0452	0.1578	—	—	—
Fluvastatin	– or +	3.7700	1.1604–12.2478	0.0273	4.3296	1.2489–15.0093	0.0209

*Multivariate analysis was performed with factors significantly associated with relapse by univariate analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; LDL-C, low density lipoprotein-cholesterol; SNPs, single nucleotide polymorphisms.

post-hoc analysis of data obtained from a randomized controlled trial[11] clarified the reason why fluvastatin significantly improves SVR rate. Interestingly, no difference was detected in both cEVR and ETR rates between the fluvastatin and non-fluvastatin groups. Fluvastatin was unlikely to influence viral kinetics aggressively from 12 weeks to the end of treatment. Fluvastatin appeared to inhibit virological relapse, thereby increasing SVR rate. This is the first report to clearly indicate the inhibition of viral relapse with concomitant use of fluvastatin, although the molecular biologic mechanism remains to be determined.

In various institutions, factors contributing to virological relapse are being investigated. A previous study reported that the deterioration of liver fibrosis and decrease in the dose of ribavirin increased relapse rate,[13] and that the prolongation of treatment period decreased relapse rate in patients who achieved LVR.[14,15] The number of mutations at the interferon sensitivity determining region (ISDR) was associated with the virological response[16–18] and contributed to relapse.[19] The relation between amino acid substitutions in the core region and relapse was controversial.[20,21] In this post-hoc analysis, neither the number of ISDR mutations nor core amino acid substitutions were associated with relapse. It has been reported that gender is involved as a factor contributing to SVR and that relapse is likely to occur in females.[22] These findings were consistent with our findings that gender and total ribavirin dose were significantly independent factors associated with relapse.

With respect to host genetic factors, SNPs located in the locus adjacent to the *IL28B* gene of chromosome No.19, rs8099917 and rs12979860, are one of the strongest factors associated with the treatment outcome in PEG-IFN/ribavirin therapy.[23–25] In this post-hoc study, however, rs8099917 was not significantly associated with relapse. In the fluvastatin group, neither of two patients with minor *IL28B* SNPs showed relapse, while two patients with relapse had major *IL28B* SNPs. *IL28B* SNPs was unlikely to contribute to relapse.

However, there were some limitations in this post-hoc analysis. The number of patients was too small to strictly identify factors contributing to relapse. Moreover, we did not confirm hepatic fibrosis by biopsy, which may influence relapse. It is unclear whether or not add-on fluvastatin significantly reduced the relapse rate in patients with advanced liver fibrosis.

Lipids play an important role in the HCV replication process, and lipid metabolism of the host is closely associated with the replication process. We assume that intervention in HCV replication-related lipid metabolism by fluvastatin may influence the HCV replication process, resulting in the inhibition of relapse. It has been reported that SVR can be more readily achieved by PEG-IFN/ribavirin therapy in patients with a high serum low density lipoprotein-cholesterol (LDL-C) level.[26] In this post-hoc analysis, however, serum LDL-C level was not associated with virological response and relapse. In the fluvastatin group, serum LDL-C level decreased during the treatment course in all but two patients (data not shown). A statin that reduces the LDL-C level more strongly than fluvastatin is now available. However, such a statin may not necessarily exhibit strong anti-HCV activity in clinical practice. Anti-HCV activity of various stains has not been investigated in any clinical study.

Table 3 Univariate and multiple logistic regression analyses of baseline and on-treatment factors associated with virological relapse in patients who achieved complete early virological response (cEVR) and late virological response (LVR)

		Univariate			Multivariate*		
Factor	Category	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Age (years)	By 1 year up	1.0337	0.9784–1.0922	0.2375	—	—	—
Gender	Female/male	3.2411	1.0574–9.9339	0.0396	—	—	—
BMI (kg/m^2)	By 0.1 kg/m^2 up	0.9242	0.7691–1.1105	0.4001	—	—	—
AST (IU/L)	By 1 IU/L up	0.9981	0.9807–1.0157	0.8275	—	—	—
ALT (IU/L)	By 1 IU/L up	0.9989	0.9894–1.085	0.8214	—	—	—
Serum Albumin level (g/dL)	By 0.1 mg/dL down	2.8216	0.4131–19.2678	0.2900	—	—	—
Total bilirubin (mg/dL)	By 0.1 mg/dL up	4.1684	0.3974–43.6681	0.2339	—	—	—
Gamma-glutamyltransferase (IU/L)	By 1 IU/L up	1.0001	0.9890–1.0121	0.9304	—	—	—
White blood cells (/mm^3)	By 100/mm^3 down	1.0158	0.9803–1.0527	0.3867	—	—	—
Hemoglobin (g/dL)	By 1 g/dL down	1.7446	1.0838–2.8090	0.0220	—	—	—
Platelets (× 10^5/μL)	By 1 × 10^5/μL down	1.0601	0.9495–1.1836	0.2993	—	—	—
LDL-C (mg/dL)	By 1 mg/dL down	1.0128	0.9913–1.0346	0.2462	—	—	—
Glucose (mg/dL)	By 1 mg/dL up	0.9988	0.9728–1.0276	0.9912	—	—	—
Triglyceride (mg/dL)	By 1 mg/dL up	0.9988	0.9866–1.0111	0.8424	—	—	—
Serum HCV-RNA level (Log IU /mL)	By 0.1 LIU/mL up	1.0041	0.4446–2.2677	0.9921	—	—	—
ISDR mutation	$ 2/0,1	2.3571	0.7693–7.2220	0.1334	—	—	—
Core aa70 substitution	Mutant/wild	2.0879	0.5639–7.7310	0.2704	—	—	—
Core aa91 substitution	Mutant/wild	0.3023	0.3023–4.3202	0.8440	—	—	—
IL28B SNPs (rs8099917)	TG/GG or TT	2.3071	0.6876–10.0452	0.1578	—	—	—
Fluvastatin	– or +	3.7700	1.1604–12.2478	0.0273	3.9809	1.0487–15.1060	0.0424
cEVR	– or +	1.7274	0.5267–5.6657	0.3671	—	—	—
PEG-IFN (μg/kg per week)	1 μg/kg per week down	1.2864	0.0535–30.9459	0.8766	—	—	—
Ribavirin (mg/kg per day)	By 1 mg/kg per day down	2.3540	1.3843–4.0032	0.0016	2.4050	1.3793–4.1929	0.0020

*Multivariate analysis was performed with factors significantly associated with relapse by univariate analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; LDL-C, low density lipoprotein-cholesterol; PEG-IFN, Pegylated interferon; SNPs, single nucleotide polymorphisms.

In the near future, combination therapy with PEG-IFN/ribavirin and new agents, such as protease,[27–30] polymerase[31–33] and NS5A inhibitors,[34] may become a standard-of-care treatment. The introduction of protease inhibitors could significantly increase the virological response rate.[12] Addition of statins to such next-generation combination therapy may be useful in reducing the relapse rate, resulting in the further increase of SVR rate.

In conclusion, the concomitant addition of fluvastatin reduced virological relapse in PEG-IFN/ribavirin therapy for chronic hepatitis C patients with genotype 1b and high viral load. Fluvastatin may consolidate the inhibition of intrahepatic HCV replication through the intervention of lipid metabolism.

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