Table 6 Supplements to guidelines for type C cirrhosis

1 To start with, IFN for compensated cirrhosis is desired at 6 MIU daily for 2–8 weeks, as far as possible, and to continue for 48 weeks or longer, as for chronic hepatitis C.
2 In patients with compensated cirrhosis who fail to clear HCV RNA within 12 weeks on IFN, long-term therapy at 3 MIU should be considered for preventing HCC.
3 In patients with platelet counts $<50 \times 10^3/mm^3$, splenectomy or embolization of splenic artery is recommended before re-treatment, and after thorough evaluation has been made on the response to IFN to be expected.
4 For the prevention of HCC, not only IFN, but also liver supportive therapy (SNMC, UDCA, etc.), phlebotomy and branched chain amino acids, either alone or in combination, are recommended for improving ALT/AST and AFP levels.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MIU, million international units; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

1 For treatment of type C cirrhosis with IFN, the initial dose of 6 million international units (MIU) daily is continued as long as possible (2–8 weeks). Thereafter, long-term IFN for 48 weeks or longer is desired as in the treatment of chronic hepatitis C.
2 In the treatment of type C cirrhosis, patients who fail to achieve EVR with the clearance of HCV RNA from serum within 12 weeks should receive long-term IFN at a dose of 3 MIU.
3 For patients with type C cirrhosis who have platelet counts of less than $50 \times 10^3/mm^3$, splenectomy or embolization of the splenic artery is desirable before commencing IFN therapy, after the efficacy of IFN has been evaluated thoroughly.[12]
4 For preventing the development of HCC, improvement in ALT, AST and AFP levels are aimed. Toward this end, not only IFN, but also liver supportive therapy (SNMC and UDCA), phlebotomy and BCAA are used, either alone or in combination.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HCV ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. The principal aim of these guidelines is to decrease the incidence of HCC due to HCV infection in Japan. In accord with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic hepatitis C. They are prepared on evidence-based data that have been accumulated by members and cooperators of the study group. It is necessary to improve these guidelines in the next fiscal year and thereafter, in accordance with many pieces of new evidence that are expected to emerge through enduring efforts of members and cooperators of the study group.

In the treatment of chronic hepatitis C, the duration of antiviral treatments is extended to 72 weeks, which has been approved as of the fiscal year 2008, and criteria for the eligibility of extended treatment duration are clearly defined. Long-term antiviral treatments, extended up to 72 weeks, are hoped to increase the SVR even further. In addition, comprehensive guidelines for the treatment of cirrhosis have been improved with substantial additions, and their criteria for the indication made explicit.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has drafted, and also displayed online (www.jsh.or.jp/medical/index.html [in Japanese]), guidelines for a spectrum of liver diseases due to HCV, from chronic hepatitis to cirrhosis of various severities. In view of the eventual goal of decreasing the incidence of HCC due to HCV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been constructed for the treatment of cirrhosis due to HCV infection. As a general rule, antiviral treatments constitute the main body of guidelines for the treatment of chronic hepatitis C. Furthermore, the fundamental concept of these guidelines would need to be kept in mind always. It is our sincere hope that, for the treatment of each patient, readers will base their clinical practice on these guidelines, and refer to appropriate individual guidelines, when they make a decision on the treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in treatment choice.

It is our sincere desire that treatment of patients with chronic hepatitis and cirrhosis due to HCV will proceed following these guidelines. Efforts along these lines will rectify a wide gap in medical treatment served to the nation and raise substantial and efficient interest in the medical economy on the national basis. In practicing treatment according to these guidelines, it will be nec-

essary to evaluate their therapeutic efficacy, and revise or add necessary supplements to them as required in the future.

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Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Affect Very Early Viral Dynamics During Treatment With Telaprevir, Peginterferon, and Ribavirin

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Substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the rate of HCV RNA loss following 12-week triple therapy, and determine the effect of aa substitutions on very early (within 48 hr) viral dynamics. Sixty-seven patients infected with HCV genotype 1b (HCV-1b) and high viral load who received 12-week triple therapy were studied. RNA loss could be achieved in 2%, 34%, 80%, 92%, 95%, 94%, and 90% of the patients after 1, 2, 4, 6, 8, 10, and 12 weeks of triple therapy, respectively. After 24-hr treatment, the proportion of patients with Arg70 and Leu91 substitutions with ≥3.0 log fall in HCV RNA was significantly higher than those with <3.0 log fall ($P = 0.008$). However, the aa substitution patterns in the core region did not influence the fall in HCV RNA after 48-hr treatment. Multivariate analysis identified substitutions of aa 70 and 91 ($P = 0.014$) and level of viremia at baseline (≥7.0 log IU/ml; $P = 0.085$) as independent parameters that determined the ≥3.0 log fall in HCV RNA level after 24-hr triple therapy. It is concluded that 12-week triple therapy achieved high rates of loss of HCV RNA in Japanese patients infected with HCV-1b and high viral load, and that the aa substitution pattern in the core region seems to influence very early viral dynamics. ***J. Med. Virol. 82:575–582, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: HCV; core region; NS5A-ISDR; telaprevir; peginterferon; ribavirin; very early viral dynamics

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999]. At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) with high viral loads (>100 KIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Iino et al., 2005; Tsubota et al., 2005]. Such background calls for efficient treatment of patients with chronic HCV infection.

Even with pegylated interferon (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b with high viral loads [Manns et al., 2001; Fried et al., 2002]. Recently, a new strategy was introduced for the treatment of chronic HCV infection by inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these drugs, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [Lin et al., 2006]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, results in a robust antiviral activity [Modi and Hoofnagle, 2007; Zeuzem, 2008]. Specifically, HCV RNA disappears in

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almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin [Lawitz et al., 2008; Suzuki et al., 2009]. However, patients resistant to treatment who do not achieve sustained virological response by the triple therapy, have been reported [Lawitz et al., 2008; Hézode et al., 2009; McHutchison et al., 2009]. The underlying mechanism of the response to the treatment is still not clear.

It is useful to evaluate treatment efficacy based on viral dynamics as an early predictor of PEG-IFN plus ribavirin combination therapy. Previous reports showed that decreases in HCV RNA levels were significantly greater in patients with than without sustained virological response from 24 hr to 12 weeks after the start of PEG-IFN plus ribavirin combination therapy in patients infected with HCV-1b and high viral load. Very early dynamics within 48 hr of such treatment is particularly important for early prediction of response to therapy [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Accordingly, the pretreatment predictors of very early dynamics during triple therapy of telaprevir with PEG-IFN and ribavirin were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with genotype 1b and high viral load are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009], and also affect the clinical outcome, including insulin resistance and hepatocarcinogenesis [Akuta et al., 2007c, 2009b; Fishman et al., 2009; Nakamoto et al., 2009]. However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict the very early dynamics and response to triple therapy of telaprevir with PEG-IFN and ribavirin.

The present study included 67 patients with HCV-1b and high viral load, who received triple therapy of telaprevir with PEG-IFN plus ribavirin and followed-up for 12 weeks or more after the start of treatment. The aims of the study were to determine the rate of loss of HCV RNA during treatment, and to identify the pretreatment factors that could predict very early viral dynamics (within 48 hr) after the start of treatment, including aa substitutions in the HCV core, the NS3, and the NS5A regions.

PATIENTS AND METHODS

Study Patients

Between May 2008 and May 2009, 67 patients infected with HCV were recruited to the study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (30%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN, and ribavirin], and 47 patients (70%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks. All patients were followed-up for at least 12 weeks after the start of triple therapy.

All patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C; (2) HCV-1b confirmed by sequence analysis; (3) HCV RNA levels of $\geq$5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese (Mongoloid) ethnicity; (5) age at study entry of 20–65 years; (6) body weight $\geq$35 and $\leq$120 kg at the time of registration; (7) lack of decompensated cirrhosis; (8) absence of hepatitis B surface antigen (HBsAg) in serum; (9) no history of HCC; (10) no previous treatment for malignancy; (11) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C; (12) no history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of $\leq$50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of $\geq$110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline; and (13) hemoglobin level of $\geq$12 g/dl, neutrophil count $\geq$1,500/mm^3, and platelet count of $\geq$100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study.

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at a dose of 750 or 500 mg three times a day at an 8-hr (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, NJ) was injected subcutaneously with a median dose 1.5 μg/kg (range: 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1,000 mg). All participating patients received these three drugs in the initial 12 weeks of the study.

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutrophil count below 750/mm^3, or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3, or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800–600, and 1,000–600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir

(MP-424) remained the same throughout the 12-week protocol, though the drug was discontinued altogether following the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at nine time points: 24 hr, 48 hr, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 12 weeks after the commencement of treatment. HCV RNA levels during treatment was evaluated in 66 (99%), 66 (99%), 65 (97%), 67 (100%), 64 (96%), 60 (90%), 58 (87%), 50 (75%), and 58 (87%) of the 67 patients, at the above time intervals, respectively. 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, and the undetectable samples were defined as negative. Reduction in HCV RNA levels at 24 and 48 hr relative to the baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core, NS3, and NS5A Regions

In the present study, aa substitutions of the core, NS3, and NS5A-ISDR regions were analyzed by direct sequencing. AA sequences in the upstream site (1027–1318 aa) of the NS3 region, including aa positions reported as resistance for telaprevir [Lin et al., 2005; Forestier et al., 2007; Zhou et al., 2007], were determined. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by PCR using the following primers. (a) Nucleotide sequences of the core region: the first-round PCR was performed with CE1 (sense: 5′-GTC TGC GGA ACC GGT GAG TA-3′; nucleotides: 134–153) and CE2 (antisense: 5′-GAC GTG GCG TCG TAT TGT CG-3′; nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense: 5′-ACT GCT AGC CGA GTA GTG TT-3′; nucleotides: 234–253) and CE6 (antisense: 5′-GGA GCA GTC GTT CGT GAC AT-3′; nucleotides: 934–953) primers. (b) Nucleotide sequences of NS3 region: the first-round PCR was performed with NS33F (sense: 5′-ACT TCT AGG ACC GGC CGA TA-3′; nucleotides: 3359–3378) and NS34R (antisense: 5′-GCT CGT CAC ACT TCT TCT TG-3′; nucleotides: 4517–4536) primers, and the second-round PCR with NS33F (sense) and NS36R (antisense: 5′-GTC TGT GAA GAC CGG AGA CC-3′; nucleotides: 3946–3965) primers. (c) Nucleotide sequences of NS5A-ISDR: the first-round PCR was performed with ISDR1 (sense: 5′-ATG CCC ATG CCA GGT TCC AG-3′; nucleotides: 6662–6681) and ISDR2 (antisense: 5′-AGC TCC GCC AAG GCA GAA GA-3′; nucleotides: 7350–7369) primers, and the second-round PCR with ISDR3 (sense: 5′-ACC GGA TGT GGC AGT GCT CA-3′; nucleotides: 6824–6843) and ISDR4 (antisense: 5′-GTA ATC CGG GCG TGC CCA TA-3′; nucleotides: 7189–7208) primers ([a,c]; nested PCR. [b]; hemi-nested PCR). All samples were denatured initially at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed on 67 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region, ISDR) reported by Enomoto et al. [1995, 1996] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (≤ 1) or mutant-type (≥ 2).

Statistical Analysis

Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to very early viral dynamics. The odds ratios and 95% confidence intervals (95% CI) were also calculated. All P-value <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with very early dynamics included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, and aa substitution in the core, NS3, and the NS5A-ISDR regions. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL).

TABLE I. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon, and Ribavirin Triple Therapy in Japanese Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	67
Sex (M/F)	36/31
Age (years)*	54 (23–65)
History of blood transfusion	19 (28.4%)
Family history of liver disease	11 (16.4%)
Body mass index (kg/m^2)*	22.7 (16.0–32.4)
Laboratory data*	
Serum aspartate aminotransferase (IU/l)	34 (15–118)
Serum alanine aminotransferase (IU/l)	43 (12–175)
Serum albumin (g/dl)	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase (IU/l)	35 (9–194)
Leukocyte count (/mm^3)	4,900 (3,000–8,100)
Hemoglobin (g/dl)	14.2 (12.1–16.8)
Platelet count ($\times 10^4$/mm^3)	17.4 (10.4–33.8)
Level of viremia (log IU/ml)	6.8 (5.1–7.6)
Alpha-fetoprotein (μg/L)	4 (2–38)
Total cholesterol (mg/dl)	184 (112–276)
High-density lipoprotein cholesterol (mg/dl)	46 (20–79)
Low-density lipoprotein cholesterol (mg/dl)	106 (47–191)
Triglycerides (mg/dl)	99 (49–215)
Fasting plasma glucose (mg/dl)	92 (66–107)
Treatment	
PEG-IFNα-2b dose (μg/kg)*	1.5 (1.3–2.0)
Ribavirin dose (mg/kg)*	11.5 (7.2–15.8)
Telaprevir dose (1,500/2,250 mg/day)	10/57

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.

RESULTS

Table I summarizes the profiles and laboratory data of the 67 patients at the commencement of treatment. They included 36 males and 31 females, aged 23–65 years (median, 54 years). The frequencies of Arg70 and Gln70 (His70) in the core region were 61% (41/67) and 39% (26/67), respectively. The frequencies of Leu91 and Met91 were 55% (37/67) and 45% (30/67), respectively. However, frequencies of wild-type and mutant-type in NS5A-ISDR were 96% (64/67) and 5% (3/67), respectively.

Rates of Loss of HCV RNA During Treatment

The disappearance rate of HCV RNA during treatment was 0% (0/66), 0% (0/66), 2% (1/65), 34% (23/67), 80% (51/64), 92% (55/60), 95% (55/58), 94% (47/50), and 90% (52/58) at 24 hr, 48 hr, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 12 weeks, respectively.

According to the substitution of core aa 70, the rate of HCV RNA loss at each time point was not significantly different between Arg70 and Gln70(His70) (Fig. 1A). According to the substitution of core aa 91, the rate at each time point was not significantly different between Leu91 and Met91 (Fig. 1B).

Very Early Dynamics According to Amino Acid Substitutions in the Core, the NS3, and the NS5A Regions

After 24 hr of commencement of the triple therapy, the proportion of patients who showed ≥2.0 log fall in HCV

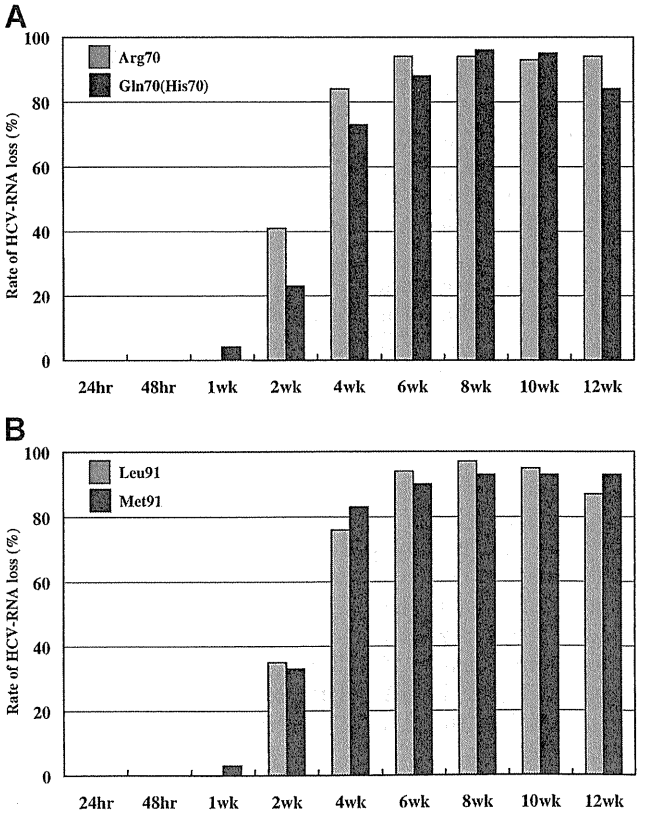

Fig. 1. Rates of HCV RNA loss according to substitutions of the core aa 70 and 91 at different time points after commencement of the triple therapy. At each time point, the rate of HCV RNA loss was not significantly different between Arg70 and Gln70(His70) (**A**) or between Leu91 and Met91 (**B**).

TABLE II. Falls in HCV RNA Levels From Baseline After 24 and 48 hr of Triple Therapy of Telaprevir, Peginterferon, and Ribavirin According to the Amino Acid Substitutions in the Core Region and NS5A Region in Patients Infected With HCV Genotype 1b

	Fall in HCV RNA[a] (log IU/ml)	≥2.0 log (n = 64)	<2.0 log (n = 2)	*P*	≥3.0 log (n = 21)	<3.0 log (n = 45)	*P*
(A) Fall after 24 hr of triple therapy							
Arg70 and Leu91	3.0 (1.8–4.0)	26 (40.6%)	2 (100%)	NS	14 (66.7%)	14 (31.1%)	0.008
Gln70(His70)	2.7 (2.3–3.5)	26 (40.6%)	0 (0%)	NS	5 (23.8%)	21 (46.7%)	NS
Met91	2.7 (2.0–3.3)	30 (46.9%)	0 (0%)	NS	4 (19.0%)	26 (57.8%)	0.004
Gln70(His70)andMet91	2.7 (2.3–3.3)	18 (28.1%)	0 (0%)	NS	2 (9.5%)	16 (35.6%)	0.037
ISDR wild-type	2.8 (1.8–4.0)	61 (95.3%)	2 (100%)	NS	2 (100%)	42 (93.3%)	NS
	Fall in HCV RNA[a] (log IU/ml)	≥3.01og (n = 62)	<3.0 log (n = 4)	*P*	≥4.0 log (n = 21)	<4.0 log (n = 45)	*P*
(B) Fall after 48 hr of triple therapy							
Arg70 and Leu91	3.8 (2.6–4.4)	27 (43.5%)	1 (25.0%)	NS	12 (57.1%)	16 (35.6%)	NS
Gln70(His70)	3.5 (2.8–4.3)	23 (37.1%)	3 (75.0%)	NS	6 (28.6%)	20 (44.4%)	NS
Met91	3.8 (2.8–4.5)	28 (45.2%)	2 (50.0%)	NS	8 (38.1%)	22 (48.9%)	NS
Gln70(His70) and Met91	3.5 (2.8–4.3)	16 (25.8%)	2 (50.0%)	NS	5 (23.8%)	13 (28.9%)	NS
ISDR wild-type	3.7 (2.6–4.5)	59 (95.2%)	4 (100%)	NS	20 (95.2%)	43 (95.6%)	NS

[a]Data are denoted by the median (range) values.

RNA level were not significantly different from that of patients who showed <2.0 log fall for all aa substitutions (Table II). However, a significantly higher proportion of patients with Arg70 and Leu91 substitutions showed ≥3.0 log drop in HCV RNA level than that of patients who showed a fall of <3.0 log (Table II, $P = 0.008$). In contrast, significantly fewer patients with Met91 showed ≥3.0 log drop in HCV RNA level than those who showed a fall of <3.0 log ($P = 0.004$). Likewise, significantly fewer patients with Gln70(His70) and Met91 showed a fall of ≥3.0 log in HCV RNA level than those who showed a fall of <3.0 log (Table II, $P = 0.037$). Thus, the fall in HCV RNA level at 24 hr was influenced by aa substitution patterns in the core region. Figure 2 shows the sequences of aa 61–110 of the core region in patients at the commencement of treatment.

At 48 hr, the proportion of patients who showed ≥3.0 log fall in HCV RNA was not significantly different from that of patients who showed <3.0 log drop for all aa substitutions (Table II). Similar results were noted in those patients who showed ≥ or <4.0 log fall in HCV RNA levels. Thus, the fall in HCV RNA level at 48 hr was independent of the aa substitution patterns in the core and NS5A regions.

Thus, the results did not identify aa substitution patterns in the upstream site of the NS3 region that influenced the fall in HCV RNA level from baseline after 24 and 48 hr of the commencement of the triple therapy. Furthermore, the frequency of the mutant-type in NS5A-ISDR was only 5%, and thus ISDR was not identified as a predictor of very early viral dynamics.

Predictive Factors Associated With ≥3.0 Log Fall in HCV RNA Level at 24 hr

Univariate analysis identified two parameters that correlated with a fall of ≥3.0 log in HCV RNA level after 24 hr of commencement of triple therapy either significantly or marginally: substitution of aa 70 and 91 (Arg70 and Leu91; $P = 0.008$) and level of viremia at baseline (≥7.0 log IU/ml; $P = 0.054$). Both these factors were also identified by multivariate analysis as independent parameters that either significantly or marginally influenced the ≥3.0 log fall in HCV RNA level after 24 hr of commencement of the triple therapy (Arg70 and Leu91; $P = 0.014$, HCV RNA ≥7.0 log IU/ml at baseline; $P = 0.085$, Table III).

DISCUSSION

Two previous studies (PROVE1 in USA, and PROVE2 in Europe) showed that 12-week triple therapy of telaprevir, PEG-IFN, and ribavirin could achieve undetectable HCV RNA levels in 70–80% of patients, and sustained virological response rates of 60–70% [Hézode et al., 2009; McHutchison et al., 2009]. In the present study, the rate of HCV RNA loss at 12 weeks were higher than those of the above two studies. The discrepancy between the present study and the above studies may be due to one or more factors. The first reason is probably the small number of Japanese patients infected with genotype 1b in the present study. The second could be the difference in body mass index. Body mass index of the patients studied (median; 23 kg/m^2) was much lower than that of the participants of the previous study by McHutchison et al. (median; >25 kg/m^2). The third reason is probably related to the type of PEG-IFN. PEG-IFN in the above reports was used at a fixed dose of PEG-IFNα-2a, but that of the present study was a body weight-adjusted dose of PEG-IFNα-2b. The present study was not designed to evaluate the sustained virological response and none of the patients was studied at 24 weeks after the end of the treatment protocol. Further studies of a larger number of patients matched for background are required to investigate the

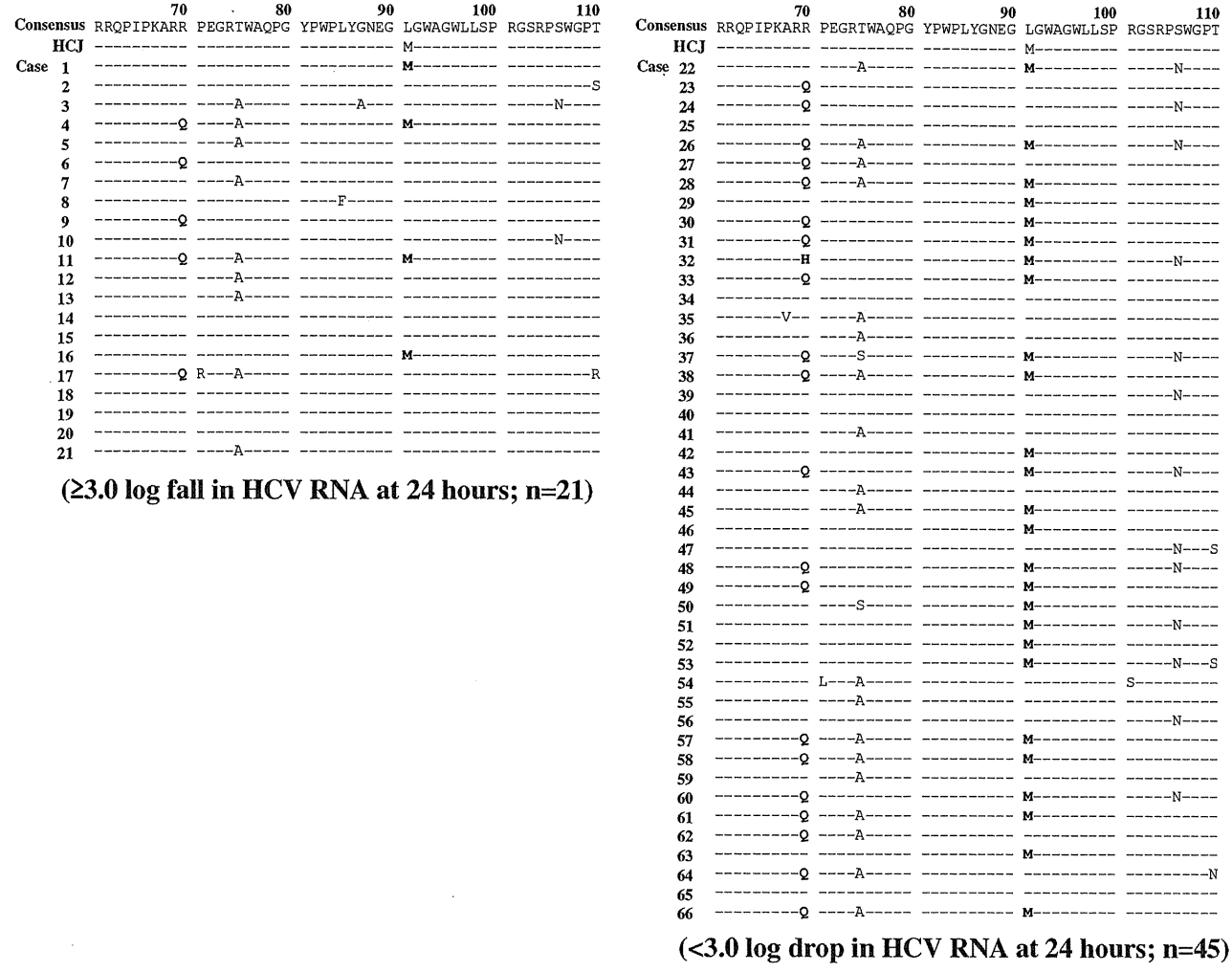

Fig. 2. Sequences of amino acids 61–110 in the core region at commencement of triple therapy in patients infected with HCV genotype 1b and high viral load. Dashes indicate amino acids identical to the consensus sequence of genotype 1b, and substituted amino acids are shown by standard single-letter codes. The amino acid patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters.

rate of HCV RNA loss during triple therapy and the sustained virological response rate.

A previous study based on a small number of 20 patients showed that the aa substitution pattern of the core region did not affect the virological response at 1 and 2 weeks after the start of triple therapy [Suzuki et al., 2009]. The present study is the first to report that the aa substitution pattern of the core region affect significantly very early viral dynamics (within 48 hr) during triple therapy. Previous reports showed that very early dynamics (within 48 hr) after the start of IFN and ribavirin combination therapy was important for early prediction of treatment efficacy including sustained virological response [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Hence, the finding of the present study of aa substitution patterns

TABLE III. Multivariate Analysis of Factors Associated With ≥3.0 Log Fall in HCV RNA After 24-hr Triple Therapy of Telaprevir, Peginterferon, and Ribavirin Therapy in Japanese Patients Infected With HCV Genotype 1b

Factor	Category	Odds ratio (95% CI)	*P*
Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1	
	2: Arg70 and Leu91	4.13 (1.33–12.8)	0.014
Level of viremia (log IU/ml)	1: <7.0	1	
	2: ≥7.0	2.73 (0.87–8.56)	0.085

95% CI, 95% confidence interval.
Only variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on multivariate logistic regression analysis are shown.

of the core region as pretreatment predictor of very early viral dynamics could be also useful for early prediction of sustained virological response following triple therapy. Amino acid substitution patterns of the core region are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009]. Previous studies reported that the core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melén et al., 2004; de Lucas et al., 2005]. The present result could be also interpreted to mean that aa substitutions of the core region might be associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which is known to inhibit IFN-α-induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2′,5′-OAS and MxA [Vlotides et al., 2004]. Furthermore, the result also indicates that aa substitutions of the core region might serve as a surrogate marker for other proteins associated with resistance to the antiviral actions of IFN. Further large-scale studies designed to examine the structural and functional impact of aa substitutions in the core region during each of monotherapy (PEG-IFN, ribavirin, and telaprevir), dual therapy (PEG-IFN/ribavirin and PEG-IFN/telaprevir), and triple therapy (PEG-IFN/ribavirin/telaprevir) should be conducted to confirm the above finding.

Another limitation of the present study was that aa substitutions in areas other than the core, the NS3, and the NS5A-ISDR regions of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR, e.g., V3 of NS5A region) [El-Shamy et al., 2008; Muñoz de Rueda et al., 2008], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [Lin et al., 2005], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [Forestier et al., 2007; Zhou et al., 2007]. Thus, viral factors before and during triple therapy should be investigated in future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, a 12-week course of triple therapy of telaprevir, PEG-IFN, and ribavirin in patients infected with HCV-1b and high viral load achieved high rates of HCV RNA loss. The aa substitution pattern of the core region seems to affect the very early viral dynamics. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy.

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Original Article

Prolonged treatment with pegylated interferon α 2b plus ribavirin improves sustained virological response in chronic hepatitis C genotype 1 patients with late response in a clinical real-life setting in Japan

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Aim: This study was conducted to clarify the factors related to sustained virological response (SVR) to pegylated interferon α 2b (PEG-IFN) plus ribavirin (RBV) combination therapy administered for 48 weeks in patients with chronic hepatitis C virus (CHCV) and to evaluate the usefulness of prolonged treatment in patients with late virological response (LVR).

Methods: Of 2257 patients registered at 68 institutions, those with genotype 1 and high viral load were selected to participate in two studies. Study 1 (standard 48-week group, $n = 1480$) investigated SVR-determining factors in patients who received the treatment for ≤52 weeks, whereas study 2 compared SVR rates between patients with LVR who received treatment for either 36–52 weeks (48-week group, $n = 223$) or 60–76 weeks (72-week group, $n = 73$).

Results: In study 1, SVR rate was 44.9%; that in male subjects (50.4%) was significantly ($P < 0.0001$) higher than in female subjects (36.4%). SVR rate significantly ($P < 0.0001$) decreased with 10-year age increments in both sexes. Multivariate logistic regression analysis revealed that age, F score, platelet count, and HCV load were SVR-related factors. In study 2, SVR rate in the 72-week group (67.1%) was significantly ($P = 0.0020$) higher than in the 48-week group (46.2%).

Conclusions: Patients with CHCV genotype 1 infection should be treated with PEG-IFN plus ribavirin combination therapy as early as possible, and 72 weeks' treatment is recommended in patients with LVR regardless of age.

Key words: chronic hepatitis C virus, elderly patients, pegylated interferon, prolonged treatment, ribavirin

INTRODUCTION

THE TOTAL NUMBER of patients infected with the hepatitis C virus (HCV) is estimated at 170 million worldwide, of whom 1.5–1.7 million are Japanese. Treatment of HCV infection began with interferon (IFN) monotherapy before the discovery of HCV in 1989. At that time, responders to treatment were mostly limited to patients with HCV genotypes 2 or 3 infection, which is highly sensitive to IFN. The sustained virological response (SVR: HCV-RNA negative at 24 weeks after end of treatment) to IFN monotherapy in genotype 1 patients known from that time to be difficult to treat was only about 5%. SVR rate has since increased thanks to concomitant administration of the antiviral drug ribavirin (RBV), and with the development of the long-acting

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IFN product pegylated interferon (PEG-IFN) it has increased to 50%.[1–4] Today, PEG-IFN plus ribavirin regimen is internationally recognized as a standard therapy for chronic hepatitis C virus (CHCV) infection.[5,6] Early clinical trials of this regimen focused on specific patient populations. Subsequently, several multinational studies such as WIN-R,[7] HALT-C,[8] EPIC3,[9] and REPEAT Study[10] have been conducted in the general clinical setting. The results of the IDEAL Study[11] directly comparing PEG-IFN α 2a versus PEG-IFN α 2b have also been published. From these studies, variables predictive of SVR have been identified, including ethnicity, sex, age, and weight as demographic parameters, staging and hepatic steatosis as histological parameters, viral load, genotype, NS5A, and core mutation as virologic parameters, alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) as biochemical parameters, and even the timing of viral negativity as a treatment variable.[12–15] More recently, the SVR rate was reported to increase in association with decrease in the relapse rate with 72-week treatment in patients with delayed HCV-RNA negativity.[15,16] However, the majority of patients participating in previous studies in western countries were aged in their 40s on average, and the influence of aging of the patient population has not been studied adequately.

We therefore examined SVR-determining factors with 48-week PEG-IFN α 2b plus RBV combination therapy in the prevailing Japanese clinical setting characterized by increasing numbers of elderly patients. We also compared SVR rate between 48-week and 72-week treatment in patients with late virological response (LVR) defined as achieving HCV-RNA negativity in the period from weeks 13 to 24 after the start of treatment so as to examine the significance of prolonged treatment.

METHODS

Patients

A MULTICENTER STUDY was conducted at 68 institutions in Tokyo and Yamanashi prefectures (PERFECT Study Group; see Appendix I) to survey the actual state of combination therapy with PEG-IFN α 2b (PegIntron; Schering Plough, Kenilworth, NJ) and RBV (Rebetol, Schering Plough) in 2008. A total of 2257 chronic hepatitis C virus (CHCV) patients seen from December 2004 who completed combination treatment by September 2007 were registered regardless of genotype, history of IFN treatment, and ALT levels. The presence of HCV in serum had to be confirmed by Cobas Amplicor HCV Monitor, version 2.0 (Roche Diagnostic, Tokyo) for registration.

Excluded from this study were pregnant or possibly pregnant and lactating women, and patients with severe heart disease, chronic kidney failure or creatinine clearance of ≤50 mL/min, current or history of severe psychiatric disorder, and autoimmune hepatitis.

Demographic characteristics examined included age, sex, height and weight, the presence or absence of diabetes mellitus, hypertension, heavy drinking, and history of IFN therapy and hepatic cancer. Hepatic histological data recorded were stage (F0–F4) and grade (A0–A3). Laboratory tests recorded were ALT, platelet count, albumin, and α-fetoprotein (AFP) before the start of PEG-IFN α 2b plus RBV combination therapy.

As indicated in Figure 1, of the total 2257 patients registered, patients with genotype 1 and high viral load (>100 KIU/mL: Amplicor PCR quantitation) who satisfied the following conditions were included in this study: patients who received treatment for ≤52 weeks (standard 48-week treatment group, $n = 1480$) in study 1, and patients with LVR who received treatment for either 36–52 weeks (48-week treatment group, $n = 223$) or 60–76 weeks (72-week treatment group, $n = 73$) in study 2.

This multicenter study was approved by IRB at each participating institution. The study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient.

Treatment

PEG-IFN α 2b was administered subcutaneously once weekly at a dose of 1.5 μg/kg. Dose reduction and treatment discontinuation followed the instructions given in the package insert, i.e., the dose was reduced by half if WBC decreased to <1500/mm^3, neutrophils to <750/mm^3 or platelet count to <80000/mm^3, and treatment was discontinued if WBC decreased to <1000/mm^3, neutrophils to <500/mm^3 or platelet count to <50000/mm^3. RBV was administered in two divided doses of 600, 800, or 1000 mg/day in patients weighing <60, 60–<80, and ≥80 kg, respectively. Dose reduction and treatment discontinuation followed the package insert, i.e., dose was reduced from 600 mg/day to 400 mg/day, from 800 mg/day to 600 mg/day, or from 1000 mg/day to 600 mg/day if hemoglobin (Hb) concentration decreased to <10 g/dL, and administration was discontinued if Hb decreased to 8.5 g/dL. Duration of treatment was 48 weeks as a rule. In LVR patients who did

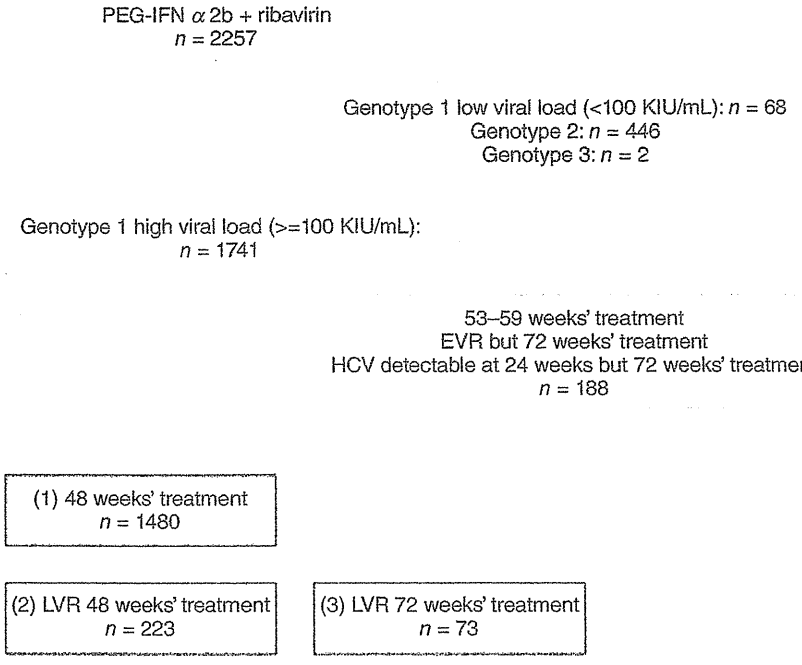

Figure 1 Flow-chart of study subjects. (1) 48 weeks' treatment (48-week standard therapy group): patients with genotype 1 and high viral load who received pegylated interferon α 2b (PEG-IFN α 2b) + ribavirin (RBV) for 52 weeks. Multiple logistic regression analysis was used to evaluate the response to PEG-IFN α 2b + RBV in this group (2) Late virological response (LVR) 48 weeks' treatment: patients with genotype 1 and high viral load who received PEG-IFN α 2b + RBV for 36–52 weeks (3) LVR 72 weeks' treatment: patients with genotype 1 and high viral load who received PEG-IFN α 2b + RBV for 60–76 weeks. SVR rate was compared between LVR 48 weeks' treatment group (2) and LVR 72 weeks' treatment group (3). EVR, early virologic response; HCV, hepatitis C virus.

not achieve HCV-RNA negativity by week 12, treatment could be extended for 48 weeks or longer based on individual patients' desire and investigators' judgment.

Evaluation of response to treatment

Determination of genotype and measurement of HCV-RNA levels were performed at each center. Pre-treatment HCV-RNA levels were determined by Amplicor PCR quantitation. Viral negativity was defined as HCV below detection limit (<50 IU/mL) by Amplicor qualitative analysis (Roche Molecular Systems, NJ).

SVR was defined as HCV below detection limit at 24 weeks after the end of PEG-IFN α 2b plus RBV combination therapy by Amplicor HCV qualitative analysis.

Statistical analysis

All statistical analyses were performed using SAS, version 9.13 (SAS Institute, Cary, NC). Intergroup comparison of SVR rate was performed by Fisher's exact test; that of background variables by Fisher's exact test and Mann–Whitney *U*-test. Trend of SVR rate by age was assessed by Cochran–Armitage test, and intergroup comparison after adjustment of stratification factors was conducted by Mantel–Haenstzel method. Determination of factors associated with SVR was conducted by a stepwise procedure using the results of logistic univariate analysis ($P < 0.2$) into logistic multivariate analysis. All tests were two-sided, with significance level set at $P < 0.05$.

RESULTS

Study 1: SVR-related factors in patients receiving standard 48-week treatment

AS INDICATED IN Table 1 and Figure 1, 1480 subjects (male, $n = 898$ [60.7%]; median age, 57 [range, 13–79] years) were eligible for analysis. SVR rate based on ITT was 44.9%. SVR rate in subjects who completed and who discontinued treatment was 56.5% ($n = 1110$) and 10.3% ($n = 370$), respectively, a statistically significant difference ($P < 0.0001$). SVR rate in male subjects (50.4%; 453/898) was significantly ($P < 0.0001$) higher than in female subjects (36.4%; 212/582). SVR rate significantly ($P < 0.0001$) decreased as age increased by 10 years in both male and female subjects (Fig. 2); the odds ratio for SVR decreasing with 10-year increase in age was 0.688 (95% CI, 0.604–0.784; $P < 0.0001$) in male subjects and 0.546 (0.449–0.663; <0.0001) in female subjects, indicating that the influence of aging was greater in female than in male subjects. There was no bias of older versus younger age among patients who had and had not previously

Table 1 Pretreatment characteristics of chronic hepatitis C virus (CHCV) patients with HCV-1b RNA who received pegylated interferon α 2b + ribavirin standard therapy for 48 weeks

Characteristic	Value ($n = 1480$)
Sex (male/female)	898/582
Age (years)	57 (13–79)
History of HCC (yes/no/unknown)	8/1405/67
Previous IFN treatment (yes/no/unknown)	459/688/333
Diabetes (yes/no/unknown)	44/480/956
Hypertension (yes/no/unknown)	105/417/958
Ongoing alcohol use (yes/no/unknown)	157/456/867
Grade (A0/A1/A2/A3/unknown)	14/499/478/55/434
Stage (F0/F1/F2/F3/F4/unknown)	36/469/316/176/48/435
ALT (IU/L)	63 (8.4–910)
Platelets ($\times 10^4/\mu L$)	16.6 (4.3–47.7)
Viral load (KIU/mL)	1900 (100–5100)

Data expressed as median (range). HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; IFN, interferon.

received IFN. Whereas, multivariate logistic regression analysis revealed that older age (<55/≥55 years), degree of progression of hepatic fibrosis (F0–1/2–4), low platelet count (≥16/<16×$10^4/\mu L$), and high viral load (<1900/≥1900 KIU/mL) are resistance factors to SVR (Table 2). In multivariate logistic regression analysis, sex was not selected.

Study 2: usefulness of prolonged treatment in LVR patients

Of the patients who completed standard 48-week treatment, 223 patients (20.0%) showed LVR (Fig. 1), and median duration of treatment was 48 weeks. Compared with patients who exhibited early virologic response (EVR) defined as HCV-RNA negative within 12 weeks after the start of treatment, those with LVR were older (median age, 58 vs 55 years; $P = 0.0043$) and had higher viral load (median, 2700 vs 1620 KIU/mL; $P < 0.0001$) and lower platelet count (median, 16.5 vs $17.3 \times 10^4/\mu L$; $P = 0.0162$). SVR rate based on treatment analysis was 56.5 in all, 79.2% in EVR and 46.2% in LVR, respectively. In multivariate logistic regression analysis of SVR-related factors in LVR patients who completed standard 48-week treatment, age (10-year groups) was selected as a significant factor.

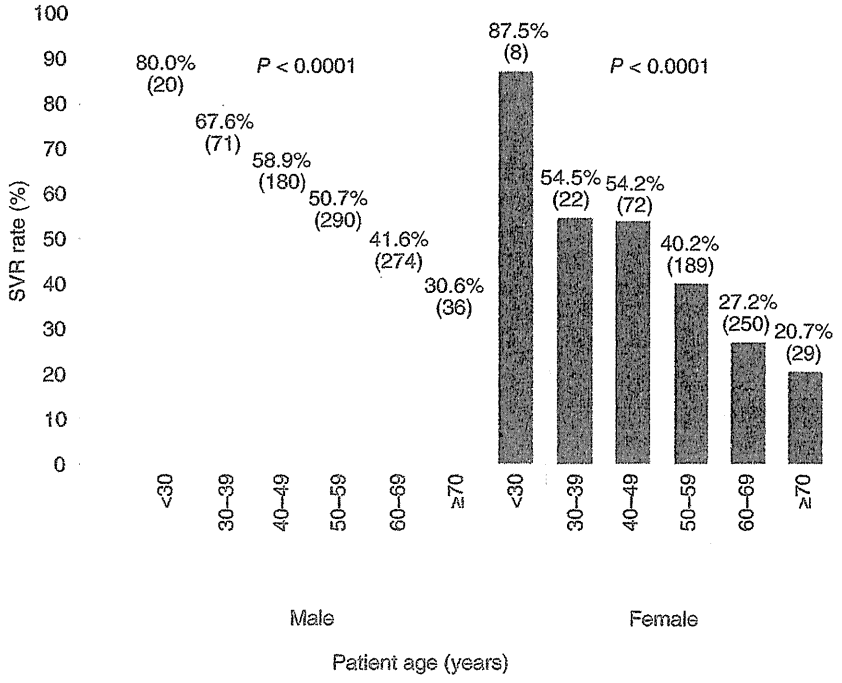

Figure 2 Sustained virological response (SVR) rate to 48 weeks' standard treatment with pegylated interferon α 2b (PEG-IFN α 2b) + ribavirin in male and female patients stratified by age. Cochran–Armitage test was used to study the underlying trend.

Table 2 Independent factors associated with sustained virological response in genotype 1 chronic hepatitis C virus patients who received pegylated interferon α 2b + ribavirin standard therapy for 48 weeks

	Odds ratio	95% confidence interval	*P*-value†
Age <55/≥55 years	0.414	0.293–0.585	<0.0001
Stage 0–1/2–4	0.633	0.442–0.906	0.0124
Platelets <16/≥16 × 10^4/μL	1.876	1.305–2.696	0.0007
Viral load </≥1900 KIU/mL	0.663	0.471–0.935	0.0192

†Multiple logistic regression analysis.

Prolonged treatment was conducted in 73 LVR patients (Fig. 1), with mean duration of 72 weeks. As shown in Table 3, whereas among LVR patients there were significantly ($P = 0.0061$) more female subjects in 72-week group than 48-week group, no intergroup difference of other factors was observed. Overall, SVR rate based on treatment analysis was significantly ($P = 0.0020$) higher in 72-week treatment group than in 48-week treatment group (67.1% [49/73] vs 46.2% [103/223]; Fig. 3A).

When stratified by sex, SVR rate with 48-week and 72-week treatment was 51.4% and 68.6% ($P = 0.0809$) in male subjects and 37.3% and 65.9% ($P = 0.0039$) in female subjects, with SVR in 72-week treatment being significantly higher in female subjects and indicating that, in LVR patients, efficacy comparable to male subjects is achieved in female subjects with 72-week treatment.

In patients aged <55 years SVR rate in the 48- and 72-week treatment groups was 57.6% and 78.9% ($P = 0.1100$) in male subjects and 40.0% and 76.9% ($P = 0.0724$) in female subjects, respectively, with higher SVR rates for the 72-week treatment group (Fig. 3B). In patients aged ≥55 years this parameter was 44.6% and 53.8% ($P = 0.5619$) in male subjects and 37.1% and 60.7% ($P = 0.0425$) in female subjects, respectively, with higher SVR rates for the 72-week treatment group than for the 48-week treatment group as in the case of the younger age group (Fig. 3C).

DISCUSSION

Study 1: SVR-related factors in patients receiving standard 48-week treatment

SVR RATE WITH standard 48-week treatment in this study was 44.9%, roughly equal to the 45% reported in previous clinical trials in Japan.[4,17–19] The present results are also similar to those of clinical trials conducted in patients aged in their mid-40s in western countries and in the general clinical setting.[1–4] Age was

Table 3 Comparison of clinical and virological characteristics between groups receiving pegylated interferon α 2b + ribavirin therapy for 48 and 72 weeks among patients showing late virological response

	48 weeks' group ($n = 223$)	72 weeks' group ($n = 73$)
Sex (male/female)	140/83*	32/41*
Age (years)	58 (21–75)	56 (22–71)
History of HCC (yes/no/unknown)	1/221/11	0/73/0
Previous IFN treatment (yes/no/unknown)	68/113/42	29/32/12
Diabetes (yes/no/unknown)	11/71/141	1/34/38
Hypertension (yes/no/unknown)	18/62/143	6/29/38
Ongoing alcohol use (yes/no/unknown)	17/75/131	6/27/40
Grade (A0/A1/A2/A3/unknown)	2/66/82/6/67	0/21/26/4/22
Stage (F0/F1/F2/F3/F4/unknown)	7/68/45/32/5/66	2/16/20/12/2/21
ALT (IU/L)	61.5 (14–550)	52 (17–254)
Platelets (×10^4/μL)	16.5 (8.5–43.2)	16.6 (4.3–40.2)
Viral load (KIU/mL)	2700 (160–5100)	2100 (130–5000)

Data expressed as median (range). *$P = 0.006$. ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; IFN, interferon.

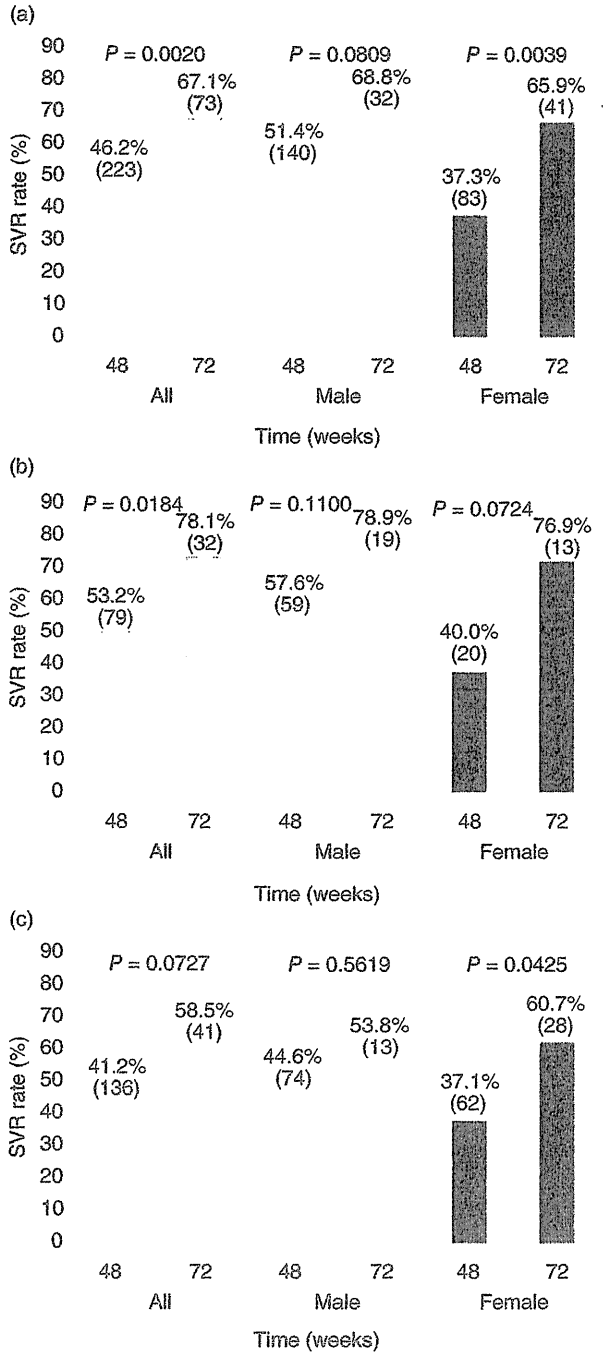

Figure 3 Sustained virological response (SVR) rate based on treatment analysis between groups receiving pegylated interferon α 2b (PEG-IFN α 2b) + ribavirin therapy for 48 and 72 weeks who exhibited late virological response (LVR). (A) Overall; (b) patients aged <55 years; (c) patients aged ≥55 years. Data on age not available for 7 male patients and 1 female patient.

selected among factors for SVR with PEG-IFN plus RBV combination therapy in an aging patient population, the examination of which was the objective of this study, and SVR rate decreased stepwise with 10-year age increase. Of particular note was the greater impact of aging observed in female than male subjects.

Lower efficacy in elderly female patients infected with HCV genotype 1 has already been reported in Japan.[20] A low SVR rate was also observed in elderly female subjects in this study. Although female sex was considered a favorable prognostic factor in some Western studies, there is no established opinion on sex difference. Change associated with aging of the patient population in Japan is considered to account for this phenomenon observed in the present study. This may be due to decrease in compliance among elderly women; on the other hand, however, there was no difference between male and female subjects aged ≥55 years in the rate of completion of treatment. Although the rate of dose reduction of RBV tended to be slightly higher in female subjects (data not shown), the difference was not significant. These findings suggest the influence of factors other than adherence to treatment for the low SVR rate among elderly women. One possible factor for reduced SVR rate among these individuals may be the effect of menopause. In women, insulin resistance begins to worsen after the age of 50 years,[21,22] and this is reported more closely associated with the effect of menopause than age itself.[23]

The presence of insulin resistance has been reported to lower efficacy of PEG-IFN and RBV combination therapy.[24–27] Insulin resistance is also a cause of advanced fibrosis and fatty change of the liver.[28–31] It is possible that such changes combined with other factors associated with metabolic syndrome interact in a complex way to reduce the efficacy of this therapy.[32–35] In fact, the incidence of non-alcoholic fatty liver disease (NAFLD) among elderly Asians was reported higher in women as compared with that in men.[36–38] However, while older age, advanced fibrosis, low platelet count and high HCV load were selected as factors for reduction of SVR rate in our multivariate logistic regression analysis, sex was not selected. It is therefore necessary to examine further the confounding of these selected factors with sex. It also should be taken into consideration that, due to limitations imposed by the retrospective nature of this study, data on factors affecting the efficacy of PEG-IFN plus RBV therapy such as insulin resistance, steatosis, and core mutation are lacking. A large-scale prospective study is

required to examine the lower efficacy observed in elderly women.

Study 2: usefulness of prolonged treatment in LVR patients

EVR (viral load reduced by 2 log or undetected in week 12) has been used for determining continuation or discontinuation of treatment in western countries. Recently, however, EVR was divided into complete EVR (HCV RNA <50 IU/mL at week 12) and partial EVR (>2 log drop in HCV RNA but still detectable [>50 IU/mL]). Fried *et al.*[15] and Berg *et al.*[16] reported that the SVR rate was a high 68–84% in patients showing complete EVR but only 17–29% in those with partial EVR with treatment for 48 weeks. They also reported that treatment for 72 weeks was effective in patients with partial EVR. In the clinical study for health registration in Japan, the SVR rate by timing of HCV-RNA negativity at 4, 12, and 24 weeks was 100%, 71.1%, and 36.4%, respectively, and no patient with HCV-RNA negativity after 25 weeks achieved SVR.[4] With these studies as reference, patients with LVR were defined as those who were positive (>50 IU/mL) at week 12 and became negative (<50 IU/mL) by week 24. To minimize the influence of treatment discontinuation, only patients who completed the standard duration of treatment were selected as subjects in this study. In the comparison of patient background, there was no significant intergroup difference except for a significantly greater number of female subjects in the 72-week treatment group. This finding might be related to the observation that it was already widely believed that efficacy in elderly women in Japan is low and that duration of treatment was at the discretion of individual physicians. Nevertheless, it is noteworthy that the SVR rate was significantly higher in the 72-week treatment group than in the 48-week treatment group and that a high 60% SVR rate was achieved with 72-week treatment in elderly female patients, a population in whom a relatively low SVR was observed with standard 48-week treatment.

This retrospective study had the limitation that duration of treatment was at the sole discretion of each participating physician. A prospective study is necessary to demonstrate whether 72-week treatment in elderly women with LVR is more efficaous than 48-week treatment in male patients. Although the number of younger subjects examined was rather low, it is noteworthy that an SVR rate of >75% was observed with 72-week treatment in both male and female patients. This also should be confirmed by prospective study.

CONCLUSIONS

PATIENTS WITH CHCV genotype 1 infection should be treated with PEG-IFN and ribavirin combination therapy as early as possible. Seventy-two weeks' treatment is recommended in patients with LVR, regardless of age.

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APPENDIX I

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