switched to the combination of PEG-IFN and RBV in recent years, it is important to know if a larger dose of IFN is beneficial to patients with chronic hepatitis C.

Many molecular mechanisms through which HCV evades host innate immunity have been reported to date. HCV core, E2 and NS5A proteins have been reported to inhibit the IFN signaling system [Gale et al., 1997; Taylor et al., 1999; Blindenbacher et al., 2003; Bode et al., 2003; Foy et al., 2003; Lin et al., 2006; Ciccaglione et al., 2007]. Variations of amino acid (aa) sequences in the E2 and the NS5A region have been reported to correlate with the effect of IFN therapy [Enomoto et al., 1996; Chayama et al., 1997, 2000; Polyak et al., 1998, 2000; Hashimoto et al., 1999; Puig-Basagoiti et al., 2001; Pascu et al., 2004; Gaudy et al., 2005; Brillet et al., 2007; Torres-Puente et al., 2008]. Recently, Akuta et al. [2005, 2006, 2007a, b] reported that substitution of aa 70 and/or 91 in the core region is an independent and significant predictor of non-virological response.

The aim of the present study was to evaluate the therapeutic efficacy and safety of a large dose of IFN-α-2b combined with RBV. For this purpose, a randomized trial was conducted to compare the therapeutic effects of high-dose (10 MU) versus standard dose (6 MU) of IFN-α-2b combined with RBV in patients with high HCV viral titers. The second endpoint of this study was to analyze the predictive factors associated with virological response including aa substitutions in the core region and the NS5A region.

PATIENTS AND METHODS

Patient Selection

Two hundred adult patients enrolled into the study. The inclusion criteria were positivity for antibody to HCV, HCV RNA levels higher than 100 KIU/ml, and the diagnosis of chronic hepatitis C was confirmed by liver biopsy. The liver biopsy specimens were evaluated as described by Desmet et al. [1994], and classified into F0 to F3. None of the patients included in this study had liver cirrhosis (F4). Other exclusion criteria included leukocytopenia (leukocyte $<4{,}000/mm^3$) and anemia (hemoglobin concentration <10 g/dl). Patients with human immunodeficiency or hepatitis B super infection, previous organ transplantation, other causes of liver disease, poorly controlled diabetes, de-compensated renal disease, pre-existing psychiatric disease, seizure disorders, cardiovascular disease, hemophilia or auto-immune type diseases were also excluded.

Study Design

The double-blind, multi-center randomized clinical trial was conducted in 23 centers in Hiroshima city (The Hiroshima Liver Study Group). The study was approved by the Ethics Committee of Hiroshima University. Written informed consent was obtained form all participants. Eligible patients were assigned randomly into either of the two groups without further stratification using sequentially numbered cards in sealed envelopes. Patients were randomized to treatment with combination of IFN-α-2b (Intron A, Shering Plough, Kenilworth, NJ) at a dose of 6 MU (Group A) or 10 MU (Group B) plus RBV (Rebetol, Shering Plough). IFN-α-2b was administered intramuscularly daily over the initial 2 weeks and three times weekly in the remaining 22 weeks. The dose of RBV was adjusted according to body weight (600 mg/day for ≤60 kg, 800 mg/day for >60 kg). Adverse events were monitored clinically by careful interview and hematological examination throughout the study. The dosage of RBV was reduced in patients who experienced a decrease in hemoglobin concentration to <10 g/dl.

Blood samples were taken 2 and 4 weeks after the beginning of therapy and every 4 weeks thereafter. Biochemical and hematological tests were performed in each center, including alanine amino transferase (ALT). Part of the serum samples were kept frozen at −80°C until further analysis. Viral genotypes were determined by phylogenetic analysis after reverse transcription (RT)-polymerase chain reaction (PCR) and direct sequencing.

Assessment of Efficacy

Serum HCV RNA was detected by nested PCR assay (Cobas Amplicor HCV test v 2.0, Roche Diagnostics, Tokyo, Japan; limit of detection, 50 IU/ml) at weeks 2, 4 and every 4 weeks during treatment and 24 weeks after the cessation of therapy. Positive samples were analyzed further by quantitative assay (Cobas Amplicor HCV monitor v 2.0, Roche Diagnostics; limit of detection, 500 IU/ml).

The primary endpoint of this study was sustained virological response, defined as undetectable serum HCV RNA by qualitative PCR test and normalization of ALT 24 weeks after the treatment. Non-virological response was applied to those patients with positive qualitative HCV RNA PCR tests in all examinations. Virological response was used to define the remaining patients who became PCR negative at least once during the treatment.

Nucleotide Sequencing of the Core and NS5A Gene

The core aa 61–110 and NS5A aa 2209–2248 (IFN-sensitive determining region [ISDR] [Enomoto et al., 1996]) sequences were determined by direct sequencing using stored serum samples obtained just before therapy. HCV RNA was extracted from serum samples and reverse transcribed with random primers and MMLV reverse transcriptase (Takara Bio Inc., Shiga, Japan). DNA fragments were amplified by PCR using the following primers. (a) Nucleotide sequences of the core region: The first-round PCR was performed with primers CC11 (forward, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (reverse, 5′-GGA GCA GTC CTT CGT GAC ATG-3′), and the second-round PCR with primers CC9 (forward, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (reverse) as described by Akuta et al. [2005, 2006, 2007a, b]. After denaturation at 95°C for 5 min, 35

cycles of amplification were set as follows; denaturation for 30 sec at 94°C, annealing of primers for 1.5 min at 57°C, and extension for 1 min at 72°C, followed by final extension at 72°C for 7 min. The second PCR was carried out with the same amplification conditions used in the first PCR, except that the second PCR primers were used instead of the first PCR primers. (b) Nucleotide sequences of ISDR in NS5A: PCR was performed with IM11 (forward, 5′-TTC CAC TAC GTG ACG GGC AT-3′) and 5OA2KI (reverse, 5′-CCC GTC CAT GTG TAG GAC AT-3′). After denaturation at 98°C for 30 sec, 35 cycles of amplification were set as follows; denaturation for 10 sec at 98°C, annealing of primers for 30 sec at 66°C, and extension for 15 sec at 72°C, followed by final extension at 72°C for 5 min. The amplified PCR products were separated in a 2% agarose gel and purified by GENECLEAN II kit (Q-Bio Gene, Carlsbad, CA). Nucleotide sequences were determined using Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan). Nucleotide and aa sequences were compared with the nucleotide sequences of genotype 1b HCV-J (Gene Bank accession number; D90208) [Kato et al., 1990].

Quantitation of HCV Core Antigen

HCV core antigen levels were measured using stored serum samples just before and 4 weeks after the start of the therapy as described previously [Aoyagi et al., 1999].

Statistical Analysis

The baseline characteristics of the patients in the two groups were compared and the differences were assessed by Chi-square test with Yate's correction and Mann–Whitney *U*-test. To assess the sustained virological response rates, an intention-to-treat (ITT) analysis and a per-protocol (PP) analysis were conducted. The response rates and substitutions in the core region and the ISDR were compared by Fisher's exact test. All *P* values reported are two-sided and those less than 0.05 were considered significant. To determine the predictors of sustained virological and non-virological responses, univariate and multivariate logistic regression analyses were carried out. Potential predictive factors included the following variables: age, sex, alcohol consumption, past history of IFN monotherapy, body mass index, ALT, hemoglobin, platelets, HCV RNA level, genotype, liver histology, total RBV dose (adjusted for body weight [mg/kg]) and total dose of IFN-α-2b. The odds ratio and 95% confidence intervals (95% CI) were also calculated. Variables with 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 factors. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

Patient Demographics

Patient enrollment started in January 2002, and the trial ended in March 2005. The disposition of patients throughout the trial is shown in Figure 1. A total of 200 patients were randomized to treatment, and 198 patients met the eligibility criteria and underwent

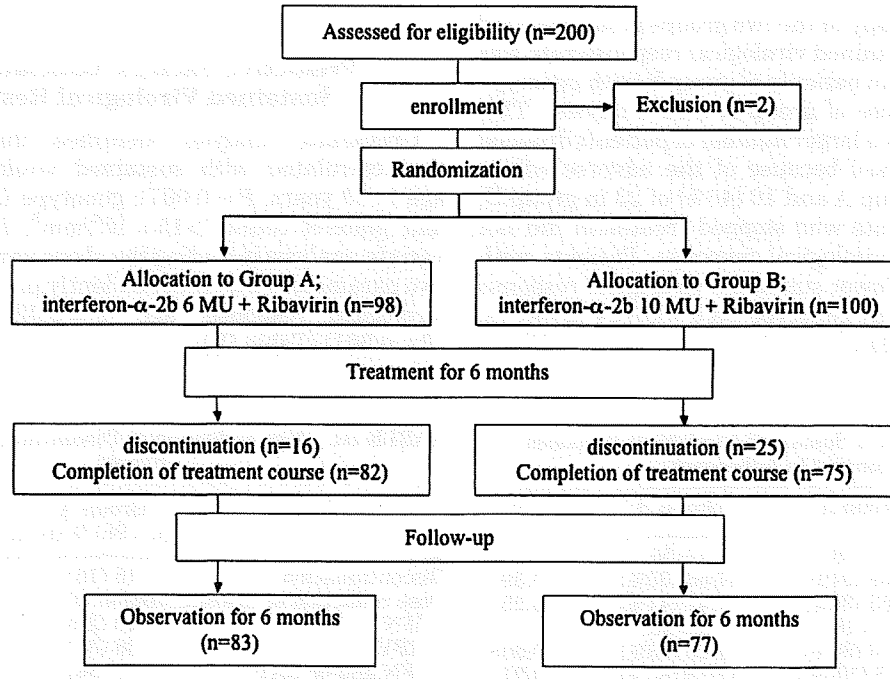

Fig. 1. Flow chart of number of patients throughout the trial. A total of 200 patients were included in this study. One hundred ninety-eight patients met the eligibility criteria and they underwent randomization, 98 patients in Group A and 100 patients in Group B.

TABLE I. Baseline Characteristics of the Patients

Characteristic	Group A (n = 98)	Group B (n = 100)	P
Age (years)[a]	55 ± 10.3	55 ± 11.0	0.43
Male sex (%)	63	75	0.07
Alcohol consumption (%)[b]	23	20	0.61
Past history of IFN monotherapy (%)	33	35	0.72
Body-mass index (kg/m^2)[a]	23.3 ± 2.9	24.2 ± 3.6	0.05
ALT (IU/L)[a]	79.2 ± 45.3	109.4 ± 111.2	0.31
Hemoglobin (g/dl)[a]	14.2 ± 1.4	14.5 ± 1.2	0.02
Platelets ($\times 10^4/mm^3$)[a]	14.8 ± 4.8	16.5 ± 5.0	<0.05
HCV RNA (KIU/ml) (%)			
100–850	49	47	
≥850	51	53	0.80
Genotype (%)			
1b	82	72	
2a/2b	17	28	0.32
3a/3b	1	0	
Liver histology[a,c]	2.0 ± 0.84	1.8 ± 0.82	0.05

ALT, alanine aminotransferase.
[a]Values are mean ± SD.
[b]Percentage of patients who consumed alcohol at >30 g/day.
[c]Liver fibrosis was scored 0 (F0), no fibrosis; 1 (F1), periportal expansion; 2 (F2), portoportal septa; 3 (F3), portocentral linkage or bridging fibrosis.

randomization. Ninety-eight patients were assigned to Group A and 100 patients to Group B. Patients were observed for 24 weeks after the treatment. Sixteen patients of Group A and 25 patients of Group B discontinued the treatment because of adverse events. Table I lists the baseline characteristics of the patients. Hemoglobin concentrations and platelet counts were higher in group B patients. The other parameters were similar between the two groups.

Overall Sustained Virological Response

The effect of therapy in the two groups is summarized in Table II. The sustained virological response rate was lower significantly in patients of group B with genotype 2a/b relative to those of group A (ITT analysis). This reflects the fact that a larger number of patients dropped out form the protocol because of the adverse effects (1 [6%] of 16 in group A and 10 [43%] of 23 in group B, $P = 0.02$). All patients who stopped treatment did not achieve sustained virological response. Patients with genotype 1b had a lower sustained virological response rate than those with genotype 2a/b (33/124 [27%] vs. 26/39 [67%], $P < 0.01$).

Dose Reduction or Discontinuation and Adverse Events

Table III summarizes the laboratory abnormalities and the dose reduction and discontinuation of IFN-α-2b and RBV due to adverse events. The overall discontinuation rate was 16% for group A and 25% for group B (not significant). The most frequent adverse event associated with dose reduction was anemia. A larger number of patients of group B developed depression ($P = 0.02$).

Predictive Factors Associated With Sustained Virological Response

Univariate analysis identified three parameters that correlated with sustained virological response: age (<60 years, $P = 0.007$); genotype (2a/b, $P < 0.001$); and platelet count ($>15 \times 10^4/mm^3$, $P = 0.01$). Multivariate analysis including the above variables identified two parameters that independently predicted sustained virological response: age ($P = 0.02$) and genotype ($P < 0.001$) (Table IV).

TABLE II. Rates of Sustained Virological Response According to Adherence

Genotype	Group A	Group B	P
1b	n = 68	n = 56	
ITT	16/68 (24%)	17/56 (30%)	0.39
PP	16/53 (30%)	17/41 (41%)	0.25
2a/b	n = 16	n = 23	
ITT	15/16 (94%)	11/23 (48%)	0.005
PP	15/15 (100%)	11/13 (85%)	0.21

ITT, intention to treatment analysis; PP, per protocol analysis; IFN, interferon; RBV, ribavirin.

TABLE III. Dose Reduction or Discontinuation and Adverse Events

	Group A (n = 98) % (n)	Group B (n = 100) % (n)	P
Discontinuation	16 (16)	25 (25)	0.13
Dose reduction or discontinuation of			
IFN	20 (20)	41 (41)	0.002
RBV	36 (35)	50 (50)	0.04
IFN and/or RBV	37 (36)	55 (55)	0.01
Depression	0 (0)	7 (7)	0.02

IFN, interferon; RBV, ribavirin.

TABLE IV. Factors Associated With Sustained Virological Response to Combination Therapy of Interferon Plus Ribavirin by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	*P*
Age (years)	0: ≥60	1	
	1: <60	2.420 (1.173–5.002)	0.020
Genotype	0: 1b	1	
	1: 2a/b	5.301 (2.401–11.702)	<0.001

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown.

Analysis of aa Sequences in the Core Gene in Genotype 1b Patients

The relationship between aa substitutions in the core region and the viral response to therapy was investigated in patients with genotype 1b using 93 available serum samples. Figure 2 shows the sequences of aa 61–110 of the HCV core region in 93 patients just before commencement of treatment. Table V summarizes the relationship between the response to IFN therapy and

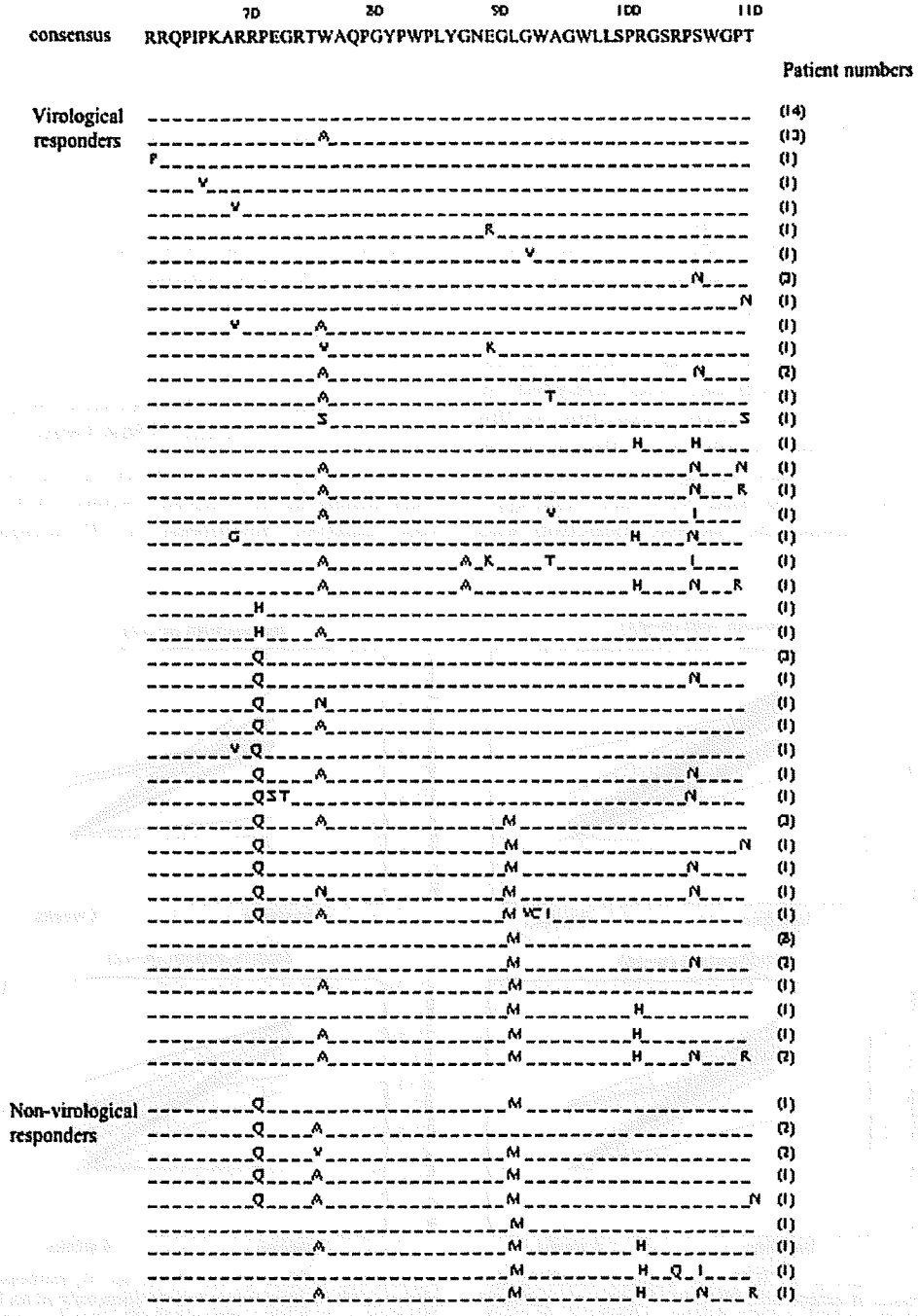

Fig. 2. Sequences of amino acids 61–110 in the core region at commencement of combination therapy in 93 patients infected with hepatitis C virus genotype 1b. Dashes indicate amino acids identical to the consensus sequence of genotype 1b, and substituted amino acids are shown by standard single-letter codes.

TABLE V. Amino Acid Substitutions in the Core Region in Non-Virologic Responders and Virological Responders in 93 Patients With HCV Genotype 1b

Presence of substitution site	Non-virological response (n = 11) % (n)	Virological response (n = 82) % (n)	*P*
aa 70	64 (7)	23 (19)	0.01
aa 75	73 (8)	45 (37)	0.11
aa 91	82 (9)	30 (25)	0.001
aa 106	27 (3)	31 (26)	1.0
aa 110	18 (2)	12 (10)	62
aa 70 and 91	45 (5)	10 (8)	0.006
aa 70 and/or 91	100 (11)	44 (36)	<0.001

aa, amino acid.

substitutions of aa. Among aa substitutions, only substitutions of aa 70 and 91 were associated with non-virological response. All non-virological responders had aa substitutions at 70 or 91, or both substitutions. In contrast, only 36 of 82 (44%) virological responders had these substitutions ($P < 0.001$, Table V). In contrast to non-virological response, these substitutions were not predictive for sustained virological response ($P = 0.11$–0.82).

Next, the effect of substitutions of aa 70 and 91 in the core region on early viral kinetics was analyzed by dividing patients into four groups according to the pattern of aa substitutions. As shown in Figure 3, the most rapid decrease in core antigen was noted in patients where both aa 70 and 91 were wild-type (double-wild). In contrast, the poorest reduction was noted in patients with both of aa 70 and 90 substitutions (double-mutant). Patients with either of the two aa substitutions (mutant/wild or wild/mutant) showed decrease in between the above two groups. HCV core antigen decreased below the detectable limit (20 fmol/L) at week 4 in 37 of 40 (93%) patients who had neither aa 70 nor aa 90 substitutions. In contrast, it decreased below the detectable limit in only 5 of 12 patients (42%) who had both aa 70 and 91 substitutions ($P = 0.031$).

Analysis of Nucleotide Sequence of the NS5A Gene

The aa sequences of ISDR in the NS5A gene were determined in 40 patients where PCR for this region was positive. Seventeen of 40 patients had no aa

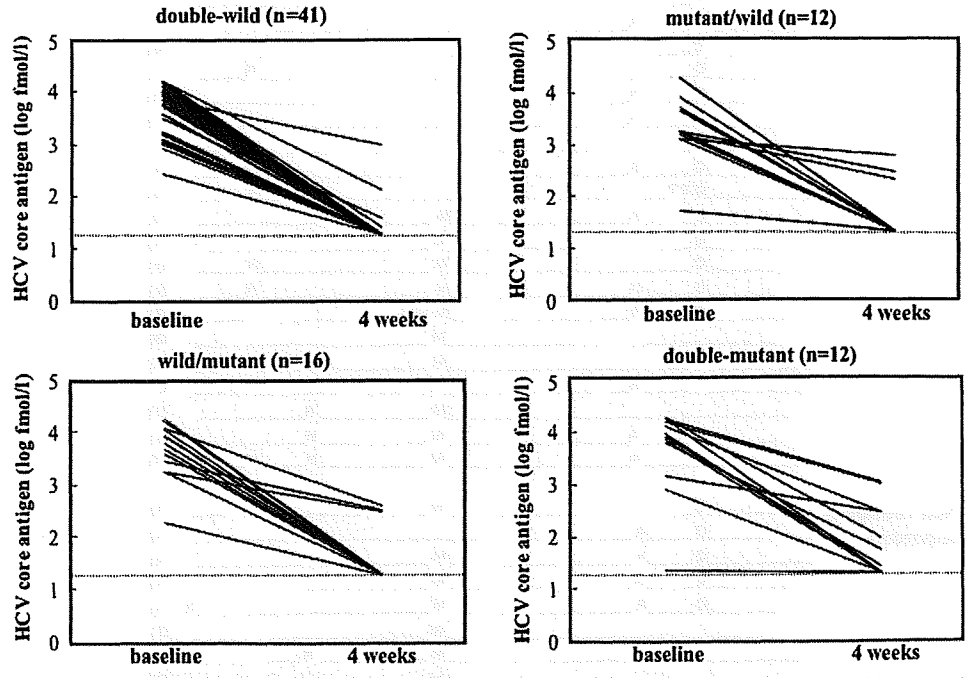

Fig. 3. Reduction of amount of HCV core antigen based on the presence of substitutions at amino acid 70 or 91. Eighty-one patients infected with hepatitis C virus were treated with combination therapy. Serum HCV core antigen was measured before treatment (baseline) and at week 4. The response was divided into four patterns based on the presence of substitution(s) at aa 70 and/or 91. Double-wild; no substitution, neither at aa 70 nor aa 91, mutant/wild; substitution only at aa 70, wild/mutant; substitution only at aa 91, double-mutant; substitutions at both aa 70 and 91. The fixed-quantity bottom value of HCV core antigen was 20 fmol/L calculated 1.3 in log, indicated by the dotted lines.

TABLE VI. Amino Acid Substitutions in the IFN-Sensitive Determining Region (ISDR) in Non-Virologic Responders and Virological Responders in 40 Patients With HCV Genotype 1b

ISDR[a]	Non-virological response (n = 8) % (n)	Virological response (n = 32) % (n)	P
Wild-type (n = 17)	36 (6)	64 (11)	
Mutant-type (n = 23)	9 (2)	91 (21)	0.012

aa, amino acid.
[a]Absence of amino acid substitutions was evaluated as wild-type, and presence of one or more amino acid substitutions as mutant-type.

substitutions in ISDR (wild-type), while the remaining 23 patients had one or more substitutions (mutant-type). The relationship between aa substitutions of ISDR and effects of treatment was analyzed. The existence of aa substitution in the ISDR was not predictive for sustained virological response ($P = 0.137$), however, such substitution was observed frequently in virological responders compared to non-virological responders (66% vs. 25%, $P = 0.012$) (Table VI). The use of a different categorization based on the number of substitutions in the ISDR (0/1 vs. ≥ 2) yielded similar results, that is, not predictive for sustained viral response but predictive for virological responders (data not shown).

HCV core antigen decreased more rapidly in patients with ISDR mutant-type compared to those with wild-type (Fig. 4). HCV core antigen decreased below the detectable limit at week 4 in only 6 of 17 (35%) patients with wild-type. In contrast, it decreased below the detectable limit in 19 of 23 (83%) in patients with ISDR mutant-type ($P = 0.006$).

Predictive Factors Associated With Sustained Virological Response and Non-Virological Response in Patients With Genotype 1b

Finally, the predictive factors associated with sustained virological response and non-virological response were analyzed in patients with genotype 1b, including aa substitutions in the core region and ISDR. Univariate analysis showed two parameters correlated with sustained virological response: age (<60 years, $P = 0.004$) and presence of aa substitutions in the core (aa 70 and/or 91, $P = 0.04$). However, multivariate analysis, including the above variables, identified no parameters that influenced sustained virological response independently (age, $P = 0.89$; core, $P = 0.07$). Univariate analysis showed two parameters correlated with non-virological response: age (<65 years, $P = 0.02$) and aa substitutions in the core (double-mutant, $P = 0.01$). Multivariate analysis including the above variables identified aa substitutions in the core as an independent factor that influenced non-virological response (age, $P = 0.40$; core, $P = 0.03$) (Table VII).

DISCUSSION

Treatment of patients with chronic HCV infection had improved by the advent of PEG-IFN and RBV combination therapy. However, a substantial number of patients do not respond to the combination therapy [Taliani et al., 2006]. Several studies described attempts to improve the sustained virological response rate in such patients. Recent trials showed that a longer treatment period results in a higher sustained virological response rate [Berg et al., 2006; Sánchez-Tapias et al., 2006]. However, there are no conclusive studies that compared a larger dose of IFN with standard dose. Although the treatment had shifted in recent years to PEG-IFN and

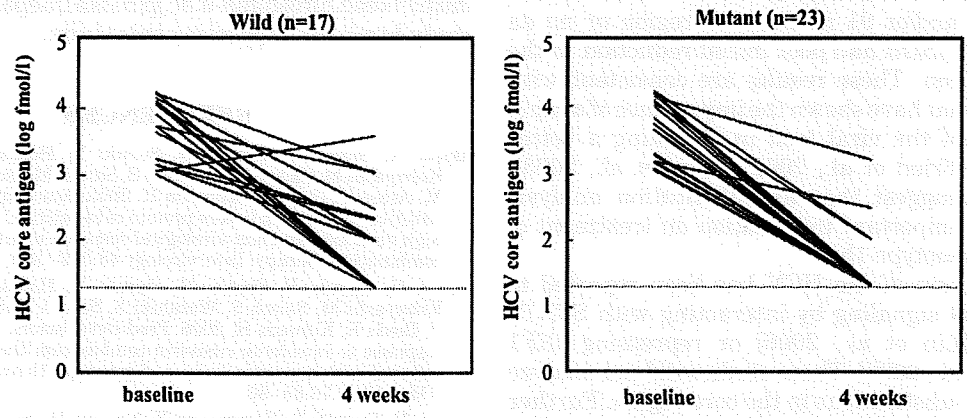

Fig. 4. Reduction of amount of HCV core antigen based on the presence of substitutions in the ISDR. Sixty-five patients infected with hepatitis C virus were treated with combination therapy. Serum HCV core antigen was measured before treatment (baseline) and at week 4. Patients were divided into two groups based on the presence of amino acid substitution(s) in the ISDR. Wild-type; absence of substitutions, mutant-type; presence of one or more substitutions. The fixed-quantity bottom value of HCV core antigen was 20 fmol/L calculated 1.3 in log, indicated by the dotted lines.

TABLE VII. Factors Associated With Non-Virological Response to Combination Therapy of Interferon Plus Ribavirin Identified by Multivariate Analysis in Patients With Genotype 1b

Factor	Category	Odds ratio (95% CI)	P
Amino acid substitutions in the core region[a]	0: No double-mutant	1	0.028
	1: Double-mutant	7.000 (1.238–39.566)	

Only the variable that achieved statistical significance ($P < 0.05$) on multivariate logistic regression is shown.
[a]The mutant aa 70 and 91 pattern was evaluated as double-mutant, and other patterns as non-double-mutant.

RBV combination therapy, a different dose of IFN was used in the present study to test whether a larger dosage of IFN improves the outcome of IFN therapy.

In this study, the larger dose did not increase sustained virological response nor decrease non-virological response. Instead, the dose reduction of IFN and/or RBV was significantly higher in the higher dose group (Table III). Furthermore, the incidence of depression was significantly higher in the high-dose group (Table III). These results suggest that a high dose of IFN is not beneficial to patients who receive IFN and RBV combination therapy, and probably who will receive the PEG-IFN and RBV combination therapy.

The predictive factors for sustained virological response and non-virological response to the combination therapy for patients with genotype 1b were analyzed. Logistic regression analyses identified pretreatment substitutions at both aa 70 and 91 in the core region (double-mutant) as a singular predictive factor for non-virological response (Table VII). Furthermore, the existence of aa substitution in the ISDR was significantly more frequent in virological responders compared to non-virological responders (Table VI), in agreement with previous reports [Puig-Basagoiti et al., 2001; Pascu et al., 2004]. It has been reported that the numbers of aa substitutions in the ISDR correlate with serum HCV RNA levels [Enomoto et al., 1996]. However, no apparent correlation was observed in this study. As shown in Figures 3 and 4, patients who had substitutions of aa 70 and/or 91 in the core region or no aa substitutions in ISDR had poor initial reduction in the HCV core antigen. These results are consistent with recent studies that have shown the importance of a rapid initial decline of the viral load in obtaining a better response rate [Fried et al., 2002; Davis et al., 2003]. These results suggest that aa substitution analysis should provide important information on treatment of patients with genotype 1b.

The core protein of the HCV has been reported to disturb the IFN signaling by interacting with STAT1 SH2 domain [Lin et al., 2006] or repressing IRF1 [Ciccaglione et al., 2007]. These studies did not analyze the effect of aa substitutions in the core region. Further study is necessary to clarify the effect of aa substitutions in the core region and to identify a molecular target to improve the therapy.

Although aa substitution in the core region was identified as an important predictor in patients with genotype 1b in this study, aa substitutions of the core region and ISDR in patients with genotype 2a/b infection were not analyzed. Although the sustained virological response rate in patients who completed the therapy was high (26/28 [93%], per protocol analysis), few patients were unable to achieve sustained virological response. Furthermore, a significant number of patients could not complete the treatment course because of adverse effects. A more effective and easy to complete therapy should be developed to treat such patients. The predictive factors in such patients should also be clarified.

The recent development of a new type of drug targeting NS3/4 protease may improve the outcome of treatment in patients with chronic hepatitis C [Reesink et al., 2006; Forestier et al., 2007; Kieffer et al., 2007; Sarrazin et al., 2007a,b]. However, drug resistant mutants might emerge against such a small molecule therapy targeting viral enzyme(s). The functions of virus proteins that resist IFN including core, ISDR and PePHD should be clarified further to develop a better therapy that can achieve a higher sustained virological response rate with fewer and milder side-effects.

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HEPATOLOGY

Dose comparison study of pegylated interferon-α-2b plus ribavirin in naïve Japanese patients with hepatitis C virus genotype 2: A randomized clinical trial

Tomokazu Kawaoka,* Yoshiiku Kawakami,* Keiji Tsuji,† Hiroyuki Ito,‡ Mikiya Kitamoto,§ Shiomi Aimitsu,¶ Hiroiku Kawakami,** Soo Cheol Jeong,* Michio Imamura,* Hiroshi Aikata,* Shoichi Takahashi* and Kazuaki Chayama*

*Department of Medicine and Molecular Science, Hiroshima University, †Department of Internal Medicine, Hiroshima City Asa Hospital, ‡Department of Internal Medicine, Saiseikai Kure Hospital, §Department of Internal Medicine, Prefectural Hiroshima Hospital, ¶Second Department of Internal Medicine, Hiroshima Red Cross and Atomic Bomb Survivors' Hospital, and **Kawakami Clinic, Hiroshima, Japan

Key words
hepatitis C virus genotype 2, low-dose pegylated interferon, ribavirin, side-effect, sustained virological response.

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Correspondence
Dr Yoshiiku Kawakami, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: kamy4419@hiroshima-u.ac.jp

Abstract

Background and Aim: To compare the efficacy and safety of pegylated interferon (PEG-I) at 1 and 1.5 μg/kg, and in combination with ribavirin (RBV) for 24 weeks in naïve Japanese patients infected with hepatitis C virus genotype 2.

Methods: The present study was an open-label, randomized trial of 55 patients receiving PEG-I (1 or 1.5 μg/kg body weight [BW], subcutaneously, once a week) and RBV for 24 weeks. The patients were followed up for 24 weeks without treatment.

Results: The intention-to-treat analyses showed that the proportion of patients with a sustained virological response (SVR) in the 1-μg/kg PEG-I–RBV group (38.5%, 10/26) was lower than that of the 1.5-μg/kg PEG-I–RBV group (74.1%, 20/27; $P = 0.013$). The PEG-I dose was reduced in two of the 26 patients of the 1-μg/kg PEG-I–RBV group (one because of thrombocytopenia at 2 weeks, and one because of generalized fatigue at 20 weeks), and four of the 27 patients of the 1.5-μg/kg PEG-I–RBV group (one because of neutropenia at 20 weeks, and three because of generalized fatigue at 1, 5, and 8 weeks). The multivariate analysis identified age (< 60 years) and dose of PEG-I (1.5 μg/kg) as significant determinants of SVR.

Conclusion: The dose of PEG-I to be used at the start of therapy should be 1.5-μg/kg BW in naïve Japanese patients infected with hepatitis C virus genotype 2.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, with an estimated 170 million chronic carriers worldwide.[1] Chronic HCV infection is causally associated with liver cirrhosis (LC) and hepatocellular carcinoma (HCC).[2–6] In Japan, 60–70% of patients with HCC or LC are HCV carriers.[7]

Pegylated interferon (PEG-I)-α plus ribavirin (RBV), the current standard treatment for chronic HCV infection, can increase the sustained virological response (SVR) rate.[8–15] In this regard, a small scale, non-randomized study by the Hepatitis C Intervention Therapy Group on the use of PEG-I-α2b plus RBV reported that the SVR rate of patients infected with HCV genotype 2 treated for 24 weeks at a dose of 1.4 μg/kg once per week (83%) was equivalent to that of patients (100%) treated with 0.7 μg/kg once per week.[16] Moreover, Lindsay *et al.*[12] reported that the SVR rate of patients infected with HCV genotype 2 on 48-week PEG-I-α2b monotherapy (dose: 1.5 μg/kg once per week, 41%) was similar to that of patients (42%) treated with 1 μg/kg once per week. Meyer-Wyss *et al.*[17] reported that SVR rates in patients infected with HCV genotype 2 or 3 were similar to those if patients treated with 1 or 1.5 μg/kg PEG-I body weight [BW], that is, SVR rates were achieved in 39 of 55 (71%) and 29 of 36 (81%) patients, respectively (P = ns). Mangia *et al.*[18] reported that the SVR rate of patients infected with HCV genotypes treated with PEG-I at 1 μg/kg BW was 80%.

However, there are no reports on whether 1 and 1.5 μg/kg doses of PEG-I plus RBV for 24 weeks have similar efficacies and safety in Japanese patients infected with HCV genotype 2. It is important to study the response to such low-dose interferon because some Japanese patients who receive treatment are older than 60 years. In addition, it is possible that the SVR rate to interferon is better in patients infected with HCV genotype 2.

The aim of the present study was to determine whether 1 and 1.5 μg/kg doses of PEG-I plus RBV for 24 weeks have similar efficacies and safety in naïve Japanese patients infected with HCV genotype 2. For this purpose, we conducted a randomized clinical trial to evaluate the efficacy and safety of 1 μg/kg versus 1.5 μg/kg

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PEG-I combined with RBV for 24 weeks in naïve-infected patients with HCV genotype 2.

Methods

Patients and study design

This study was an open-label, randomized clinical trial conducted in six centers across Japan. Enrolment spanned from February 2006 to October 2007. The inclusion criteria were male and female patients with chronic hepatitis C who were than 20 years. Naïve cases were infected with HCV genotype 2.

The exclusion criteria were as follows: (i) patients treated with Shosaiko-to, a Japanese herbal medicine considered to improve liver function; (ii) patients with autoimmune hepatitis; (iii) patients with a history of hypersensitivity to PEG-I-α-2a or other interferons; (iv) patients with a history of hypersensitivity to biological products, such as vaccines; (v) patients with decompensated liver cirrhosis (LC); (vi) patients with hepatocellular carcinoma (HCC) or malignant tumors in other tissues; (vii) patients with or without a history of severe psychosis, such as being severely depressed and/or suicidal; (viii) women who were pregnant or lactating or who were suspected of being pregnant; and (ix) patients judged by the investigator not to be appropriate for inclusion in this study.

The patients were randomly allocated (1:1, groups of four, central randomization) to one of the following two parallel treatment groups: the 1-μg/kg PEG-I–RBV group and the 1.5-μg/kg PEG-I–RBV group. The patients of the former group received 1 μg/kg BW PEG-I subcutaneously once a week. The RBV dose was adjusted according to BW: 600 mg for ≤ 60 kg BW, 800 mg for > 60 kg BW, but ≤ 80 kg BW and 1000 mg for > 80 kg BW, based on the drug information for RBV supplied by the manufacturer. These durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare.

A lower dose of RBV was selected by the Japanese Ministry of Health, Labor and Welfare. Patients of the latter group were treated with 1.5 μg/kg BW PEG-I subcutaneously once a week. The RBV dose was also adjusted according to BW as described earlier. The daily dose of RBV was reduced by 200 mg when hemoglobin (Hb) fell below 10 g/dL, there was an acute decrease followed by the stabilization of Hb concentrations at more than 3 g/dL from baseline, or the appearance of clinical symptoms of anemia (e.g. palpitation, dyspnea on efforts, and fatigue) associated with a decrease in Hb of > 2 g/dL from baseline. Once the RBV dose was reduced, it was maintained at that level throughout the rest of study when patients complained of anemia-related symptoms of fatigue or pallor. However, RBV was discontinued when Hb fell below 8.5 g/dL or when patients manifested more severe anemia, including orthostatic hypotension. After the end of the 24-week active treatment, the patients were followed up for a further 24 weeks without treatment.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committees of all of the participating centers. Written informed consent was obtained from all participating patients.

Data collection

Visits were scheduled at baseline, after 1, 2, 3, 4, and 8 weeks of treatment, at 4-week intervals until the end of treatment, and finally, 4 and 24 weeks after the completion of treatment. At each visit, blood samples were analyzed for hematology and blood chemistry at the local hospital laboratory using standard methodology. Serum HCV-RNA was determined at baseline, after 4, 8, 12, 16, and 20 weeks of treatment, at the end of treatment, and at the end of the 24-week, drug-free follow-up period. HCV-RNA was centrally assessed by qualitative reverse transcription–polymerase chain reaction. The histopathological stage was conducted before treatment and determined based on the histological scoring system of Desmet *et al.*[19]

At each visit, information on possible side-effects was obtained by questioning the patients in a structured manner about specific, commonly observed, and expected side-effects of the study medication, such as flu-like symptoms, fatigue, nausea, vomiting, diarrhea, dizziness, depression, and hair loss.

Data management and statistical analysis

The primary objectives of the study were to show the efficacy and safety of PEG-I at 1 μg/kg versus 1.5 μg/kg. The primary study end-point was SVR, defined as HCV-RNA below the detection limit at the end of the follow-up period, that is, 24 weeks after the completion of treatment. The secondary end-points were initial and end-of-treatment virological responses at weeks 4 and 24, and virological breakthrough and relapse, that is, the reappearance of HCV-RNA during therapy and follow up, respectively. Safety and tolerability, as reflected by clinical and laboratory side-effects, were analyzed descriptively.

Non-parametric tests were used to compare variables between groups (Mann–Whitney *U*-test, two-tailed test, and Fisher's exact probability test). Missing HCV-RNA values were treated on a worst-case basis, that is, they were treated as if they would have remained above the detection limit. Thus, patients with missing values and those who abandoned the study prematurely were classified as treatment failures at the time points following withdrawal, regardless of the reason for discontinuation. The intention-to-treat (ITT) analyses for efficacy and safety were performed based on the patients who received at least one dose of the study medication.

Univariate and multivariate logistic regression analyses were used to determine the predictors of SVR. We also calculated the odds ratios and 95% confidence intervals (95%CI). All *P*-values less than 0.05 by two-tailed tests were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) upon the univariate analysis were entered into the multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following variables: sex, age, body mass index, genotype (2a or 2b), aspartate aminotransferase, alanine aminotransferase, platelet count, serum iron, serum ferritin, hyaluronic acid, viremia level, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), pathological staging, dose of PEG-I, RBV dose/BW, > 80% of RBV total dose, and reaching undetectable levels by week 4. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

Results

A total of 55 patients were enrolled. Of these, two patients were excluded from randomization because of decompensated cirrhosis.

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A total of 53 patients were randomized. Thus, 53 patients ($n = 26$, for the 1-μg/kg PEG-I–RBV group, and $n = 27$ for the 1.5-μg/kg PEG-I–RBV group) received at least one course of treatment (Fig. 1). Table 1 summarizes the baseline characteristics of the 53 patients. These were similar in the two treatment groups; the majority of patients were males, with a median age of ≥ 50 years and median BW of > 50 kg.

Efficacy

ITT analysis

The proportion of patients in the 1-μg/kg PEG-I–RBV group who exhibited a rapid decrease in HCV-RNA to undetectable levels (HCV-RNA ≤ 100 copies/mL) by week 4 (57.7%, 15/26) was not

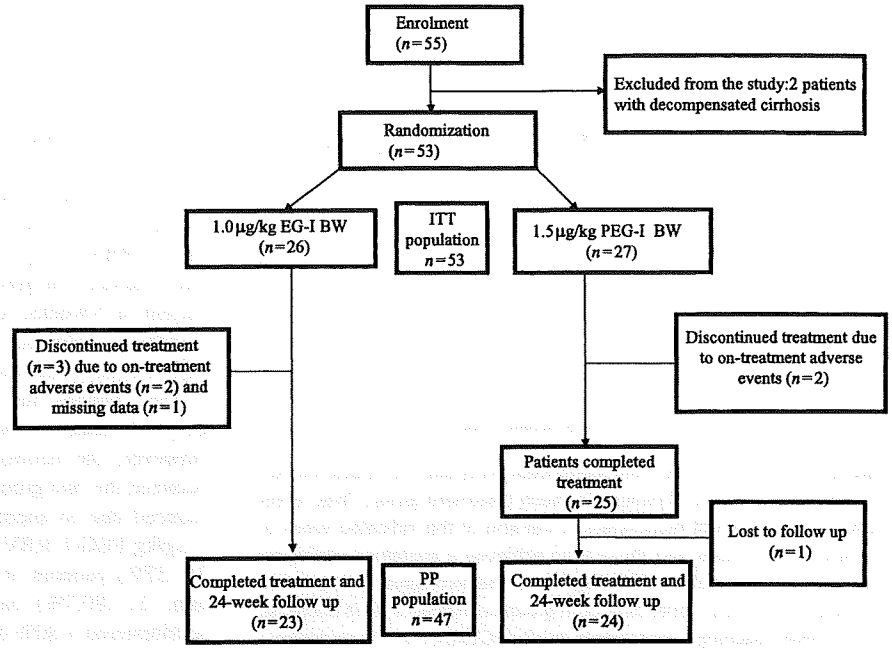

Figure 1 Flow diagram showing the number of patients who enrolled in the study and those who withdrew from the study. BW, body weight; ITT, intention to treat; PP, per protocol.

Table 1 Baseline characteristics

	1 μg/kg pegylated interferon + ribavirin ($n = 26$)	1.5 μg/kg pegylated interferon + ribavirin ($n = 27$)	*P*-value
Age (years)*	57 (31–77)	55 (41–75)	NS
Sex (male/female)	9/17	15/12	NS
Body weight (kg)*	53 (40–85)	61 (38–83)	NS
Body mass index (kg/m^2)*	22.1 (16.9–34.0)	23.9 (16.1–28.9)	NS
Genotype (2a/2b)	13/13	13/14	NS
White blood cell (×10^3/μL)*	5.8 (4.0–7.2)	5.2 (3.9–6.4)	NS
Neutrophil cell (×10^3/μL)*	3.0 (2.0–4.0)	2.8 (1.8–3.5)	NS
Hemoglobin (g/dL)*	13.3 (9.8–16.4)	13.7 (11.3–17.6)	NS
Platelet count (×10^4/mm^3)*	20.9 (15.5–27.9)	20.1 (16.2–26.9)	NS
Serum aspartate aminotransferase (IU/L)*	32 (18–164)	37 (18–203)	NS
Alanine aminotransferase (IU/L)*	31 (16–164)	34 (17–180)	NS
Serum iron (μg/dL)*	129 (12–246)	107 (60–275)	NS
Serum ferritin (μg/dL)*	75.3 (4.9–389.3)	92 (4.9–671)	NS
Total cholesterol (mg/dL)*	198 (134–249)	175 (117–279)	NS
High-density lipoprotein cholesterol (mg/dL)*	55 (25–98)	55 (32–78)	NS
Low-density lipoprotein cholesterol (mg/dL)*	124 (63.8–176.4)	104.6 (44.2–188.2)	NS
Triglycerides (mg/dL)*	93 (12.8–210)	95 (46–210)	NS
Hyaluronic acid*	51 (9–411)	47 (10.3–411)	NS
Hepatitis C virus viremia (KIU/mL)*	1100 (200–> 5000)	1700 (300–> ,000)	NS
Histological stage† (F0/F1/F2/F3)	1/14/8/3	0/13/9/5	NS

*Values are median (range); †as assessed by the local pathologist. NS, not significant.

Table 2 Adherence to therapy

Treatment	1 μg/kg pegylated interferon + ribavirin ($n = 26$)	1.5 μg/kg pegylated interferon + ribavirin ($n = 27$)	*P*-value
> 80% of pegylated interferon dose/ < 80% of pegylated interferon dose†	22/1	24/1	NS
Premature withdrawal of pegylated interferon	3	2	NS
Ribavirin dose (mg/kg)*	11.5 (9.4–15.0)	11.6 (10.0–16.0)	NS
> 80% of ribavirin dose/ < 80% of ribavirin dose†	21/ 2	22 / 3	NS
Premature withdrawal of ribavirin	3	2	NS

*Values are median (range); †actual dose was > 80% of prescribed pegylated interferon and ribavirin dose. Patients who received full-length treatment, but required dose reductions (< 80% of the originally assigned dose). NS, not significant.

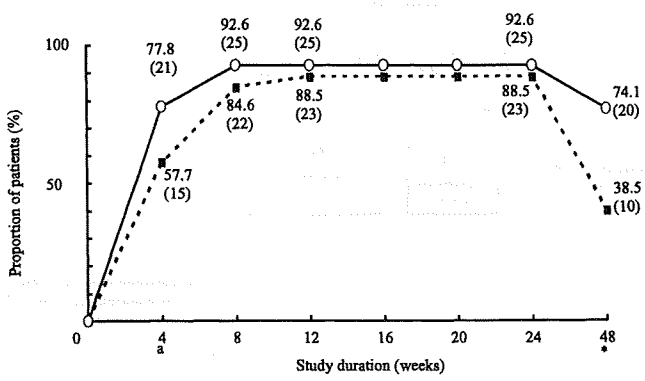

Figure 2 Results of the intention-to-treat analyses. Numbers are percentage and (number) of patients of each treatment group. Data represent the proportion of responders at the end of the indicated week of therapy (0–24 weeks) and those who achieved a sustained virological response (at 48 weeks). With regard to the virological response to combination therapy, patients of both groups exhibited a rapid decrease in HCV-RNA, reaching undetectable levels (HCV-RNA ≤ 100 copies/mL) each week. a*P* = 0.13; **P* = 0.013. -●-, 1 μg ($n = 26$); -○-, 1.5 μg ($n = 27$).

significantly different from that of the 1.5-μg/kg PEG-I–RBV group (77.8%, 21/27; $P = 0.13$). Further analysis showed that 10 of 15 (66.7%) patients of the 1-μg/kg PEG-I–RBV group who exhibited a rapid decrease in HCV-RNA to undetectable levels by week 4 achieved SVR. Twenty of 21 (95.2%) patients of the 1.5-μg/kg PEG-I–RBV who exhibited a rapid decrease in HCV-RNA to undetectable levels by week 4 achieved SVR (Fig. 2).

The proportion of end-of-therapy responders of the 1-μg/kg PEG-I–RBV group (88.5%, 23/26) was similar to that of the 1.5-μg/kg PEG-I–RBV group (92.6%, 25/27) (Fig. 2). The proportion of patients of the 1-μg/kg PEG-I–RBV group who showed SVR (38.5%, 10/26) was significantly lower than that of the 1.5-μg/kg PEG-I–RBV group (74.1%, 20/27; $P = 0.013$). Furthermore, the proportion of patients of the 1-μg/kg PEG-I–RBV group who developed viral relapse after the end of treatment (50%, 13/26) was significantly higher than that of the 1.5-μg/kg PEG-I–RBV group (18.5%, 5/27; $P = 0.047$; Fig. 2).

Tolerance of therapy and adverse events

There was no difference in the proportion of drop-out patients from the 1-μg/kg PEG-I–RBV group (7.7%, 2/26, one for depression, and one for generalized fatigue) and that of the 1.5-μg/kg PEG-I–RBV group (7.4%, 2/27, one for excitability, and one for generalized fatigue).

The dose of PEG-I was reduced in two of the 26 (7.7%) patients of the 1-μg/kg PEG-I–RBV group (one patient for thrombocytopenia at 2 weeks, and one patient for generalized fatigue at 20 weeks), and four of the 27 (14.8%) patients of the 1.5-μg/kg PEG-I–RBV group (one patient for neutropenia at 20 weeks, and three patients for generalized fatigue [one patient at 3 weeks, one patient at 5 weeks, and one patient at 8 weeks]). There was no significant difference in the proportion of patients who required a PEG-I dose reduction ($P = 1.0$).

The changes in leukocyte and platelet counts during the 24-week treatment period were similar between the two groups. However, the neutrophil cell count was significantly different between the two groups at 1 and 24 weeks. The dose of RBV was reduced due to anemia in 15 of the 26 (57.7%) patients of the 1-μg/kg PEG-I–RBV group, and for the same reason in 10 of the 27 (37%) patients of the 1.5-μg/kg PEG-I–RBV group. In addition, 21 (80.7%) patients of the 1-μg/kg PEG-I–RBV group administered > 80% of the prescribed RBV dose, while the > 80% dose was administered by 22 (81.5%) of the 1.5-μg/kg PEG-I–RBV group (P = not significant). There was no significant difference between the two groups with regard to the number of patients who required a RBV dose reduction (Table 2).

Predictors of SVR

The univariate analysis identified nine parameters that influenced SVR: age (< 60 years; $P = 0.001$), Hb (> 13 g/dL; $P = 0.021$), serum iron (< 120 μg/dL; $P = 0.044$), triglycerides (≥ 100 mg/dL; $P = 0.034$), LDL-C (< 120 mg/dL; $P = 0.061$), dose of PEG-I (1.5 μg/kg; $P = 0.009$), total RBV dose (> 80%; $P = 0.003$), and reaching undetectable levels of HCV-RNA (HCV-RNA ≤ 100 copies/mL) by week 4 (≤ 100 copies/mL; $P = 0.028$). The multivariate analysis identified two parameters that independently influenced the SVR: age (< 60 years; odds ratio 11.93, 95%CI 1.75–81.19; $P = 0.011$), and the dose of PEG-I (1.5 μg/kg; odds ratio 5.502, 95%CI 1.248–24.26; $P = 0.024$; Table 3). These results indicated that age and the dose of PEG-I are significant and independent predictors of SVR.

Discussion

Although the number of patients in this clinical trial was relatively small, our results showed a significantly lower SVR in patients of

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Table 3 Multivariate analysis of factors associated with sustained virological response to pegylated interferon–ribavirin combination therapy in patients infected with hepatitis C virus

Factors	Category	Odds ratio (95% confidence interval)	*P*-value
Age (years)	1 ≥ 60	1	
	2 < 60	11.93 (1.75–81.19)	0.011
Dose of pegylated interferon	1 μg/kg	1	
	1.5 μg/kg	5.502 (1.248–24.26)	0.024

the 1-μg/kg PEG-I–RBV group than that of the 1.5-μg/kg PEG-I–RBV group in the ITT analysis. Furthermore, the frequency of viral relapse at the end of treatment was higher in the lower PEG-I dose group than in the higher dose group. The cause of the high relapse rate was probably a result of the slower viral response of HCV-RNA in the 1-μg/kg PEG-I–RBV group. Although Meyer-Wyss *et al.*[17] reported that the virological response rates towards the commencement of treatment at week 8 and at the end of treatment at week 48 were not significantly different between the two treatment groups, in the present study, the proportion of patients of the 1-μg/kg PEG-I–RBV group who showed viral response (57.7%, 15/26) at 4 weeks tended to be lower than that of the 1.5-μg/kg PEG-I–RBV group (77.8%, 21/27; $P = 0.13$). This in agreement with the results of Rumi *et al.*,[20] who reported that failure of PEG-I therapy could be predicted by the lack of a rapid virological response in patients infected with HCV genotype 2. Moreover, patients with an early virological response seemed to have a high rate of SVR.[21–23] Accordingly, the time of viral response in the 1-μg/kg PEG-I–RBV group will be achieved later than that of the 1.5-μg/kg PEG-I–RBV group. This suggests that if an early virological response is not evident, treatment with PEG-I should probably be extended to 48 weeks in order to increase viral clearance and improve the SVR rate. In this regard, treatment with PEG-I–RBV for 16 weeks in patients infected with HCV genotype 2 or 3 is reported to achieve a lower overall SVR rate than the standard 24-week regimen.[24]

The SVR rate of the 1-μg/kg PEG-I–RBV group was lower than that reported in previous studies.[17,18,25–27] It is possible that these differences are related to differences in race, age of studied patients, and the dose of RBV. The mean age of our patients was 50 years, but has been reported to be 30–40 years in previous studies.[7,18,25,26] In addition, while previous studies evaluated patients infected with HCV genotypes 2 and 3, the number of patients infected with genotype 2 was small.[17] Although Meyer-Wyss *et al.*[17] reported that although SVR rates in patients infected with HCV genotypes 2 and 3 were similar between patients treated with 1 or 1.5 μg/kg PEG-I BW, were differences between the virological response (85%) at the end of treatment and the virological response (71%) at the end of follow up, in patients treated with 1-μg/kg PEG-I. This means that a high proportion of patients of the 1-μg/kg PEG-I–RBV group developed viral relapse after the end of treatment.

The dose of RBV used in the present study was lower than that used in previous studies.[17,18,25–27] The above durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare. A lower dose was selected by the Japanese Ministry of Health, Labor and Welfare. In this regard, 21 (80.7%) patients of the 1-μg/kg PEG-I–RBV group were administered > 80% of the prescribed RBV dose, while the > 80% dose was used by 22 (81.5%) patients of the 1.5-μg/kg PEG-I–RBV group (P = not significant). There was no significant difference between the two groups with regard to the number of patients who required RBV dose reduction. In addition, there was no significant difference between the two groups with regard to the concentration of RBV at 8 weeks (data not shown).

In our study, the proportion of patients of the 1.5-μg/kg PEG-I–RBV group who showed SVR was significantly higher than that of the 1-μg/kg PEG-I–RBV group, despite the lack of a significant difference in the exposure to RBV. The proportion of end-of-therapy responders of the 1-μg/kg PEG-I–RBV group was similar to that of the 1.5-μg/kg PEG-I–RBV group. Although the proportion of patients who exhibited a rapid decrease in HCV-RNA to undetectable levels (HCV-RNA ≤ 100 copies/mL) by week 4 was similar in the two treatment groups, the proportion of patients of the 1-μg/kg PEG-I–RBV group who showed viral response (57.7%, 15/26) at 4 weeks tended to be lower than that of the 1.5-μg/kg PEG-I–RBV group (77.8%, 21/27; $P = 0.13$). This finding suggests the clinical importance of the time period during which HCV-RNA is at undetectable levels (≤ 100 copies/mL).

In the IDEAL (Individualized Dosing Efficacy Versus Flat Dosing To Assess Optimal Pegylated Interferon Therapy) study, the proportion of 1-μg/kg PEG-I–RBV patients who developed SVR was similar to that of the 1.5-μg/kg PEG-I–RBV group.[27] We believe that there was no significant difference in the exposure to PEG-I between the IDEAL study and the present one with respect to SVR. However, no information was provided in the IDEAL study on the rapid virological response and end-of-therapy response. Therefore, we could not compare our rapid virological and end-of-therapy responses with those of that study.

Based on the above results, we believe that the time period during which HCV-RNA is at undetectable levels (≤ 100 copies/mL) is more important than the dosage of PEG-I in 24-week treatment regimens, that is, we prefer to use the 1.5-μg/kg PEG-I–RBV regimen since it is more likely to achieve a rapid virological response than the 1-μg/kg PEG-I–RBV regimen.

Our study showed no significant difference between the two treatment groups in the tolerance of therapy and adverse events. At the start of the study, we believed that the number of patients of the 1.5-μg/kg PEG-I–RBV group who would require a PEG-I dose reduction or termination of such therapy would be greater than that of the 1-μg/kg PEG-I–RBV group. However, the data analysis showed no significant difference in the proportions of such patients between the two groups. Furthermore, there were no significant differences in the rate of change in leukocyte and platelet counts over 24 weeks of therapy between the two groups, and neutrophil cell counts of the 1.5-μg/kg PEG-I BW group at 1 and 24 weeks were significantly lower than those of the 1-μg/kg PEG-I BW group. These results indicated that 1.5 μg/kg PEG-I BW is a safe regimen.

The multivariate analysis showed that SVR was dependent on the age of the patient and the dose of PEG-I. Other studies reported a higher SVR rate for young patients than older patients.[28–30] Interestingly, the dose of PEG-I was a significant and independent predictor of SVR. Furthermore, other studies reported that no or mild hepatocyte steatosis was a significant factor associated with SVR.[31] However, we did not investigate hepatocyte steatosis because of the small sample used in this study.

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In conclusion, the dose of PEG-I to be used at start of therapy of naïve Japanese patients infected with HCV genotype 2 should be 1.5 μg/kg BW.

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C型肝炎 難治例の治療の実際
―治療効果向上のための工夫をどう行うか

高齢者・女性のC型肝炎患者に対する治療

高橋祥一*

Summary

高齢者・女性のC型肝炎患者はペグインターフェロン/リバビリン併用療法に対し，忍容力が低く減量中止例が多い点，また治療完遂しても早期のウイルス陰性化が得られにくい点から奏効率が低くなっている。奏効率改善のためには72週の長期投与が有用で，特に高齢女性の奏効率を有意に改善する。ただし長期間の治療を余儀なくされるため，適切な投薬量のコントロールが必要である。

Key Words　PegIFN/RBV 併用療法／高齢者／女性／72週投与

はじめに

わが国におけるC型肝炎患者新規感染は，第2世代C型肝炎ウイルス（HCV）抗体がスクリーニング検査として使用されるようになり，輸血などの医原性感染がほとんど認められなくなった1992年以降は激減した。これに伴い，わが国のC型肝炎患者は年々高齢化し，その平均年齢は60歳代半ばに達している。日本人の平均寿命の延長も相まって，以前はインターフェロン（IFN）治療の対象とは考えられていなかった65歳以上のC型肝炎患者もその対象と考えられるようになってきている。C型肝炎患者が高齢化し，加齢と肝細胞癌（HCC）の発現との関係が明らかになる一方で，最近のIFN療法による発癌予防効果の報告は[1,2]，高齢者に対するIFN療法の動機づけをさらに強めている。

2004年にわが国においても処方可能になったペグインターフェロン（PegIFN）/リバビリン（RBV）併用療法は，それまでのIFNの治療効果を劇的に改善し，難治性のgenotype 1b/高ウイルス量（1b/high）症例のIFNの著効率（sustained viral response：SVR）が約50%，比較的効果の出やすい2a，2b型のSVR率は90%程度まで見込めるようになった。多くのC型肝炎患者がこのPegIFN/RBV併用療法の恩恵を受ける一方で，治療終了後に再燃したり，治療を途中で中止する症例も散見された。これらの再燃中止例の大部分は高齢者や女性のC型肝炎患者であった。本項では高齢者・女性のC型肝炎患者の特徴とその治療成績，今後の対策について概

*広島大学大学院分子病態制御内科学／広島大学病院消化器・代謝内科講師

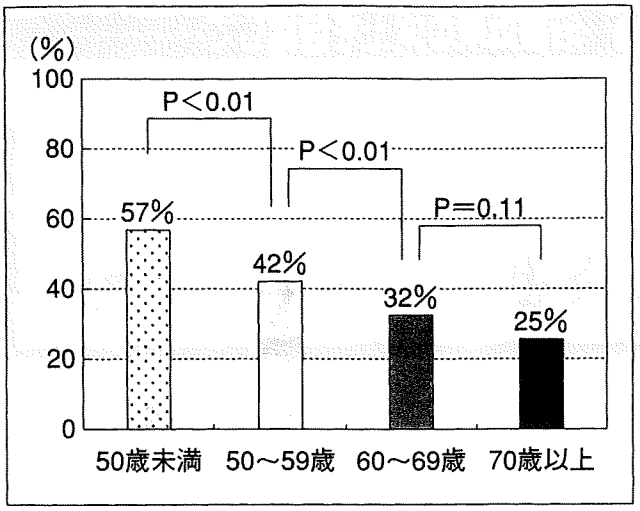

図1　SVR率（1b/high）—年齢別

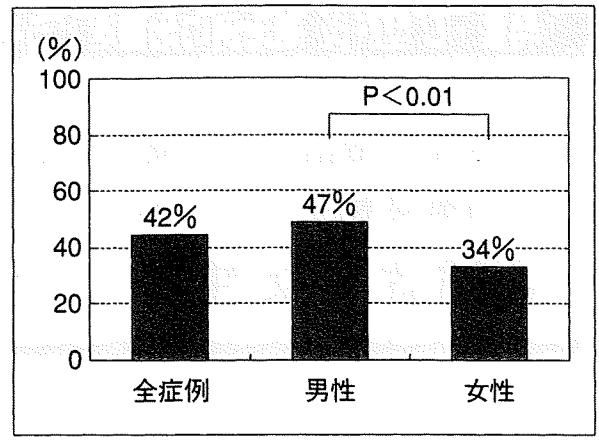

図2　SVR率（1b/high）—男女別

説する。

高齢者・女性のC型肝炎に対するIFN治療の成績

高齢者や女性に対するIFNの治療効果が低いことは以前から知られていたが，我々も，以前PegIFNが開発されるまでに頻用された，従来型のIFNとRBVの併用療法時代（24週投与）の高齢者（70歳以上）と非高齢者の治療効果について比較した。1b/high症例におけるSVR率は非高齢者の29%に対し，高齢者は6%と有意に低く，また治療完遂率も非高齢者の81%に対し，高齢者では62%と有意に低い結果であった。1b/high以外の症例（others）ではSVR率は非高齢者で67%，高齢者で50%と有意差を認めず，高齢者でも比較的高値であったため，1b/highの症例では高齢者の根治治療を目指すのは困難とした一方で，othersではSVRを目指して高齢者にも積極的にIFN治療を勧めるべきであると結論づけた[3]。一方で，現在のゴールデンスタンダードと考えられているPegIFN/RBV併用療法における高齢者のSVR率は，若年者に比べて明らかに低く，当科および広島肝臓スタディーグループでの集計（n=1,082）によると，1b/highで48週間標準投与症例の50歳未満，50歳代のSVR率が57%，42%である一方で60歳代，70歳以上のSVR率は32%，25%と低値であり，以前の従来型IFNとRBV併用療法の成績に比べると良好ではあるが，やはり高齢者のSVR率は低い結果であった（**図1**）。また男女間のSVR率の差も顕著であり，男性のSVR率 47%に対して，女性のSVR率は34%と有意に低値であった（**図2**）。そこでこの両方を掛け合わせると，50歳未満は男女ともに50%以上と高いSVR率を認めるも，年齢が進むにつれ徐々にSVR率は低下し，特に60歳代の女性ではSVR率 25%，70歳以上の女性ではSVR率は16%と60歳以上の高齢女性で有意なSVR率の低下を認めた（**図3**）。一方で1b/high以外のothersでは，50歳未満，50歳代で81%，80%のSVR率，60歳代，70歳以上で62%，61%とやはり高齢者ではSVR率は低下する傾向にあったが，比較的良好な成績であった。

高齢者・女性では，IFNに対する忍容性の問題や副作用出現による薬剤の減量や中止が多く，いわゆる治療の完遂率が低いことが指摘されている。では，高齢者・女性では治療が完遂できないためにSVRが低いのか，あ

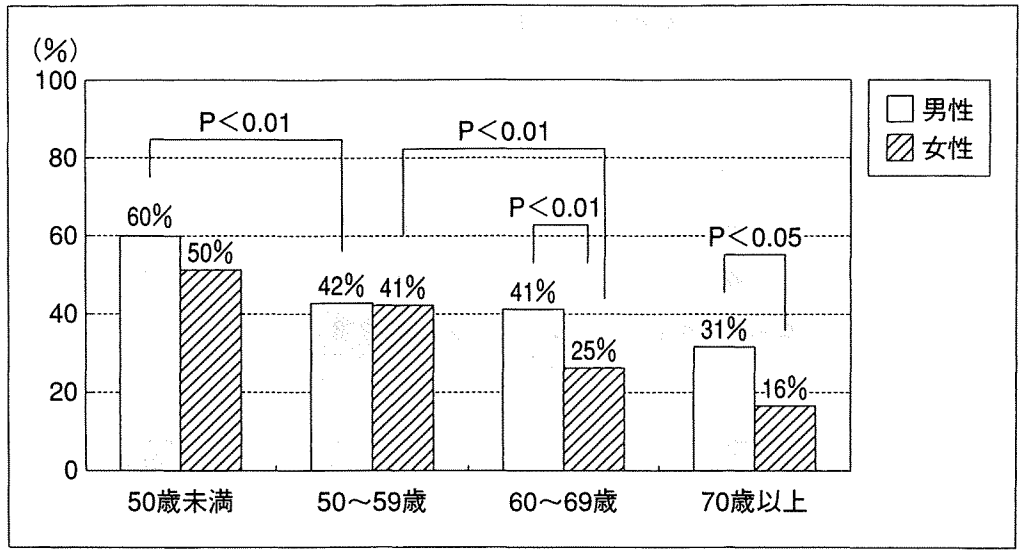

図3　SVR率（1b/high）—年齢別・男女別

るいはもともとIFNに対する感受性が低いのかを検討した。まず当科における従来型のIFNとRBVの併用療法の完遂率は，非高齢者 83%に対し高齢者 62%と高齢者で有意に低い結果であった（$p<0.005$）。また比較的副作用が低いと考えられるPegIFN/RBV併用療法での完遂率は，非高齢男性で78%，非高齢女性で75%であったのに対し，高齢男性で69%，高齢女性で67%と，こちらも非高齢者より高齢者の方で完遂率が低い結果であった（$p=0.03$）。次に完遂した症例のみで検討するper protocol studyでみてみると，従来型のIFNとRBV併用療法では，非高齢者のSVR率が35%である一方で高齢者ではSVR率はわずかに9%であった。またPegIFN/RBV併用療法における完遂症例でのSVR率は，男性では50歳代，60歳代，70歳以上で55%，54%，46%と治療完遂できればあまりSVR率に違いを認めなかったが，女性では50歳代，60歳代，70歳以上でそれぞれ52%，35%，28%と60歳を過ぎると仮に治療完遂したとしてもSVR率が低下していた（**表1**）。すなわち，高齢男性でSVR率が低いのは，副作用などで治療中止を余儀なくされる症例が多いためであり，高齢女性でのSVR率の低下は，治療中止が多いことに加えて，仮に治療完遂したとしても，もともと非高齢者に比してIFNの効果が低いことが原因であると考えられた。

表1　治療完遂例における男女別・年齢別SVR率

SVR率	50歳未満	50～59歳	60～69歳	70歳以上
男性	74%	55%	54%	46%
女性	64%	52%	35%	28%

高齢者・女性のC型肝炎治療に対する治療の工夫

高齢者・女性ではなぜ非高齢者・男性に比べてIFNに対する効果が低いのか？　この理由の一つは，高齢者・女性ではPegIFN/RBV治療開始後ウイルス量が測定限界以下になる時期が遅いからと考えられている。一般にIFN治療開始後4週間以内にHCV量が測定限界以下になるrapid viral responder（RVR）症例や12週間以内にHCV量が測定限界以下になるearly viral responder（EVR）症例では，それ以外の症例に比べて明らかにSVR率が高いことが知られている。我々の検討では，RVR症例ではSVR率 94%，

表2　治療開始12週目におけるウイルス消失（EVR）率

	EVR率		EVR率
50歳未満 男性	65%	50歳未満 女性	53%
50～59歳 男性	58%	50～59歳 女性	51%
60～69歳 男性	51%	60～69歳 女性	34%
70歳以上 男性	41%	70歳以上 女性	18%

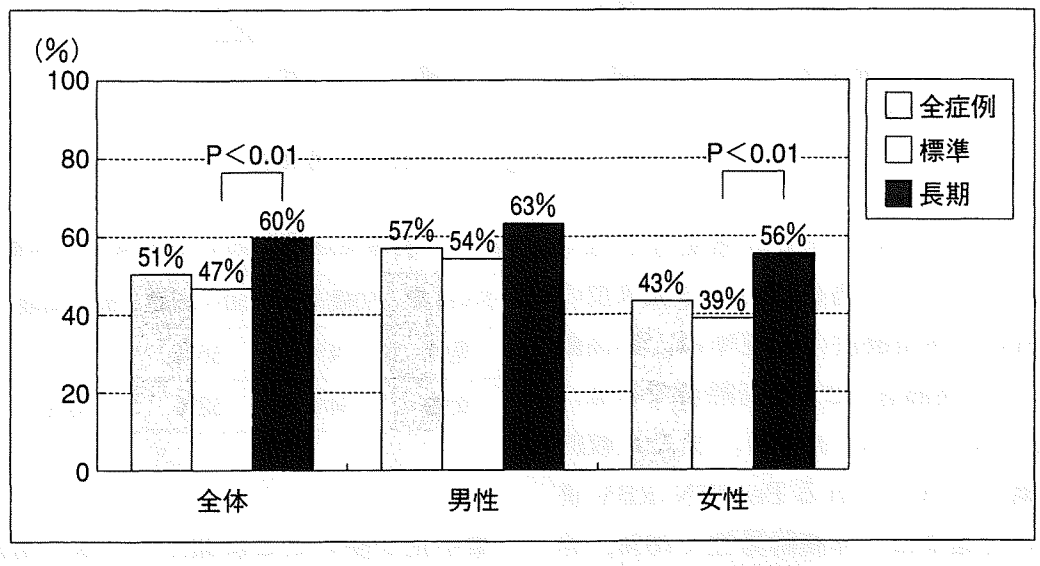

図4　SVR率（1b/high）—男女別・投与期間別

EVR症例ではSVR率が67%，非EVR症例ではSVR率は20%であった。したがって，少なくとも12週までにHCVが消失すれば，SVRが見込めることになる。そこで年齢別・男女別のIFN治療開始12週目でのウイルス消失率（EVR率）を検討したところ，男性のEVR率は50歳未満，50歳代，60歳代，70歳以上でそれぞれ65%，58%，51%，41%であった。一方女性の12週目のEVR率は53%，51%，34%，18%であり，高齢者・女性でのEVR達成率は低いことが示された（**表2**）。

ウイルス消失時期がIFN投与開始12週以降になる症例（late viral responder：LVR）では，逆にみればウイルス消失を達成してからさらにIFNを投与できる期間が，RVR症例やEVR症例に比べて短いため，どうしても治療終了後の再燃が多くなる。このようなLVR症例のSVR率を改善するために考えられたのが治療期間の延長で，具体的には72週間のPegIFN/RBV併用療法が推奨されている。Bergらは，13～24週の間に初めてウイルスが消失したLVR症例で48週の標準投与を行った群と72週の延長投与を行った症例を比較し，HCV RNAが陰性化した後治療終了後にHCV RNAが陽性になった再燃率は48週群で64%に対し，72週群で40%と有意な改善を認めたと報告した[4]。我々も同様の検討を行い，48週の標準投与と長期投与（中央値72週）を比較すると男女いずれにおいても長期投与によってSVR率の改善を認め，特に女性において有意にSVR率の改善を認めた（**図4**）。また年齢別にみても，特に高齢者で

SVR率の改善を認めている。したがって高齢者・女性でEVRが得られない症例に対しては，可能ならば72週間の長期投与が推奨される。しかしながら前述のごとく，高齢者・女性では副作用などで中止せざるをえない症例も少なくないため，適切時期での薬剤の減量が重要である。特に高齢者でのリバビリンによる貧血の進行が治療中止の原因になることが多いため，豊田らのリバビリンの全身クリアランス（CL/F）算出式の利用や平松らの2 by 2 ruleなどを用いた適切なRBV量の調節が推奨される[5,6]。

高齢女性のSVR率が著明に低いのは事実で，閉経の関与が示唆されている。このため女性ホルモン補充療法などの報告が学会などで散見されるが，まだはっきりとしたエビデンスは出ていない。また培養細胞などの実験レベルでは，HCVの複製に関与する脂質代謝を阻害するスタチン系の抗高脂血症薬やビスホスホネート製剤などの有用性が報告されており，これを応用したPegIFN/RBV併用療法との3剤併用で臨床研究が行われている。こちらも学会発表レベルでは報告が散見されるが，臨床での明らかなエビデンスはいまのところ出ていないのが現状である。

今後プロテアーゼ阻害剤などの新規薬剤が臨床応用されれば高齢者・女性のSVR率の飛躍的な改善が見込まれるかもしれないが，本邦で承認されるのはまだ数年先である。すでに高齢化したC型肝炎患者をさらに何年も肝庇護剤で待たせる愚は避け，前治療でのnull responderやHCVコア領域アミノ酸変異などをもち，null responseが強く予想される症例を除けば，現在可能なPegIFN/RBV長期投与でHCV排除を目指すのが得策と思われる。

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